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Oral Delivery of Poorly Soluble Actives – From Drug Discovery to Marketed Products

Tokyo, Japan • June 6, 2003

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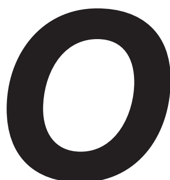
Introduction



OFFERING A PLATFORM for the exchange of scientific ideas is a very well-known and well-established Capsugel commitment. We have been doing this for 12 years and the best examples has been the series of symposia we organized around the Biopharmaceutic Classification System (BCS) and the optimization of oral drug delivery systems.

Today, we are going to look at oral delivery of poorly soluble actives, from drug discovery to marketed products. Poor aqueous solubility is an industry wide problem in ADME screening. Newer drugs are larger, more lipophilic and less permeable. Whatever is the reason for this poor solubility, excessive lipophilicity or crystal packing, formulating this kind of components is a real challenge.

Thus we are here to assess where we are today and to discuss the factors determining the bioavailability of these drugs. What can be the novel approaches to formulate class II components? As usual some industrial experts will share their experience and expertise by presenting real cases. The drug regulation of poorly soluble actives will also be reviewed.

In 2002, the market share of poorly soluble actives was 26% or 110 billion USD. They represented 58% of the NCE's launched. It is well accepted that 40 - 50% of product under development are class II products. No need to say that we need to try different approaches to circumvent the issues to formulate such components.

Some industrial examples will be presented at the end of the symposium and will demonstrate that new industrial technologies are now available to achieve an acceptable absorption.

Our ultimate goal in organizing and publishing these symposia is to provide timely input to build the science around the formulation of poorly soluble actives. Your feedback will tell us if we achieved our objectives.

Looking forward to hearing from you.

Roland Daumesnil

Director, Global Business Development - Pharmaceutical

Opening remarks – An overview

Professor Shinji YAMASHITA

Opening remarks – An overview

Professor Shinji Yamashita

Setsunan University

Noriko Yamanuchi, Capsugel Japan: Ladies and gentlemen, I am Noriko Yamanouchi of Capsugel Japan. We are very pleased to open this symposium on the Oral Delivery of Poorly Soluble Actives. I'd like to welcome you. The Masters of Ceremony for today are myself, and Roland Daumesnil of Capsugel.

As for today's program, in the morning there will be lectures and a discussion and in the afternoon our invited lecturers are scheduled for an open discussion forum. We have to go through a very tight schedule today and we really would hope for your co-operation in this. On behalf of the organizers, I would like to ask Professor Yamashita of Setsunan University to say a few opening words.

Professor Yamashita, please.

Chair, Professor Shinji Yamashita, Setsunan University: Good morning, I am very pleased to be here and on behalf of the organizers would also like to thank the audience for coming. Our topic today is the discovery and design of poorly soluble actives and this is the latest in this series of symposia. I'd like to thank Roland Daumesnil, Phil and other colleagues for Capsugel Inc.'s involvement. I would like to extend our hearty appreciation for your support of this symposium.

Before I open the symposium I must inform you that my co-organizer, Professor Gordon Amidon of Michigan University, who was very much looking forward to coming to this symposium, is unable to do so through unavoidable circumstances. He has asked me to convey his best regards and sincere apologies to you. In his place and speaking on his behalf we have another distinguished lecturer, Professor James Polli. Dr. Polli is a young professional scientist who specializes in this area and I believe you will really enjoy hearing what he has to say.

With your permission I would now just like to tell you about the purpose of the symposium and the topics we will be discussing. The title of today's symposium, as mentioned in the introduction, is the Oral Delivery of Poorly Soluble Actives: from Drug Discovery to Marketed Products, and we will be exploring the subject comprehensively. Let me give you an overview of why we have chosen this topic.

In the past 10 years, drug development strategy has undergone drastic changes. Now that we are using combinatorial chemistry or high-throughput screening methods, we have been able to produce

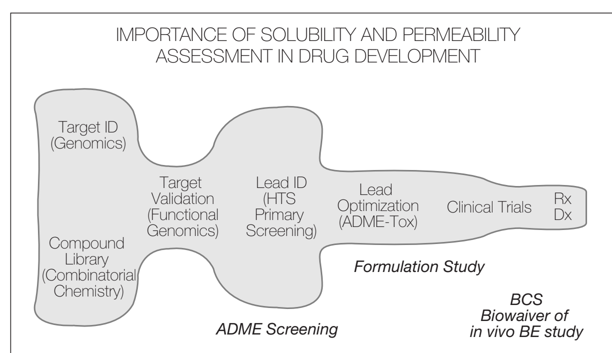


Figure 1.

numerous candidates which can be further screened (*Figure 1*). High-throughput screening (HTS) and combinatorial chemistry are targeted at pharmacological efficacy, or the activity, and so the lead optimization stage, or the ADME screening stage, could turn out to be a barrier to further development. How can these issues be resolved, particularly with oral formulation?

Of course, solubility is important. Once the drug is dissolved it then permeates through the intestinal membrane, which means both the permeabilityand solubility have to be evaluated before reaching formulation stage. Once solubility and permeability have been determined and assessed, we have to narrow down the NCE candidates during early stage development, so screening becomes important. These parameters continue to be important at formulation stage.

Guidance for Industry

Waiver of *In-Vivo* Bioavailability and Bioequivalence
Studies for Immediate-Release Solid Oral Dosage Forms
Based on a Biopharmaceutics Classification System

Additional copies are available from:
Office of Training and Communications Division of Communications
Management Drug Information Branch,
HFD-210 5600 Fishers Lane Rockville, MD 20857
(Tel) 301-827-4573 (Internet) http://www.fda.gov/cder/guidance/index.htm

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
August 2000
BP

Figure 2.

Also, when those drugs are in clinical trial or at post-marketing stage, we have to comply with regulations such as the US guidance brought out by the FDA (Food and Drug Administration), which includes the Biopharmaceutical Classification System (BCS). Where this particular standard is concerned we would, of course, like to obtain a waiver of the *in-vivo* bioequivalence study (*Figure 2*). Therefore, solubility and permeability have to be evaluated at every stage of development and by using those parameters we can achieve a shorter time to market for new chemical entities. That is the topic for today.

With both early-stage development and regulatory adherence, the parameters are the same, even though the criteria and methodology may be different.

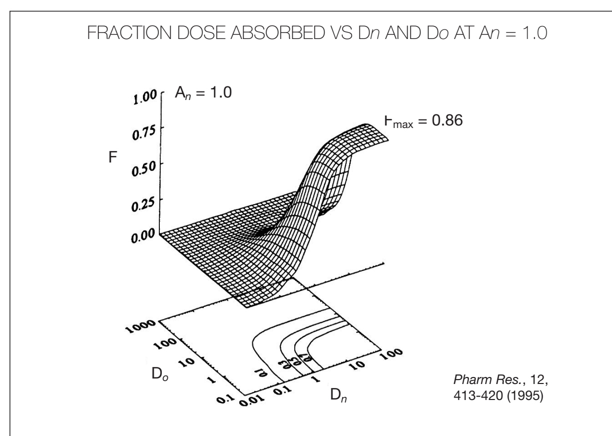


Figure 3.

In fact, it is important to look at the total scientific picture. *Figure 3* is a well-known chart showing the dissolution rate and solubility. To arrive at the permeability, three parameters have to be evaluated (An, D_o and D_n). The F_{max}, or the absorption, can then be determined by adjusting the three parameters.

At every stage of pharmacological development, specialist scientists have to look at those parameters to optimize the formulation and maximize the efficacy and safety of the drug. How to achieve this is the basis of the discussion that we are going to have.

With regard to the associated regulatory issues, Professor Lawrence Yu will be discussing the BCS, his area of expertise at the FDA regulatory agency. He will be focusing on the important parameters of solubility, permeability and dissolution and discussing their relevance and the criteria involved (*Figure 4*). Using those parameters you may be able to re-examine your candidate drugs with a view to obtaining a bio-

Class boundary in BCS

Solubility
- **Highly soluble** when the highest dose strength is soluble in < 250mL water over pH range of 1 to 7.5 at 37 °C.

Permeability
- **Highly permeable** when extent of intestinal absorption in humans is > 90% of dose.

Dissolution
- **Rapidly dissolving** when > 85% dissolves within 30 min in 0.1 HCl, pH 4.5, and pH 6.8 buffers.

Figure 4.

BCS in Drug Discovery and Development

Drug Discovery
- Scientific insights to rank order compounds
  Solubility, Permeability,
  Stability, etc……
- Selection of compounds for future evaluation

Drug Development
- Determination of causes of poor oral drug absorption
- Formulation strategies

Figure 5.

waiver of the *in-vivo* studies or you may be able to simplify the later stage of the development process.

It is important to consider these different applications from early development stage (*Figure 5*). Indeed, even at drug discovery stage. For instance, it can be useful in meeting the regulatory requirements for the compounds, or it might help in coming up with the scientific rationale behind the candidates or in selecting the most suitable compounds for future development.

At late formulation stage you will again need to use these parameters to determine the cause of poor absorption, for instance. In fact, you will be relying on those parameters and their different criteria throughout the later stages of drug development, and one of today's topics will be about the most efficient and effective ways of utilizing them at each stage of development.

Figure 6, for instance, shows the relationship between solubility and permeability. At an early stage, at least, 20 percent absorption seems to be the cut-off

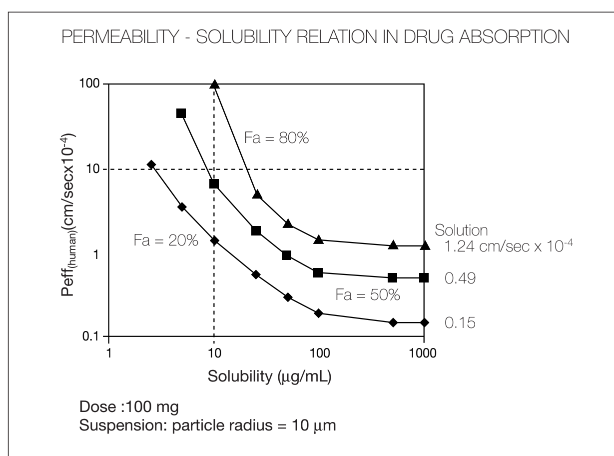


Figure 6.

PERMEABILITY - SOLUBILITY RELATION IN DRUG ABSORPTION

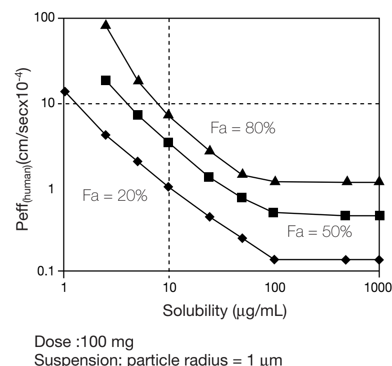


Figure 7.

value. At this point, you can choose the candidates likely to have those properties. Then at a later stage when you perhaps might want 80 percent absorption, you might select the compounds within the right-hand quadrant of *Figure 7*. Of course it depends upon particle size and so forth, and on how well the formulation technology works in terms of delivering the maximum range of drug efficacy.

Likewise, if we apply the BCS in discovery stage and find by formulation stage that it is a Class I drug, meaning that it has high solubility and high permeability, there is no problem (*Figure 8*). But if the drug is Class II, with low solubility, then a formulation study may be needed to increase absorption; even a Class II drug can be dealt with at pre-formulation stage. So these are the issues to be incorporated into your drug development strategies.

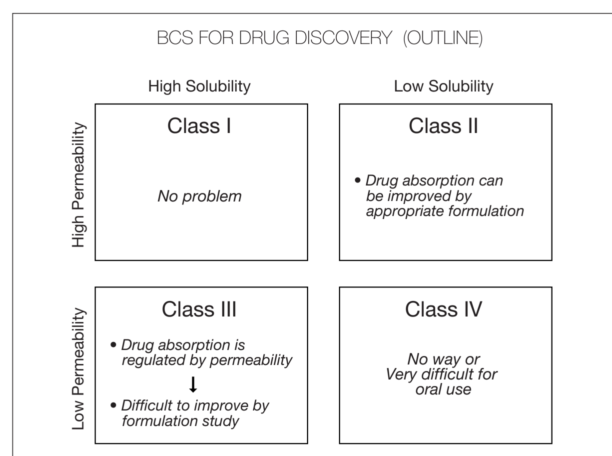
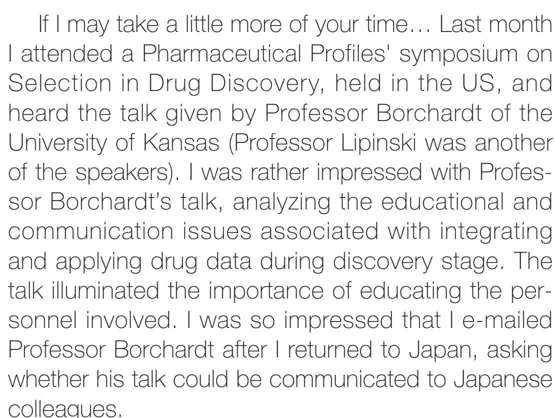


Figure 8.



If I may take a little more of your time… Last month I attended a Pharmaceutical Profiles' symposium on Selection in Drug Discovery, held in the US, and heard the talk given by Professor Borchardt of the University of Kansas (Professor Lipinski was another of the speakers). I was rather impressed with Professor Borchardt's talk, analyzing the educational and communication issues associated with integrating and applying drug data during discovery stage. The talk illuminated the importance of educating the personnel involved. I was so impressed that I e-mailed Professor Borchardt after I returned to Japan, asking whether his talk could be communicated to Japanese colleagues.

I would just like to use some of the ideas in his talk to illustrate how important the human element is in the drug development program. For instance, when we are talking about technologies such as solubility assessment and how to improve absorption, we have to start with the question: what do we need for development? And the correct answer is, what we need is the right scientists. What do we mean by 'the right scientists'? Well, Professor Borchardt drew up a list of the necessary qualifications and the two most important are, scientific depth and scientific breadth.

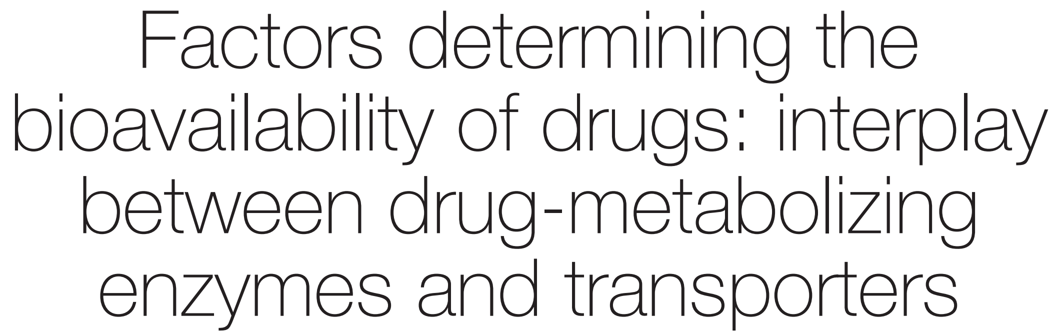
Scientific depth means having a profound understanding of your own scientific discipline, and scientific breadth means understanding related disciplines as well. So both are needed – breadth and depth. Until now, the educational focus has been solely on scientific depth. As a result, even if we have great scientists they only understand their own discipline. There are many drawbacks in this attitude which must be overcome in trying to meet the US protocol for the development program.

The current educational program fails either at discovery stage or at formulation stage. For instance, in the course of development, the way to deal with precisely the same compounds will vary. Maybe you need 80 percent purity at the initial stage but in the later stage you need 99 percent purity. Unless you understand the different criteria involved there will be conflict between scientists in different disciplines.

Professor Borchardt would therefore like scientists to have both types of understanding because, in the knowledge-based future, the focus will be on a multidisciplinary approach incorporating scientific depth and breadth. Whether your background is in chemistry, biology, or pharmaceutics, multidisciplinary training is necessary so that we can achieve a more efficient and more effective drug development program in the future.

Although this is a one-day seminar, if you are a chemist, biologist or pharmaceutical scientist, please look at the other disciplines so that your scientific breadth can be expanded. With this I conclude my opening remarks. Thank you very much for your attention.

Well, let us get into the first morning's session, which I am chairing. In this morning's session we have two special speakers. The first honorable lecturer is Professor Yuichi Sugiyama of the University of Tokyo. For his career background, please refer to the first page of your handout. Actually, he needs no introduction, therefore I will be very brief. I would like to give him plenty of time to discuss the topic, while leaving some time for discussion. His lectures are always insightful and stimulating, and his talk incorporates both scientific depth and breadth. So – Professor Sugiyama.



Factors determining the bioavailability of drugs: interplay between drug-metabolizing enzymes and transporters

Professor Yuichi SUGIYAMA

Factors determining the bioavailability of drugs: interplay between drug-metabolizing enzymes and transporters

Professor Yuichi Sugiyama

University of Tokyo

Professor Yuichi Sugiyama, University of Tokyo: Thank you very much for the introduction. First of all, let me express my gratitude to my colleague Roland Daumesnil for holding this stimulating symposium. Of course I also appreciate Professor Yamashita's work as an organizer – he is a great organizer. Also, yesterday our speakers and chair persons got together and I would like to thank all the staff from Capsugel.

As Professor Yamashita has already mentioned, intestinal absorption depends on a membrane permeability, rate and extent of dissolution and metabolic stability. In recent years, various drug transporters in the liver and the small intestine have been analyzed to determine the pharmacokinetics of the compounds – together with, of course, metabolizing enzymes. Hepatic enzymes, such as the P450, transporter play an important role. Today, I will be talking about the transporters in the gastrointestinal (GI) tract and the liver, and the interplay of these important transporters.

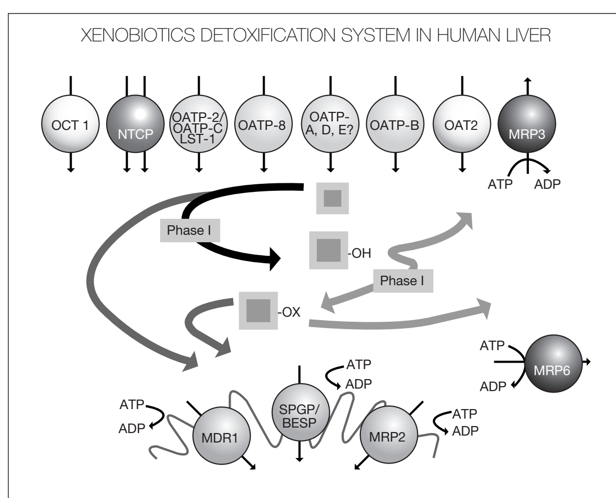
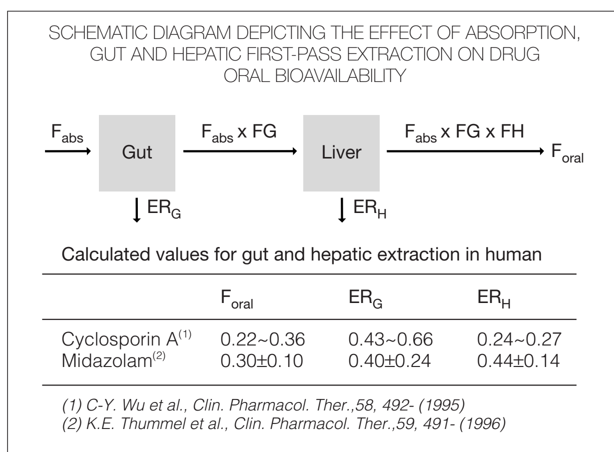


Figure 1.

I'll start with the GI tract transporters but before I do so I'll just mention xenobiotic detoxification. *Figure 1* shows the liver. Various xenobiotics come into the liver from the vessel, the parent compound is metabolized and then an efflux system comes into operation to remove them. In the GI tract the efflux transporter and the influx transporter may interplay, working together to detoxify the xenobiotics in those organs.

Now I'll talk about P-glycoprotein (P-gp) and the CYP3A4 enzyme. *Figure 2* is a very well-known schema from 1996 by Les Benet. It is a comparison of cyclosporine A and midazolam. If you run a pharmacokinetic study in human with these orally administered drugs, bioavailability is about 30 percent. But within the gut the clearance, or the metabolism, is similar to that of the liver or maybe higher. This was the first occasion that they were able to demonstrate this. Here in this case both drugs are the 3A4 substrates, and Cyclosporine is a good substrate also for P-glycoproteins. What Les Benet frequently says is that CYP3A4 and P-glycoprotein have substrate features in common; they share the same substrate and the same inhibitors. However, there are exceptions, so this is just a rule of thumb and not a universal rule.

Figure 3 shows that it can work as the substrate. From the lumen the drug is taken up by the epithelial



	F_{oral}	ER_G	ER_H
Cyclosporin A[1]	0.22~0.36	0.43~0.66	0.24~0.27
Midazolam[2]	0.30±0.10	0.40±0.24	0.44±0.14

(1) C-Y. Wu et al., Clin. Pharmacol. Ther.,58, 492- (1995)
(2) K.E. Thummel et al., Clin. Pharmacol. Ther.,59, 491- (1996)

Figure 2.

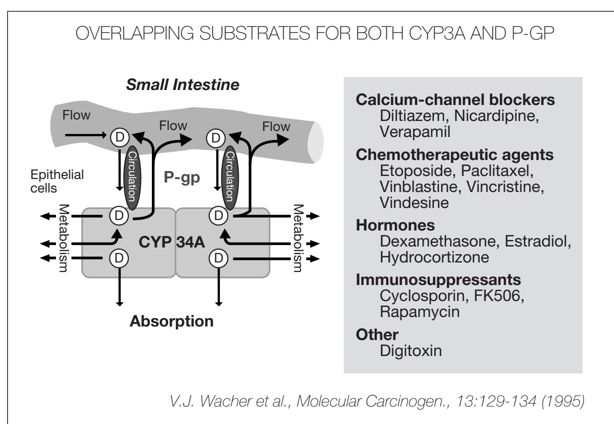


Figure 3.

cells of the GI tract and if there is P-glycoprotein the efflux system works, and then after efflux there is movement downward the intestinal lumen, and the uptake and efflux recycle many times. Without P-glycoprotein, of course, the drug would be more efficiently absorbed in the bloodstream. However, the presence of the P-glycoprotein means increased exposure with a high probability of the metabolism because of the recycling through the joint effect of the P-glygoprotein and CYP3A4.

But first of all, what is P-glycoprotein? Let me show you a rather good animation (Video not supplied). Suppose this is the layer of the epithelial cell, this is the lumen side and this is the cytosol side. The drug or the xenobiotic is transported in the lumen and then it is permeated through the lipid bilayer. Within that layer P-glycoprotein recognizes the drug and the conformational change of P-glycoprotein takes place, and then the efflux system works and the xenobiotics are taken out to the lumen. In this model, of course, the substrate binds to the P-glycoprotein within the lipid bilayer membrane and then it is taken out so the P-glycoprotein is working as a flippase here. Now I am going to talk about the work done by Dr. Kiyomi Ito, who is now at Kitasato University. She and I heard Les Benet's talk and we thought that we might be able to come up with a mathematical model to illustrate the concept which Dr. Benet described, and we started on this work. We used the partial differential equation with boundary condition shown in *Figure 4*. A boundary condition is the condition at the membrane wall of luminal side, which then incorporates the metabolism/diffusion/influx and the P-glycoprotein mediated efflux. The *Figure* shows the initial conditions and the boundary conditions which explain the interplay of these two molecules, that is P-glycoprotein and CYP 3A4. The work was published in *Pharmaceutical Research*.

To make a long story very short, if you look at the left-hand side of *Figure 5*. Only the inhibition of the P-glycoprotein affect the intestinal absorption to some extent. So did the inhibiton of only CYP 3A4. However, when both are inhibited, the change in absorption is quite large, larger than with the single inhibition, so a synergetic effect is clearly shown in our mathematical model.

Although we were able to calculate this, it is not so easy to understand intuitively so I asked Dr. Ito to come up with the schema in *Figure 6.* In simple language, the top part represents the lumen side, the bottom part the blood vessel side. After absorption within a cell you can see the diffusion. If the diffusion is slow enough, following uptake the drug is effluxed out by the P-glyco-

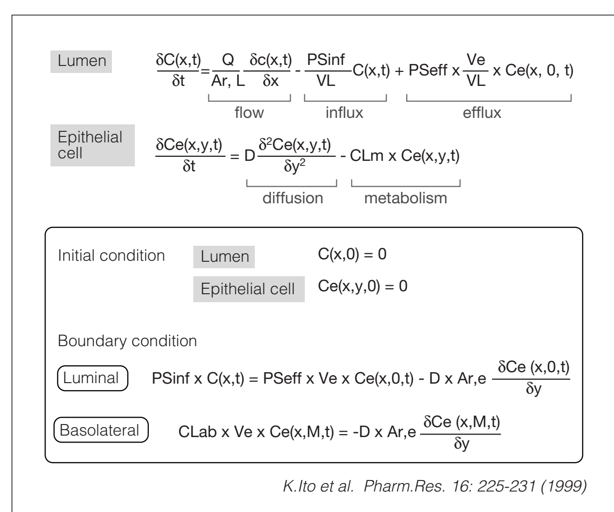


Figure 4.

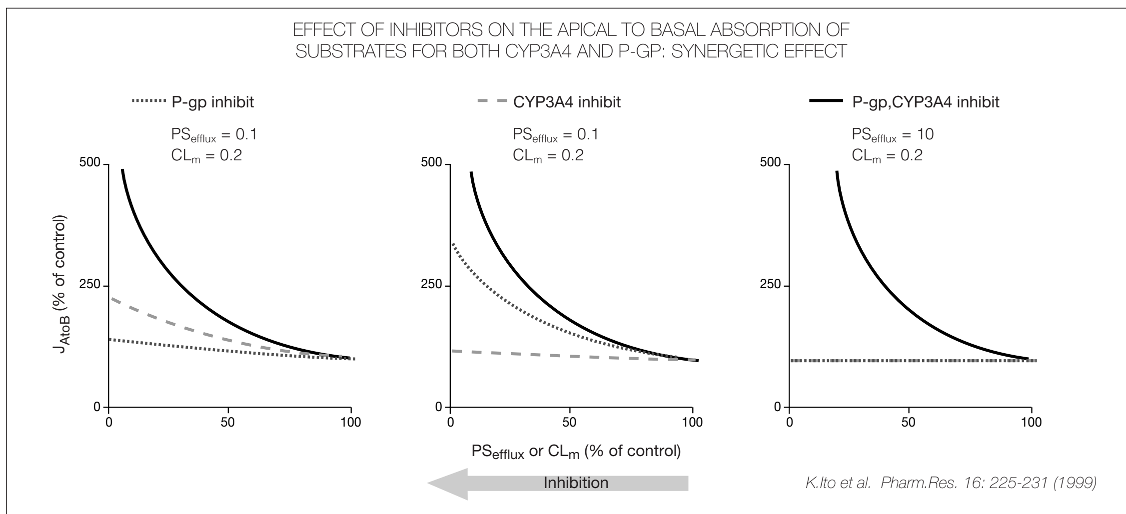


Figure 5.

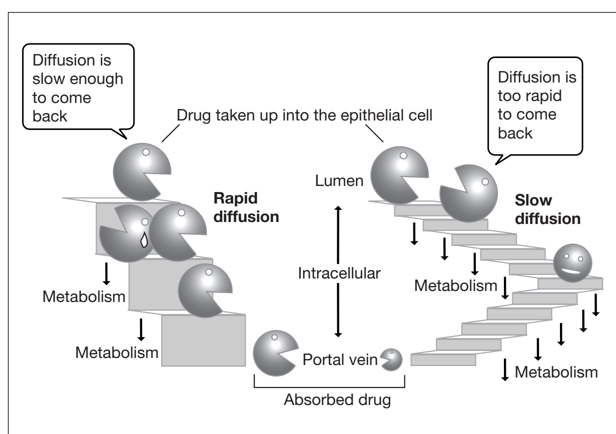


Figure 6.

protein; so the P-glycoprotein role is obvious. If the diffusion process is very fast or there is good permeability to the vessel, then once it is taken in the cells, the efflux by P-glycoprotein may not take place so efficiently. This is the interplay confirmed by the mathematical model that we have generated.

Maybe in the future we will be able to reach the same conclusions without such a difficult mathematical model. If we consider the epithelial cell as a simple compartment, then we should be able to arrive at a simpler equation. From the lumen, a compound is taken up and if the permeability is very high then total absorption is limited by the influx rate, therefore there is no metabolic effect. However, if the permeability is low enough, then not only the influx but the efflux capacity or the metabolic pathway would have a major direct effect on absorption. You can explain these phenomena with the simple mathematical model and so, perhaps, instead of using that first very complicated mathematical model, maybe we can use this simple equation to illustrate the phenomena.

The concept of "enzyme and transporter interplay" may be demonstrated by using specific inhibitors which we recently identified (*Figure 7*).

There is some supportive evidence for the idea of interplay between CYP3A4 and the P-glycoprotein. The same transcription factor regulates the expression of both 3A4 and the P-glycoprotein. It is the PXR/RXR alpha heterodimer, which has been found to be the co-

Comparison of IC50 values for CYP 3A4 and P-gp

	CYP 3A4	**P-gp**	**CYP3A4 / P-gp ratio**
Ketoconazole	0.01 - 0.04	> 1	0.01 - 0.04
Verapamil	10 - 20	> 30	0.33 - 0.67
L754, 394	0.006 - 0.04	> 3	0.002 - 0.01 CYP3A4 Specific
PSC833 (Valspodar)	4 - 7	0.03 - 0.1	40 - 233 P-gp Specific

Figure 7.

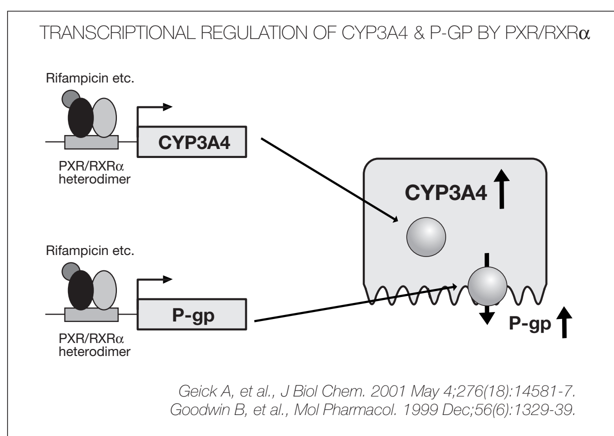


Figure 8.

upregulator of the expression of those two molecules (*Figure 8*). Therefore, both the transporter and the enzyme may be working together to accelerate detoxification, or to inhibit intestinal absorption of the compound.

Next, I'll talk about the functions of the P-glycoprotein. Well, again to make a long story short, we used a P-glycoprotein knock-out mouse, mdr1a(-/-), to look at distribution to the brain.

Using the knock-out mouse we can see the increase in drug distribution in the brain (*Figure 9*). For instance, number 12, which is quinidine, shows a 25-fold increase in BBB (blood brain barrier) transport. With number 11, digoxin, the increase in BBB transport is 11-fold but if you look at number 1, diazepam, or number 2, progesterone, they do not offer any P-glycoprotein substrate and there is no change in the brain distributon in the mdr1a(-/-), knock-out mouse.

In *Figure 10*, the Y-axis shows the *in-vitro* result of P-glycoprotein function, using an expression system. The X-axis shows the P-glycoprotein function *in-vivo* (assessed by using intestinal perfusion system), and the *in-vitro*/*in-vivo* correlation is very good. So the *in-vitro* performance can predict the *in-vivo* result.

Figure 11 is the model that I used for the quantitative analysis, and I'd just like to give you the result of this study, where we compared the performance of P-glycoprotein in the normal mouse *vis-à-vis* the knock-out mouse. Here in this model, it was assumed that the membrane permeabilities which came from other mechanisms than P-glycoprotein mediated transport are the same between normal cells/mouse and the knock-out cells/mouse. We must look at the proportion of the balance between the two functions. Even when the compund is a P-glycoprotein substrate, if passive diffusion is higher than the P-glycoprotein mediated transport, the P-glycoprotein does not function, as you may understand from the final equation shown in the bottom of the line.

Since our topic is GI tract absorption, using a knock-out mouse we looked at the intestinal perfusion result as well as the result of the normal mouse, and in *Figure 10* the X-axis shows the absorption change and the Y-axis shows the *in-vitro* results.

In general, there is some correlation; however, what I would like you to note here is number 11, quinidine. Digoxin, number 7, the maximum increased absorption in the GI tract in the knock-out mouse was just two-fold, so the change is very small. In the case of quinidine, the change is eight-fold. However, with all the other compounds, even if their transports to the brain were changed very much in the knock-out mouse, GI absorption doesn't change greatly; though there are some changes.

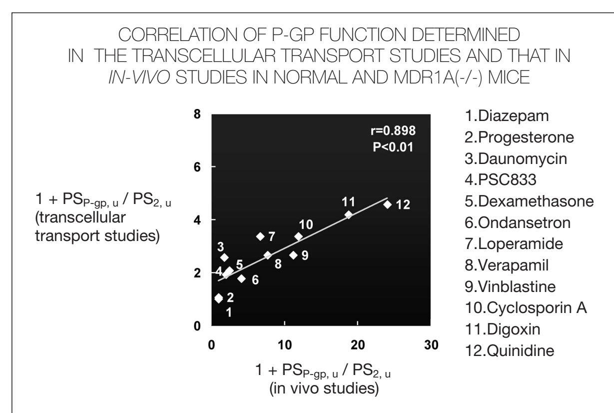


Figure 9.

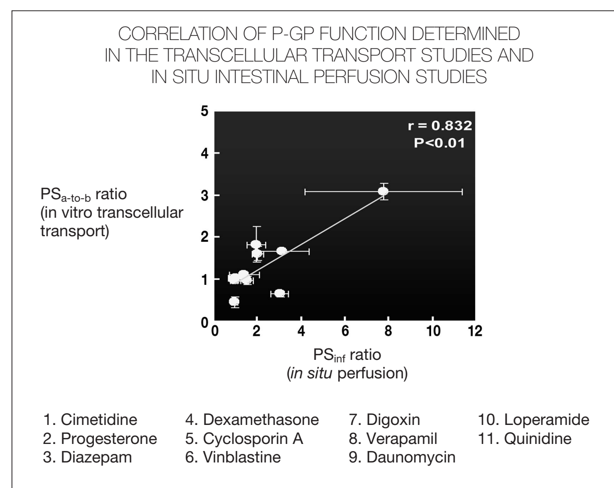
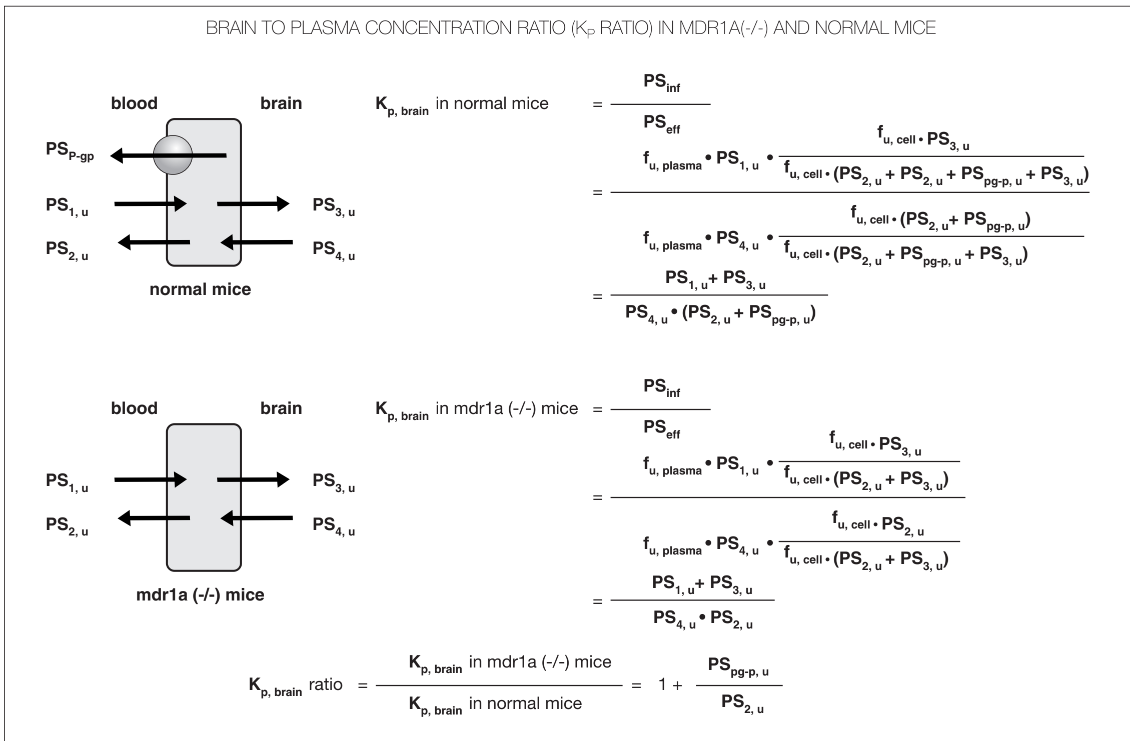


Figure 10.



$$K_{p,\,brain} \text{ in normal mice} = \frac{PS_{inf}}{PS_{eff}} = \frac{f_{u,\,plasma} \cdot PS_{1,\,u} \cdot \dfrac{f_{u,\,cell} \cdot PS_{3,\,u}}{f_{u,\,cell} \cdot (PS_{2,\,u} + PS_{2,\,u} + PS_{pg\text{-}p,\,u} + PS_{3,\,u})}}{f_{u,\,plasma} \cdot PS_{4,\,u} \cdot \dfrac{f_{u,\,cell} \cdot (PS_{2,\,u} + PS_{pg\text{-}p,\,u})}{f_{u,\,cell} \cdot (PS_{2,\,u} + PS_{pg\text{-}p,\,u} + PS_{3,\,u})}} = \frac{PS_{1,\,u} + PS_{3,\,u}}{PS_{4,\,u} \cdot (PS_{2,\,u} + PS_{pg\text{-}p,\,u})}$$

$$K_{p,\,brain} \text{ in mdr1a (-/-) mice} = \frac{PS_{inf}}{PS_{eff}} = \frac{f_{u,\,plasma} \cdot PS_{1,\,u} \cdot \dfrac{f_{u,\,cell} \cdot PS_{3,\,u}}{f_{u,\,cell} \cdot (PS_{2,\,u} + PS_{3,\,u})}}{f_{u,\,plasma} \cdot PS_{4,\,u} \cdot \dfrac{f_{u,\,cell} \cdot PS_{2,\,u}}{f_{u,\,cell} \cdot (PS_{2,\,u} + PS_{3,\,u})}} = \frac{PS_{1,\,u} + PS_{3,\,u}}{PS_{4,\,u} \cdot PS_{2,\,u}}$$

$$K_{p,\,brain} \text{ ratio} = \frac{K_{p,\,brain} \text{ in mdr1a (-/-) mice}}{K_{p,\,brain} \text{ in normal mice}} = 1 + \frac{PS_{pg\text{-}p,\,u}}{PS_{2,\,u}}$$

Figure 11.

Now I would like to show you the clinical data that matches the *in-vitro* data. *Figure 12* is Dr. Hoffmeyer's data. It shows some polymorphism and in some patients the GI tract P-glycoprotein expression level is very low, down to one-tenth. Even if the expression level is one-tenth of the normal, if you look at digoxin's AUC (the area under the curve), you can see that it has reduced only two-fold. If you remember, P-glycoprotein is defective in the knock-out mouse, and yet absorption does not change greatly. So the clinical result matches the data obtained with knock-out mouse.

Of course, the function of the P-glycoprotein is modeled in the intestinal absorption. However, P-glycoprotein would actually work best in the permeability of drugs

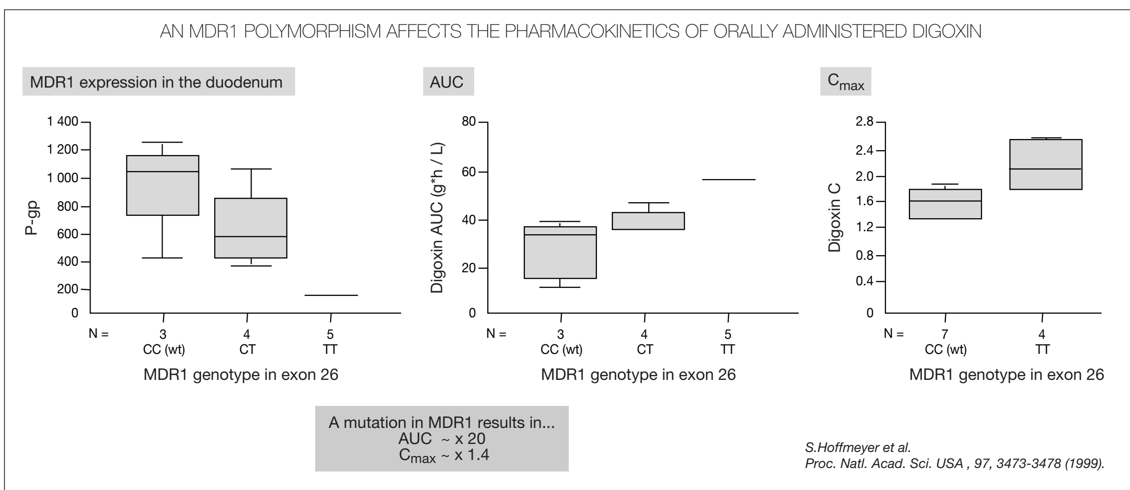


Figure 12.

in the BBB. We have to look at P-glycoprotein's different capacity in different tissues compared with the passive diffusion mediated permeabilities.

Let me talk about the interplay of these transporters.

P-glycoproteins in humans and in the rat are expressed in the luminal side of the intestine, therefore they are thought to be involved in the efflux of drugs. According to a recent study, the MRP2 transporter and the BCRP transporter are also involved and expressed on the luminal side, and involved in the efflux of the compounds. And on the basolateral side, i.e. vessel side, there is MRP3. We were the first researchers who identified this transporter. It is expressed in the basolateral side and involved in the function there.

What is most interesting are the OATP families. They are also on the lumen side and perhaps they may be working as the influx transporter. Although we have no actual evidence of this, there are suggestions of such an influx function.

Together with Dr. Rost we published a report in Am.J.Physiology in 2002 (282 : G720-726 (2002). The localization of MRP2 is the apical side as well as the P-glycoprotein.. However, MRP3 does not match, clearly showing that the localization of the MRP3 is on the basolateral side (*Figure 13*). When we looked for the localization of MRP2 using Western blots, we found it in the upper tract, in the duodenum or jejunum. However, interestingly enough, MRP3 is expressed mainly in the lower GI tract, in the ileum and colon.

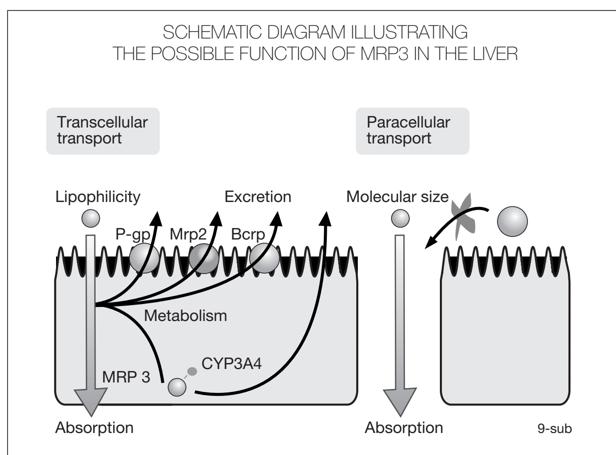


Figure 13.

One of the attendees here is Dr. Goto of Kissei Pharmaceuticals, and *Figure 14* shows the work he did while he was with us in our laboratory. We wanted to see MRP2 functioning within the intestinal tract. We have a very good rat model called EHBR;MRP2 is defective in this strain. We administered CDNB, a lipophilic compound which is taken in and conjugated to the glutathione conjugate, and excreted to the lumen side. The efflux of glutathione conjugates into the intestinal lumen is reduced in EHBR, suggesting that this efflux is mediated by MRP2. Even if we use the everted sac, a similar result is obtained. While there is a large variation, in the jejunum there is a significant difference, and the contribution of MRP2 is demonstrated.

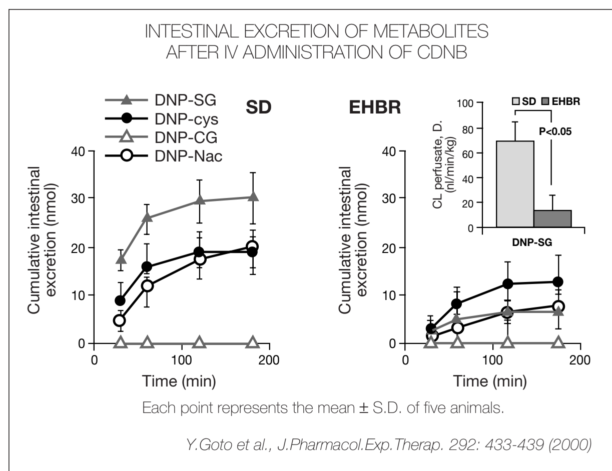


Figure 14.

In *Figure 15*, we were able to prepare the basolateral membrane from rat intestine and found that the MRP3 substrates such as glucuronide conjugate and bile acids are transported by this membrane in an ATP dependent manner. Perhaps MRP3 is working as the efflux system on the basolateral side. In the past we did not know anything about transporters at the basolateral side but

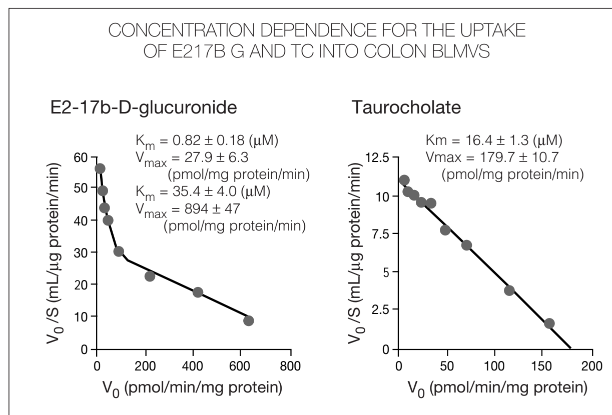


Figure 15.

MRP3 may turn out to be a good candidate for transporter involved in the efflux system at the basolateral side. As shown in the interplay schema the metabolic enzymes and the transporters may have a synergetic effect and in that sense, maybe some common regulators are working in there, too.

The MRP families that we are working on, MRP1 or MRP2, accept the conjugates such as glutathione conjugates and glucuronide conjugates as substrates. Therefore, the conjugating enzymes and also the efflux transporters, may be co-regulated. For the past two years we have been trying to demonstrate this hypothesis.

Figure 16 shows the result of work on MRP1 transcription factors, carried out by our students. The transcription factor, Nrf2 may be associated with the up-regulation of the entire MRP family. It has been already known that this transcription factor up-regulate gluthathione S-transferases.

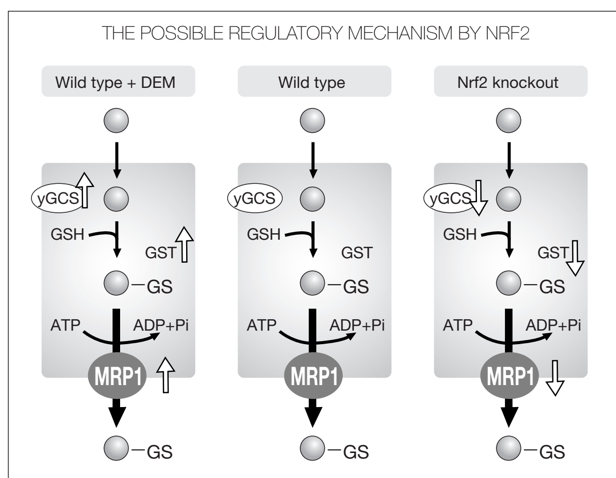


Figure 16.

If I may return to intestinal absorption. *Figure 17* is a joint study undertaken by Okudaira-San of Meiji Seika. The company had developed a compound called ME3277. It is an active form. As you can see, it has two carboxylic acids, the log-P is very small and permeability is also low. Therefore, absorption is very low *in-vivo.* The approach Meiji Seika took with this compound was to create the prodrug. Of course, the log-P becomes higher and so membrane permeability rises, and there is the chance that absorption in the GI tract will improve. The thought was that maybe it would then be released as an active form into the blood vessels. But if you look at the experimental results, actually this did not help improve absorption.

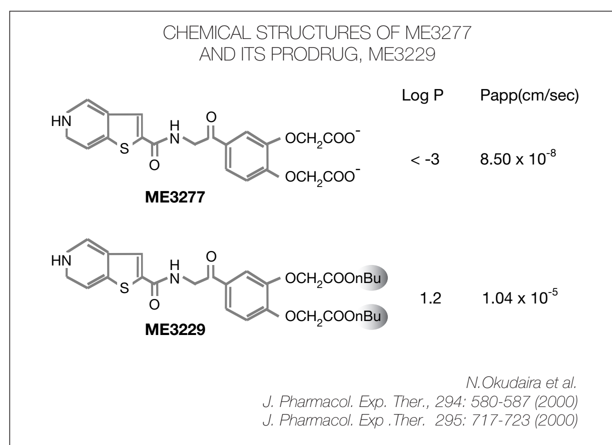


Figure 17.

That is why we started the joint research and we came to understand that the mechanism involved means that the active form produced from the prodrug in the GI epithelium is effluxed out into the lumen by an active efflux transporter. In *Figure 18*, we used an Ussing chamber and everted sac to see the results, If you look at the control, it compares the mucosal to serosal (M to S), and the serosal to mucosal (S to M) transport, and as you can see, the S to M secretory transporter capacity is higher than the other and if the ATP is decreased then the difference is narrowed down. Therefore, this transport works by an active transport system. As you may recall, this drug is the anion type; therefore, I thought, this efflux must be mediated by MRP2 so EHBR (Eisai hyperbilirubinemic rats) where MRP2 is hereditarily deficient was compared with normal rat, because the efflux should be decreased if MRP2 plays a role in the efflux process. However, we live in a world more complex than we expect.

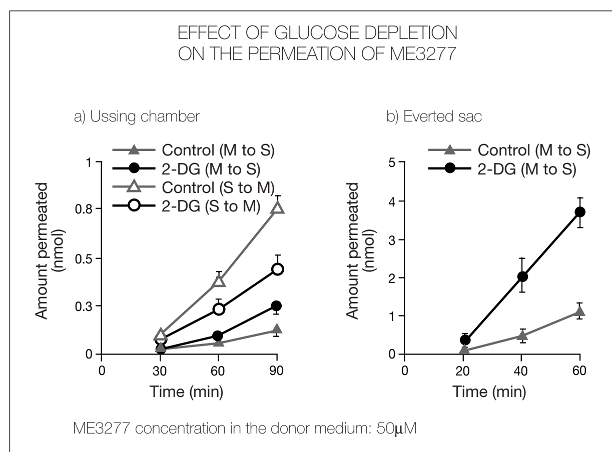


Figure 18.

As you can see from *Figure 19*, there is some directional transport. However, no difference was observed between normal and EHBR rats. Therefore, the transporter responsible for the efflux of this compound is not MRP2. I didn't know what this candidate transporter was, so this research was discontinued.

However, six months ago the study was looked at again because, according to recent research, the BCRP transporter is known to be located on the lumen side. According to our own study BCRP accepts anion as a substrate. BCRP is in the ABC transporter family but it is a half-size transporter. MDR1 and MRP2 have two binding cassettes and after ATP binds to those cassettes, the hydrolysis of ATP starts and the substrate is effluxed out into the lumen side. However, BCRP only has the one ATP binding cassette.

In reality the homo-dimer is formed and works as a dimer. In any case, the BCRP transporter is focused and several researchers in my laboratory have been working on it. Before I will show our result, let me introduce a great work by Jonker et al.

As you can see from *Figure 20,* there is another cancer drug called topotecan. It can be the substrate of the BCRP as well as the MDR1. GF120918 is a P-glycoprotein-inhibiting and BCRP-inhibiting compound

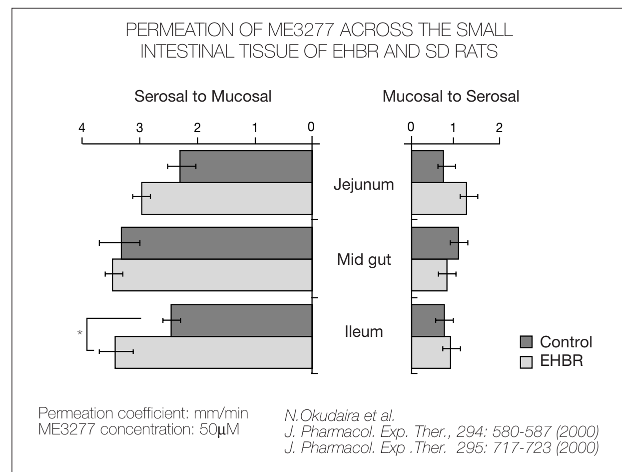


Figure 19.

In order to differentiate the two, they used a P-glycoprotein knock-out mouse. If GF compound inhibits the efflux activity in the P-glycoprotein knock-out mouse, the transporter involved ought to be BCRP. Even in a knock-out mouse with a GF compound, GI absorption is upgraded and therefore BCRP seems to be working as a transporter in the efflux system on the lumen side. The reason why topotecan was taken out to the lumen

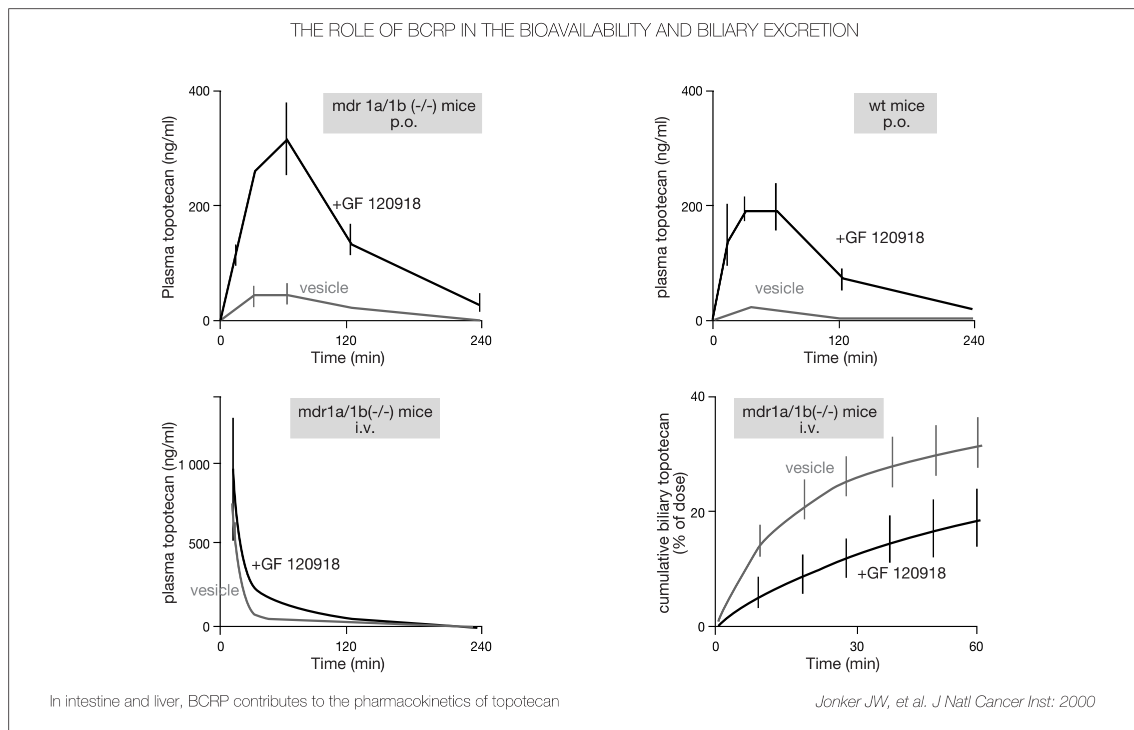


Figure 20.

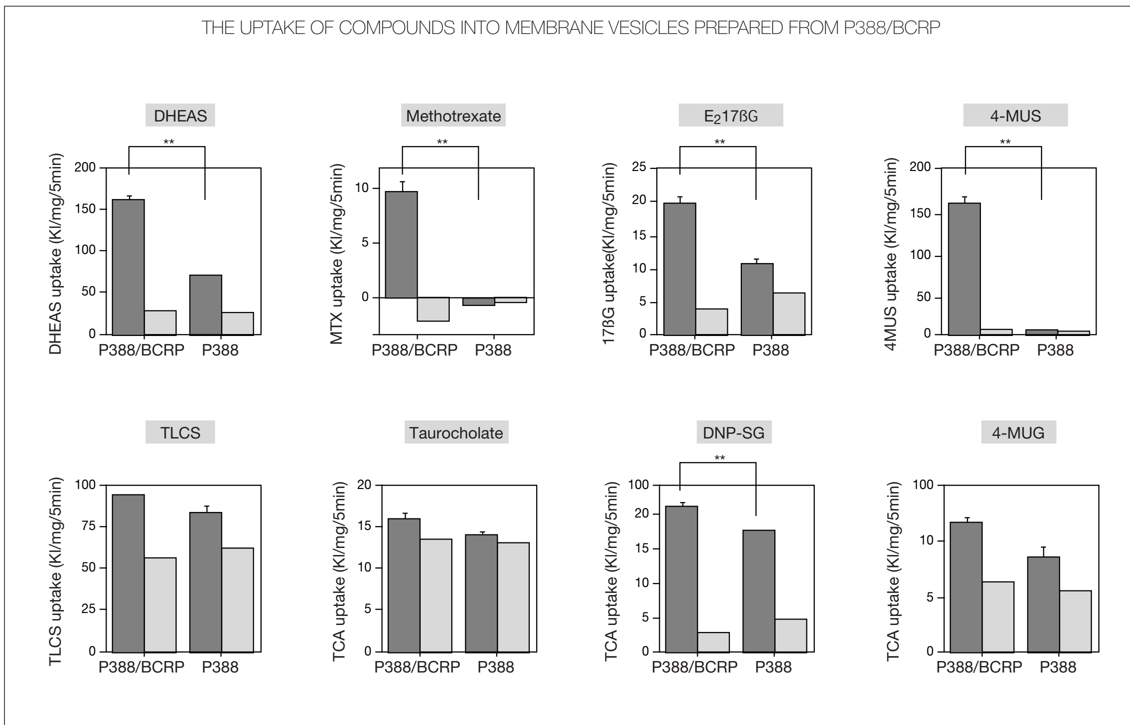


Figure 21.

side was because of the BCRP. BCRP indeed accepts topotecan as a substrate. There are many anionic compounds that can serve as the substrate for BCRP (*Figure 21*). There is another cancer drug, methotrexate, or DHEAs or the DNP conjugate, and many of them can work as the anion substrate for the BCRP transporter.

I have maybe 15 more minutes to talk about hepatobiliary transport and the functions of OATPs (organic anion transporting polypeptides) and MRP2 in relation to its role in drug disposition in the body.

The drug is taken in and is metabolized. Then ABC transporters on the lumen side are responsible for the efflux (excretion) of various kinds of anionic compounds. The uptake transporter, OATP2 and the efflux transporter,MRP2 are both deeply involved in the hepatobiliary transport of anionic compounds. A part of that is the example of OATP2 in *Figure 22*. Incidentally, OATP2 is also referred to as OATP-C or LST1. The nomenclature is very complex and I am very sorry about that, but within our laboratory it is called OATP2, and so I will call it today OATP2. From the Northern blotting system, you

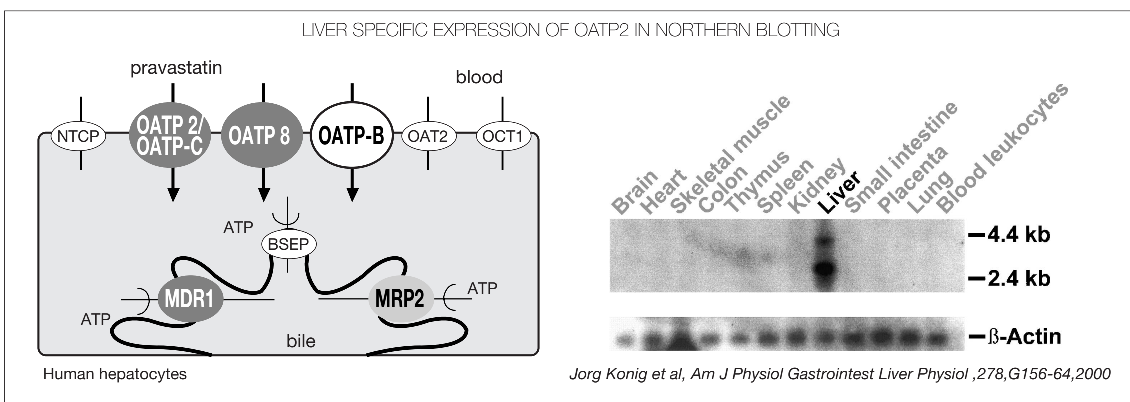


Figure 22.

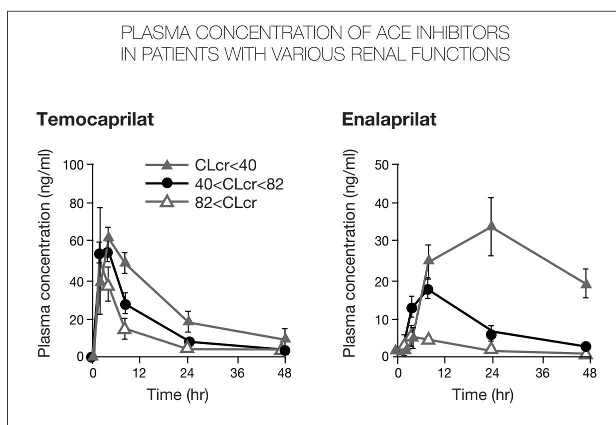


Figure 23.

can see it is a very liver-specific type of expression system. It is clear that uptake by the liver has a major impact on the pharmacokinetics of, for example, pravastatin. This transporter accepts a variety of therapeutically important drugs.

Figure 23 is a slide I refer to quite frequently. It shows temocapril from Sankyo, a compound that was launched in Japan a long time ago, but as the seventh or eighth ACE inhibitor. Now, it is one of the top-ranked inhibitors on the market, because of its good pharmacokinetic properties.

It is converted into temocaprilat, an active form which has two carboxylic acids. One of its characteristics is that, whereas with conventional ACE inhibitors the plasma concentration tends to rise steeply in people with renal failures, with temocaprilat the concentration level is not so dependent on renal properties. In that sense it has a very good *in-vivo* property. It is used mainly for hypertension in elderly people, meaning that inter-subject variability must not be too high as this would be likely to induce a lot of side-effects.

So we became involved in a study where we found that with a conventional ACE inhibitor, more than 90 % of dose is excreted into the uriner. If there is a problem within the kidney there is no way that it can get out of the blood circulation, and that is the reason why the plasma concentration level goes up. I think that makes sense (*Figure 24*).

But because temocaprilat is also involved in biliary excretion as well as in urinary excretion, there is an alternative path. Even if the kidney is not functioning well, there is another way to exit. That's the reason why the plasma concentration level of temocaprilat is not affected so much. That's one of the kinetic explanations, which has been proved experimentally.

We have also found out why it is only the temocaprilat that is excreted into the bile. It is taken up by OATP2 and is excreted into the bile by MRP2 (*Figure 24*). There are also other ACE inhibitors which use the uptake transporter and gets into the liver. However, they are not recognized by the MRP2 so that's why they are not excreted into the bile. We hope that this will become established knowledge.

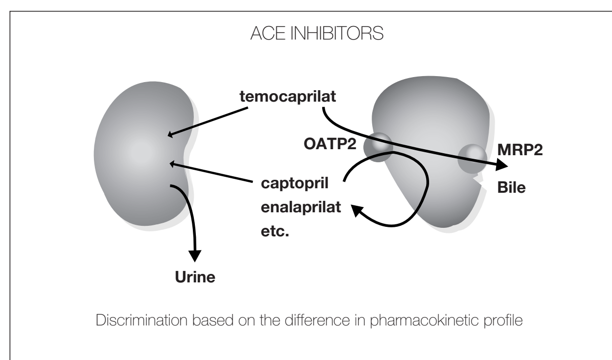


Figure 24.

As for *in silico* models, we are now working on those as well, but as we have limited time I won't go into detail about that.

Now about the HMG CoA-reductase inhibitor, pravastatin. *Figure 25* shows that pravastatin is absorbed in the intesine by a transporter, maybe by OATP-B. Then it is taken up by the liver by OATP2 from the portal vein followed by the biliary excretion by MRP2 (previously called cMOAT). Having three transporters, a very effi-

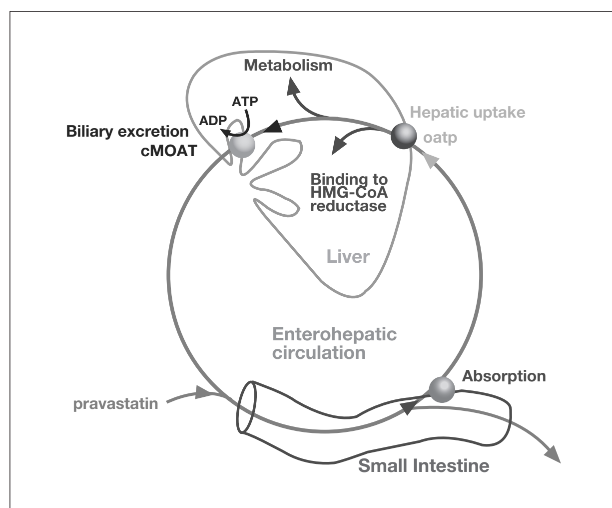


Figure 25.

Substrates for rat cMOAT and human MRP

		ratcMOAT/Mrp2	hMRP
Glutathione Conjugate	LTC4	?	?
	DNP-SG	?	?
	glutathione bimane	?	?
	glutathione disulfide (GSSG)	?	?
	monochloro-monoglutathionyl melphalan	N.D.	?
Glucuronide Conjugate	17ß-estradiol 17-(ß-D-glucuronide)	?	?
	-naphthyl glucuronide	?	N.D.
	bilirubin glucuronide	?	N.D.
	E3040 glucuronide	?	N.D.
	liquiritigenine (flavonoid) glucuronide	?	N.D.
	glycyllhizin ? flavonoid-glucuronide	?	N.D.
	grepafloxacin (new quinolone) glucuronide	?	N.D.
	SN-38 (camptothecin analogue)s glucuronide	?	N.D.
	glucuronosyl etoposide	N.D.	?
Bile acids	cholate-3-O-glucuronide	?	N.D.
	lithocholate-3-O-glucuronide	?	N.D.
	tauro/glycolithocholate 3-sulfate	?	N.D.
	taurochenodeoxycholate 3-sulfate	?	N.D.
	6α-Glucuronosylhyodeoxycholate	N.D.	?
	3α-sulfatolithocholyltaurine	N.D.	?
Others	LTE4	?	?
	LTD4	?	?
	LTE4NAc	?	N.D.
	dibromosulfophthalein	?	N.D.
	cefodizime (ß-lactam antibiotic)	?	N.D.
	grepafloxacin (new quinolone antibiotic)	?	N.D.
	methotrexate	?	N.D.
	CPT-11 acid form (camptothecin analogue)	?	N.D.
	SN-38 acid form (camptothecin analogue)	?	N.D.
	pravastatin (HMA CoA reductase Inhibitor)	?	N.D.
	temocaprilat (ACE inhibitor)	?	N.D.
	BQ-123 (cyclic peptide; endothelin antagonist)	?	N.D.

N. D.: Not Determined

Figure 26.

cient enterohepatic circulation takes place.For this drug, this is very efficient because the pharmaceutical target in this case is the HMG-CoA reductase in the liver. If we have this kind of efficient enterohepatic circulation, that means that exposure within the target site in the liver is very high.

OATP is especially active with this type of uptake, and the first pass hepatic uptake after its oral administration is very high. So only a small portion of drug goes into the blood circulation and then a severe muscle lysis which is known as the side effect caused by statins may be minimized. In this case, transporters which play a role in the enterohepatic circulation act as good players for the drug in terms of the better pharmacological effect and minimum side effects. We need to be very conscious about this example, because people have been believing that drug transporters have been working as bad players as is the case of P-glycoprotein. That's my view.

Figure 26 shows theMRP2/cMOAT substrates. There are a lot of different types of conjugates and even if it's not a conjugate some compounds may have certain anionic properties. I wanted to point out that more than 80 percent of MRP2 substrates are also substrates of OATP2, though the structures of OATP2 and MRP2 are quite different. OATP2 is an exchanger type of trans-

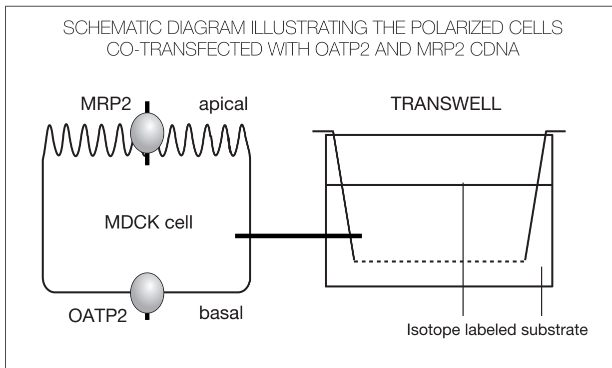


Figure 27.

porter, while MRP2 is a primary active transporter utilizing ATP hydrolysis as a driving force.

Since the substrate specificity is very similar, these two transporters may provide vectorial transport of drugs and endogenous compounds such as bilirubin glucuronide for the liver. This is very useful because if one compound is recognized by these uptake and efflux transporters, it will be transported effectively from the blood side to the bile side. We call it vectorial transport. In trying to reconstruct *in-vitro* the vectorial transport, we recently came up with MDCK2 (*Figure 27*). It is a cell line with polarity, in which we expressed simultaneously the uptake and efflux transporters on different sides and the *Figure* shows the result. I won't go into the details but we call this type of cell the double transfectant cell. Several pharmaceutical companies are interested in these cells and use them in drug development.

With this double transfectant cell we have established a system that's used for Caco-2 cells, and a type of vectorial transport which has been observed *in-vivo* has been reconstructed *in-vitro* For example, this is the result with pravastatin (*Figure 28*). This is a single expression type, or vector-control. Without the transporter, no vectorial direction is seen, but by using this double transfectant, the transport from basal to apical is much higher compared with the opposite direction. We have been able to demonstrate very clear vectorial transport here, with pravastatin.

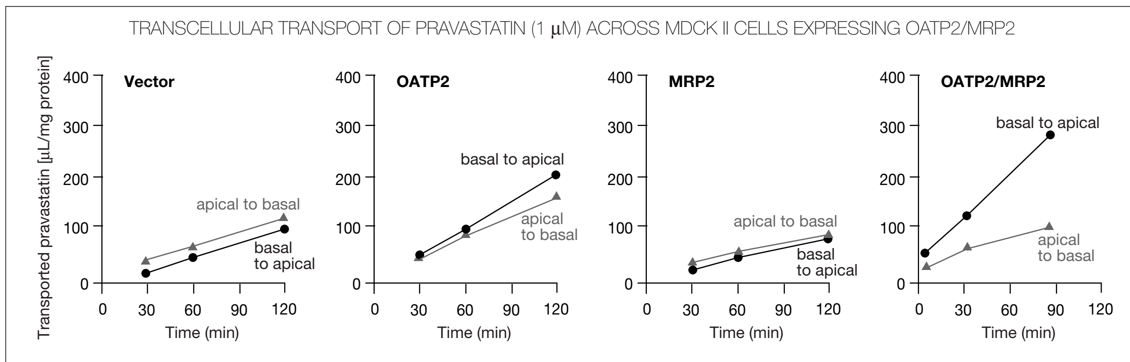


Figure 28.

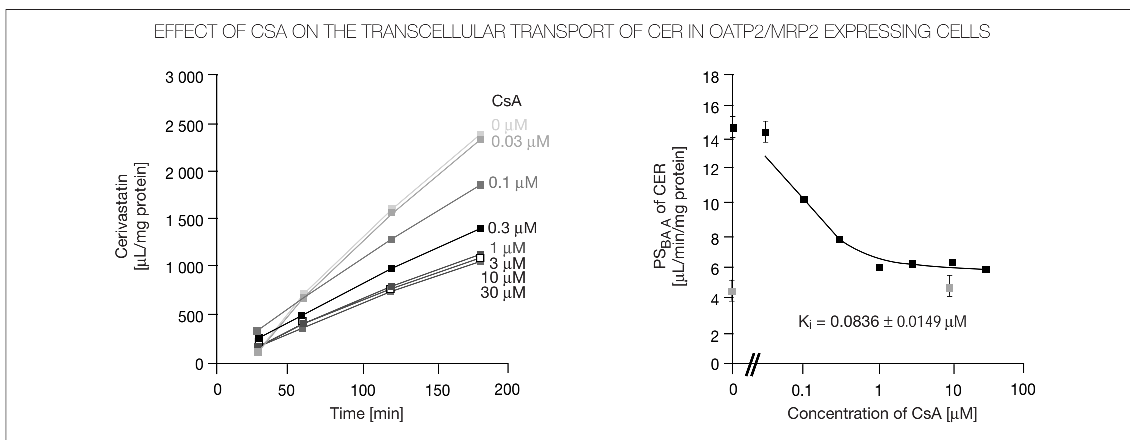


Figure 29.

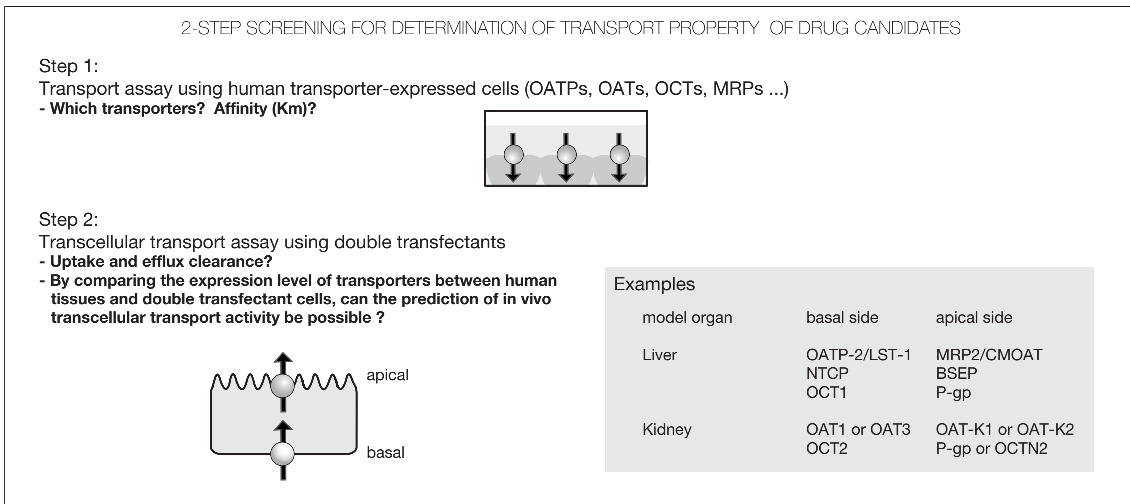


Figure 30.

Now I want to talk about the drug-drug interactions. Cerivastatin(CER) was withdrawn from the market due to the side-effects of muscle lysis. One of the major cause for this serious side effect is drug-drug interaction.

We used cyclosporine as an interacting compound and tried to see cyclosporine's inhibiting effect on the cerivastatin transport using the double transfectant cell. As you can see on the right-hand side of *Figure 29* the Ki value is about 0.1μM, therefore this is something that is clinically feasible at the cyclosporine concentration level in the plasma. Our further analysis indicated that this drug drug interaction is mainly due to the inhibition of OATP2-mediated uptake process. In this way, cyclosporin increased the plasma concentration of cerivastatin 5 folds.

Let me tell you about one of the dreams I have. As shown in *Figure 30*, in the future I would like to be able to use the double transfectant cell in drug development. In order to do that, as a first step, a single transfectant cell should be used in finding candidate transporters, such as when you use the CYPisofrm expression systems. Once you have narrowed down the number of candidates, at that stage you can refer to this double transfectant cell model. This is my proposal. The throughput is not very high, just like in Caco-2 system, so you need to have narrowed the candidates down before moving on to this kind of double transfectant cell model.

We now have about 15 targets – we are trying to come up with about 15 different types of double transfectant cells, and once they are available we can start to evaluate the transport properties of new drug candidates in various tissues, such as liver, kidney, intestine and the blood brain barrier. After that, at late-stage of drug development you can make the final decision of whether it should go into clinical trial or not.

More recently, the OATP2 transporter has been found to be genetically polymorphic (*Figure 31*), especially star 15 (*15). In this case the allel frequency is about 10 percent in Japanese population Patients with this kind of polymorphism have very high concentration levels of pravastatin.

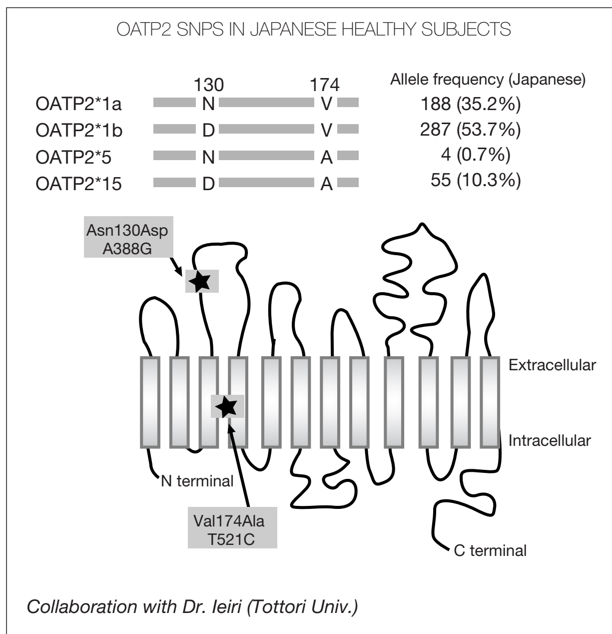
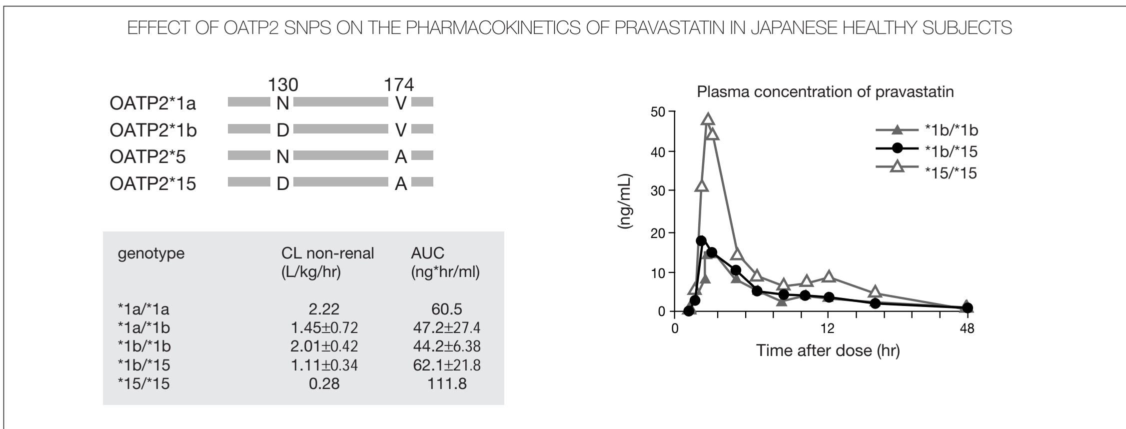


Figure 31.



genotype	CL non-renal (L/kg/hr)	AUC (ng*hr/ml)
*1a/*1a	2.22	60.5
*1a/*1b	1.45±0.72	47.2±27.4
*1b/*1b	2.01±0.42	44.2±6.38
*1b/*15	1.11±0.34	62.1±21.8
*15/*15	0.28	111.8

Figure 32.

Figure 32 is a joint study done with Dr. Ieiri in Tottori University. For those with the heterozygous polymorphism, the plasma concentration of pravastatin is increased about two times, therefore the star 15 polymorphism has some impact on the uptake of pravastatin and that would consequently make a difference to the concentration level and this has also been verified in individuals as well.

Dr. Richard Kim and his co-workers used fexofenadin. Grapefruit juice is known as an inhibitor of P-glycoprotein, so they thought that the intestinal absorption of fexofenadin must increase by adding grapefruit. However, through experiments they showed that grapefruit juice worked to reduce the concentration level, and it was the same with orange juice and apple juice. This may indicate that the transporter(s) responsible for the intersinal absorption of this drug may be inhibited by these juices.

Taking this further, they used other types of expression systems, and they also diluted various types of juice drinks and tried to find out about their inhibitory effect. The findings were that OATP uptake was inhibited by this kind of juice.

Now let me tell you about another dream that I have for the future. Maybe it will take about 5-10 years. I have about eight more years until my retirement, but by then I would like to create a huge database with a simula-

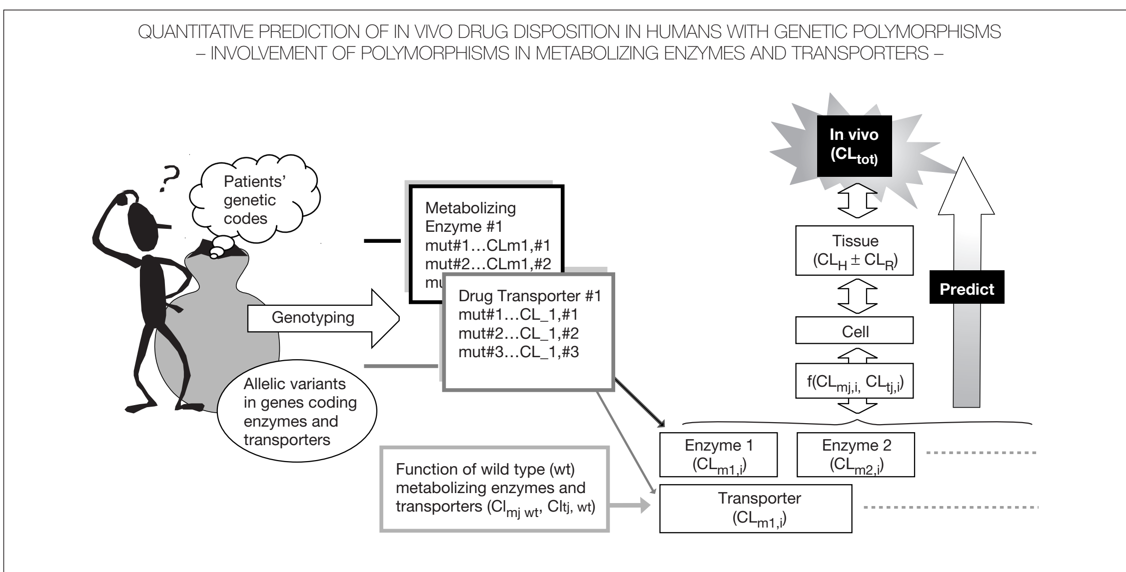


Figure 33.

tion capacity. Once we have installed that type of simulation software on a database then we could look at what happens after the administration of drug candidates, as shown in *Figure 33.*

We could look directly at polymorphism and functionalities, and also at drug-drug interaction. You could use the system during drug discovery and development or get experimental data out of it. All of the *in-vitro* information could be useful; for example, you need to take the biochemical data such as drug metabolism, intestinal absorption, membrane transport, binding to plasma and tissue proteins into consideration as well. But by using a mathematical approach to bring together information on what happens at cell level, at organ level and also at the whole-body level, it may be possible to create simulation models and to build up these kinds of statistics.

We have worked on this approach for the past decade and we have our methodology available and in place. What remains is the content of the database. We need to get good quality content, plus we need to supplement some of the -genetic polymorphism related information. Once we have achieved that we will have a very high-quality database with simulation functionality. This is one of my objectives before I retire.

If you look at my home page (http://www.f.u-tokyo.ac.jp/~sugiyama/), you will find a logo depicting a fat rat - it's a caricature of me (*Figure 34)*. There's a message in it. First, even when you are taking a nap you have to think of the research. Second is the content of our research. We are studying the molecular and cell biology of transporters, but our final goal is to integrate thus obtained information into the mathematical modeling to understand and predict the drug disposition in the body. This is what I have been stressing all the time, and that's the point of the logo.

Figure 34.

I have an announcement for you. The Second World Pharmaceutical Congress is going to be held next year in Kyoto and I am sure that Capsugel will be heavily involved in it. I will be serving as chairman. Dr. Hashida is responsible for the program and Drs Nagai and Benet are the co-chairs of the scientific advisory committee. I hope you will attend this meeting in Kyoto and that there will be a big turnout. We are expecting about 3,000 delegates and hope to see you again on that occasion.

With this, I would like to conclude my presentation. Please do visit my home page. I now have my secretary in my lab who is very artistic, so we have renewed our home page. Thank you very much for your attention.

Chair, Professor Sjinji Yamashita, Setsunan University: Thank you very much, Professor Sugiyama, for leaving plenty of time for discussion. If you have any questions or comments, would you come up to the microphone. The whole program is being recorded and will be edited for the proceedings, so would you please state your name and affiliation before you speak.

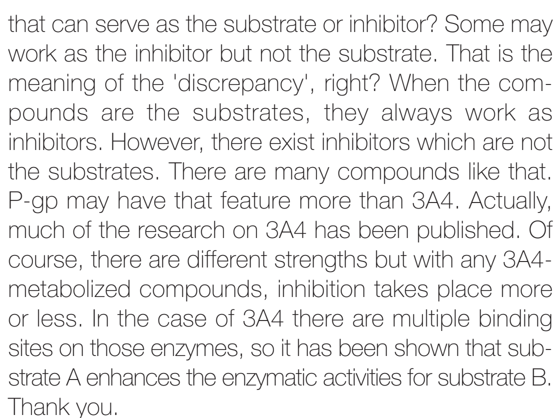
Kato of Keio University: CYP3A4 and MDR1 are known to have some common features. I have two questions. What are the common features and which are more important? Some say size is important, but how about you? And if it is the size, then if it is below 300 it cannot make a good substrate. A molecular weight of more than 400 would be a good substrate for P-glycoprotein.

Professor Yuichi Sugiyama, University of Tokyo: Lipophilicity may also play a part; some level of lipophilicity is needed to service a substrate. While in some exceptional cases the anions can be the substrate, either the neutral or the cationic substrate would be a better substrate for the P-glycoprotein.

Having said so, you may think that prediction is quite easy. However, for the past few years, people have been making *in silico* predictions of good substrates for P-glycoproteins. There are several publications which I have read and we have had discussions. I understand that the predictability of *in silico* models is quite low at the moment.

Kato of Keio University: I have a second question. For instance, we do CYP3A4 and we also have MDRI transporters as some can serve as the substrate and some may work as the inhibitors. But there are some discrepancies. What are the sizes of these discrepancies? According to my impression, MDR1 may have the larger discrepancy.

Professor Yuichi Sugiyama, University of Tokyo: What do you mean by discrepancy – whether



that can serve as the substrate or inhibitor? Some may work as the inhibitor but not the substrate. That is the meaning of the 'discrepancy', right? When the compounds are the substrates, they always work as inhibitors. However, there exist inhibitors which are not the substrates. There are many compounds like that. P-gp may have that feature more than 3A4. Actually, much of the research on 3A4 has been published. Of course, there are different strengths but with any 3A4-metabolized compounds, inhibition takes place more or less. In the case of 3A4 there are multiple binding sites on those enzymes, so it has been shown that substrate A enhances the enzymatic activities for substrate B. Thank you.

Question from the audience: The difference between inhibition and the substrate, does it come about after binding, or is it because the feature is receptor-specific?

Professor Yuichi Sugiyama, University of Tokyo: Even if the binding site is the same for two compounds, in order to obtain the transport, conformational change has to be present. Therefore, even if it binds but that binding does not lead to conformational change, that would never become the substrate; in other words, it is never transported.

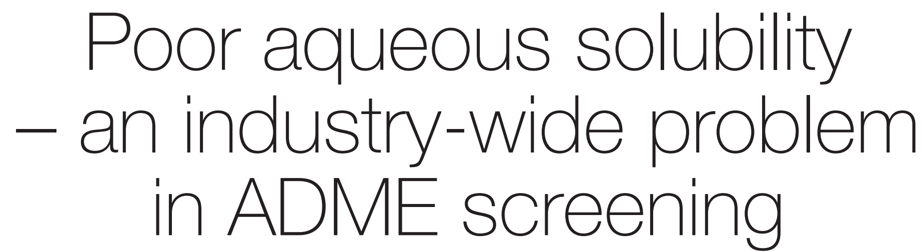
Roland Daumesnil, Capsugel Inc., North Carolina, USA: Yuichi, thank you, I always enjoy your presentations. I have a question for you. You're trying to correlate *in-vitro* permeability with *in-situ* permeability in a rat. You have a PS ratio, I assume that is a permeability ratio from apical to basal level. I'm just wondering how you determine this in numbers because in the *in- situ* I would imagine it's difficult from the basal to apical side.

Professor Yuichi Sugiyama, University of Tokyo: I used a knock-out mouse, not the rat, to look at the *in-situ* perfusion and the *in-vitro*, and there is a correlation curve. Actually, the quantification and determination of the apical to basal flux in a strict sense is very difficult. Was that your question?

I'm sorry it's not so clear. It is the *net* apical to basal flux. In other words, this is a steady-state experiment, so at the steady state of intestinal perfusion we measured the drug concentration in-rate and also out-rate. By using that difference, based on the so-called tube - model we then calculated the PS apical to basal. In other words, that PS apical to basal flux clearance is the net clearance. It's not necessarily the real unidirectional apical to basal flux. That is my answer.

Chair, Professor Sjinji Yamashita, Setsunan University: Any other questions, Roland San? Do you have any other comment or questions? Well, I am afraid the time is up. He has kept to time, and I must thank Professor Sugiyama for an informative presentation and go on to the next speaker.

I am very pleased to call upon the second speaker in this special lecture session, Dr. Christopher Lipinski. He will talk about poor aqueous solubility – an industry-wide problem in ADME screening. He is a very well-known scientist who actually pioneered this theory and he is well known in this area I believe that all of us know his name and the role that Dr. Lipinski has played. He was with Pfizer for many years. Since his retirement last year he has participated even more actively in academic society meetings world wide, and his topic today will cover the worldwide perspective. Dr. Lipinski, the floor is yours.



Poor aqueous solubility – an industry-wide problem in ADME screening

Dr. Christopher A. LIPINSKI

Poor aqueous solubility – an industry-wide problem in ADME screening

Dr. Christopher A. Lipinski

Pfizer Inc., USA

Thank you, Capsugel, for the privilege of addressing this excellent conference.

Let me outline the first part of my talk. I'm going to be talking about property changes over time. I'm going to be talking about errors, mistakes, in the early years of combinatorial libraries. I'm going to be talking about physico-chemical property changes, changes that occurred in the medicinal chemistry labs at the Pfizer location in Groton, Connecticut. I'll be talking about changes in early Pfizer Groton high-throughput screening leads and then I'm going to switch and I'm going to talk about changes, not just at Pfizer, but that are industry-wide.

Then I'm going to talk about the profiles of clinical candidates from the Pfizer Groton Laboratories and the Merck worldwide organization. I'm going to be talking about the changes in molecular weight that have come about more recently, as well as the more recent changes in lipophilicity and changes in hydrogen bonding. Then I'm going to explain how these changes for these two organizations have implications for either poor permeability or poor solubility, as problems in poor oral absorption.

So what were the errors, the mistakes, in the early years of combinatorial libraries? Well, combinatorial-like chemistry is a new technology; it's the automated synthesis of many compounds. When you're starting a new technology, you usually try to use technology that existed earlier, and the earlier technology for the automated synthesis of chemistry compounds was Merrifield solid-phase peptide synthesis. So the early combinatorial libraries had peptidic backbones and, just like Merrifield solid-phase peptide synthesis, many of these early combinatorial libraries used solid-phase synthesis.

Now, one of the problems with solid-phase synthesis is that oftentimes in the earlier years the quantities were very low, so we could not do much testing with these compounds. There is an additional problem with hydrolytic instability. We now recognize that unless you take some extra efforts with the peptide backbone, these compounds can hydrolyze, can degrade. We also now recognize that having a peptidic backbone is very bad in terms of passive intestinal permeability. You can expect many permeability problems with peptidic backbones.

This is already a series of bad problems, but in addition another problem was superimposed: an error in the application of the principle of maximal chemical diversity. Let me explain. In the period before combinatorial chemistry, it was very hard to get starting points for medicinal chemistry programs. As a result, organizations were very eager to develop methods for generating *in-vitro* actives as starting points, and so there was a tremendous emphasis on *in-vitro* activity. So the idea was – using the wrong application of the principle of maximal chemical diversity – you might take the central part of a compound and attach all kinds of interesting functionality so as to maximize the chance of getting *in-vitro* activity. And that worked.

Many of these combinatorial libraries generated many *in-vitro* active compounds. But the problem was that these compounds were very large. It was very common, in those early libraries, to get com-

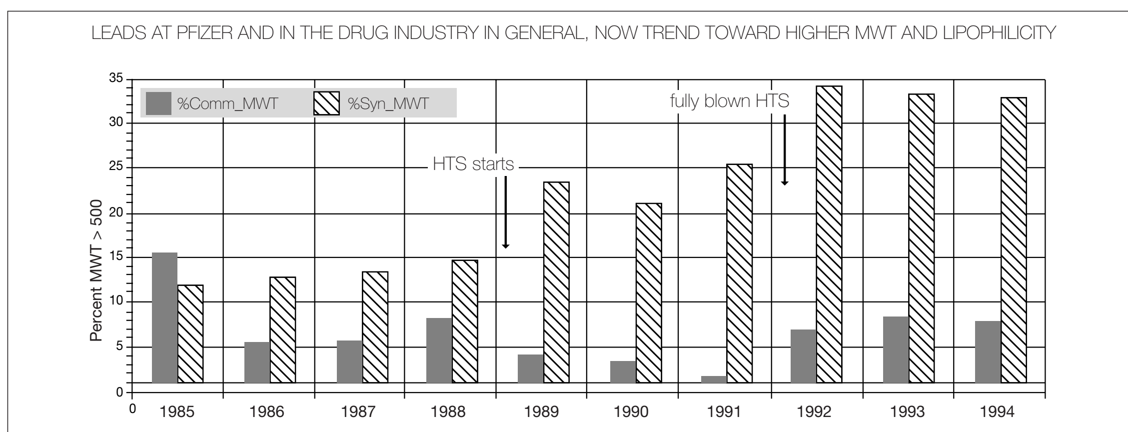


Figure 1.

pounds with molecular weight 650, and it was not realized until later that with these very large compounds it was very difficult to obtain oral activity. So many of these early libraries generated *in-vitro* actives but no orally active compounds.

That is true across the industry. Now in *Figure 1*, illustrates the trends at the Pfizer Groton Labs that result from high-throughput screening. What I have shown here are the percentage of compounds made by the Pfizer Groton medicinal chemists that had very high molecular weight, greater than 500. These are shown in cross hatched. You can see that between 1985 and 1988 the percentage of very large compounds was quite small, between 10 and 15 percent.

But all of a sudden, in 1989, something happened: our chemists were making much larger compounds. About 20 to 25 percent of them were very large. Then, in 1992, the size problem got much worse. Our chemists were now making 30 to 35 percent compounds which had a molecular weight greater than 500. What happened to cause this?

Well, from about 1988 to 1989, the Pfizer Groton Laboratories began to do high-throughput screening and from about 1992, the organization was doing a great deal of high-throughput screening. So the starting points for medicinal chemistry were coming from high-throughput screens and we began to get larger starting points. Therefore everything that the chemists made was large and that's why we see this increase in size of the compounds. For comparison, shown in grey in the *Figure* are the compounds that Pfizer purchased from academic sources. There is no trend, and that's because these academic compounds have no relationship to high-throughput screening.

Computationally comparing libraries.
Drug-like vs new drugs

- Use the presence of an INN name or a USAN name or marketed status as a flag for a compound with “drug-like” properties
- 7 483 Drugs with INN name, USAN name or approved for marketing
- Compare to 2 679 New Drugs from the Derwent World Drug Index
  - mechanism field - trial preparations
  - No CAS registry number, no INN/USAN name, abstracted in 1997, 1998, 1999

Figure 2.

Now you can learn a lot about drug-like compounds by looking through the literature (*Figure 2*). I had the idea of looking at compounds with an INN name or a USAN name – these are names that are given to a compound when it goes into Phase II studies. If you identify compounds with these names or that actually were marketed somewhere, then you have identified drug-like compounds because, if a compound has these names, you have eliminated all the compounds that failed preclinically. You have eliminated all the very insoluble compounds, all the very poorly permeable compounds, all the very toxic compounds. Now the compound with these names might not be efficacious, because that's what you discover in Phase II studies. But with respect to everything else except efficacy, these are drug-like compounds.

In the next few *Figures*, I'm going to compare these drug-like compounds, these Phase II compounds, 90 percent of which were intended for oral use, with what

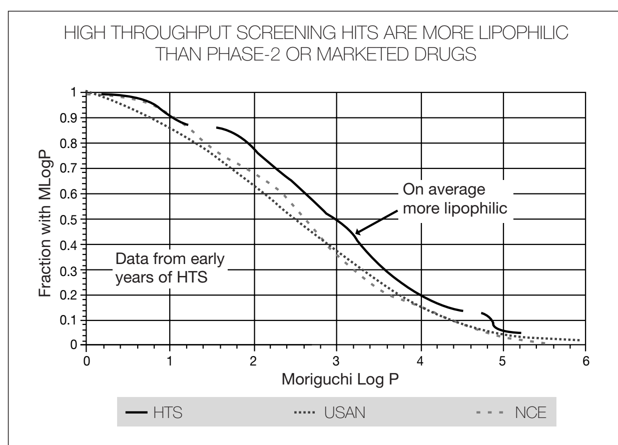


Figure 3.

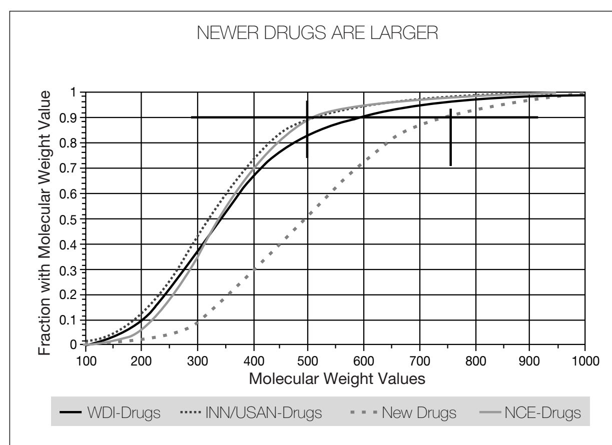


Figure 4.

I call new drugs. I've described the characteristics in *Figure 2*. Essentially, these new drugs are the kinds of compounds that medicinal chemists have been making in recent times. They would be reported in the medicinal chemistry journal, reported at the medicinal chemistry meeting… but they are not necessarily drug-like compounds. There are a lot of badly-behaved compounds among these new drugs.

Figure 3 shows a plot distribution of compounds in different collections. The Phase II drugs are shown as the broken line and the dotted line, and the unbroken line shows the first 190 high-quality, high-throughput screening hits from the Groton Laboratories. This is a distribution plot, the calculation of lipophilicity, using the Moriguchi log-P algorithm. If the curve is shifted to the right it means that on average these early high-throughput screening hits were more lipophilic. The average shift is about a half a log-P unit over the Phase II orally active drugs.

Now half a log-P unit shift may not seem like very much. But if, for example, you have 10 clinical candidates, and if you only have a few of these compounds with these more difficult properties, it creates a great problem. Our pharmaceutical sciences people tell us that for these large lipophilic compounds it takes two or three times longer, it costs two or three times more and it takes two or three times more people-effort to develop these kinds of compounds. So, just a few compounds among the clinical candidates can cause a great many problems in the development organization.

Now the second point is that this half a log-P shift only occurred in the early years of high-throughput screening. If you look at the entire high-throughput screening history of Pfizer you would not see this. Why not? Because, after the early years, we learned from our mistakes and we put filters in place to correct for this property shift.

So that was Pfizer's high-throughput screening history. But what we saw at Pfizer occurred across the entire pharmaceutical industry (*Figure 4*). The *Figure* shows a plot distribution of molecular weight. The curves to the left are the Phase II drugs and marketed drugs, while the broken line shows the newer drugs, the kinds of compounds that chemists are making nowadays. Note that this curve is shifted to the right. That means that, on average, the kinds of compounds that chemists are making across the entire pharmaceutical industry are much larger than the traditional drugs, the type of drugs that got into Phase II studies. That's molecular weight.

The same thing holds for lipophilicity (*Figure 5*). This is the Moriguchi log-P algorithm. The top curve

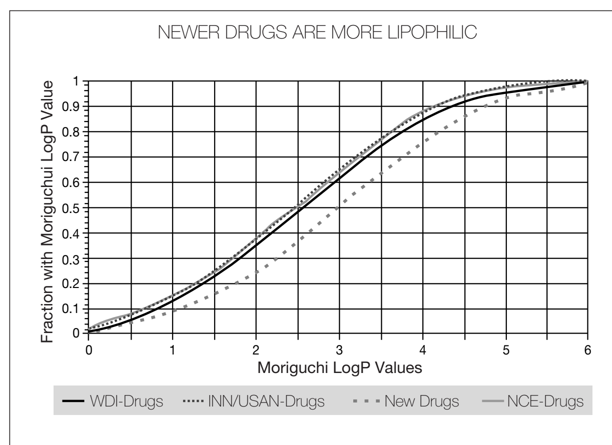


Figure 5.

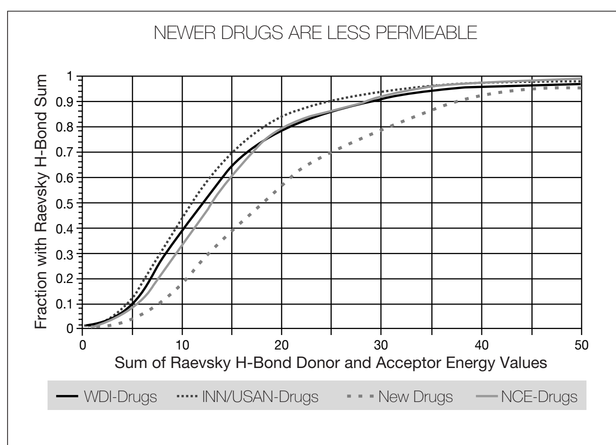


Figure 6.

shows Phase II drugs, marketed drugs. The broken line is the newer drugs that chemists are making, shifted in a right shifted downwards direction. The newer drugs that chemists are making are more lipophilic than the kinds of compounds that traditionally have reached Phase II studies.

The same thing holds for permeability (*Figure 6*). On the X-axis is a calculation of hydrogen bond donor and acceptor energy values. The further one goes to the bottom right-hand corner the more difficult it will be for a compound to cross the gastrointestinal tract for passive permeability. The two top curves are the Phase II orally active drugs. With the newer drugs, the kinds of compounds medicinal chemists are making nowadays, the dotted-line curve is shifted in a right shifted downwards direction. Chemists are making less permeable compounds now than they were a number of years ago, less permeable than the kinds of compounds that used to get into Phase II studies.

I have gone through specific Pfizer history and I have gone through the experience of the entire pharmaceutical industry. Now I'm going to go through a comparison of clinical candidates from two very productive organizations: the Pfizer Labs in Groton, Connecticut, and the Merck worldwide organization.

Let me explain *Figure 7*. This is data from the Pfizer Groton Laboratories. Each square represents a clinical candidate compound made between about 1965 and 1995. On the Y-axis you can read off the molecular weight of the compound. There are lots of clinical candidates, lots of scatter. The black line is the best straight line through those points. You see it goes up, the slope is rising, meaning that on average the clinical candidates from the Pfizer Groton Laboratories have become larger in more recent years. The next point to make here is that there are very few clinical candidates with molecular weight above about 500; most are below 500. So this is the trend with molecular weight.

Figure 8 gives a similar molecular weight plot for the Merck Organization, but there are now several differences from *Figure 7*. The slope looks larger, and there are fewer points. The slope is higher because the scale is different. The number of points has nothing to do with productivity. It simply has to do with the fact that I was only able to capture the data on the Merck compounds from the literature at a later stage of clinical candidacy. But the trend is the same with time.

The *Figure* shows the Merck Organization's clinical candidates from about 1965 to 1995. In this period the clinical candidates are larger. The plot shows lots of scatter, but do you notice there are not too many clinical candidates with molecular weight greater than 500? Most of them are below 500. So, with respect

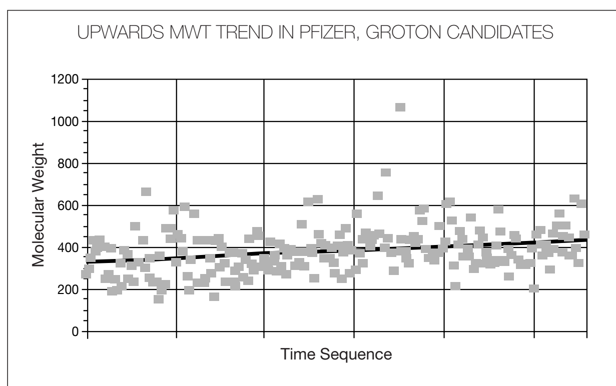


Figure 7.

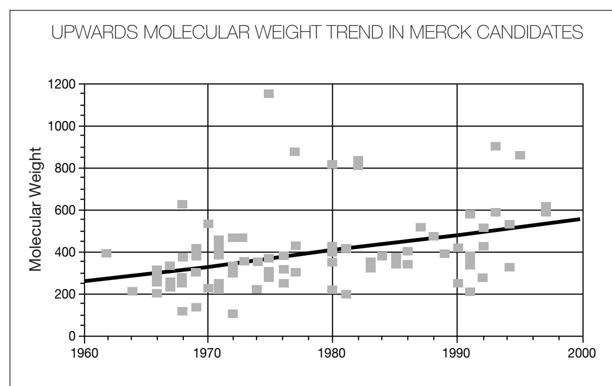


Figure 8.

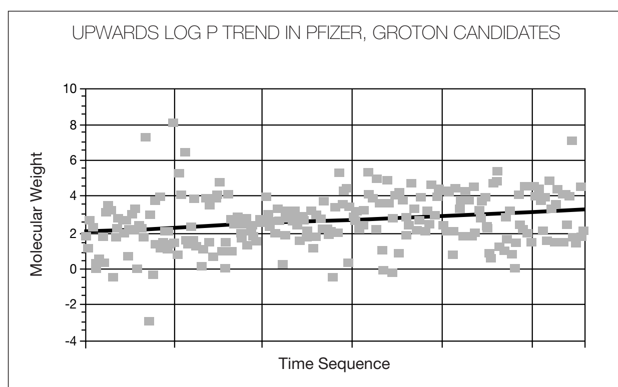


Figure 9.

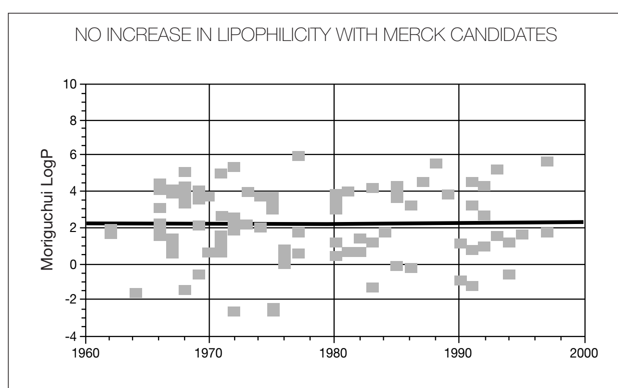


Figure 10.

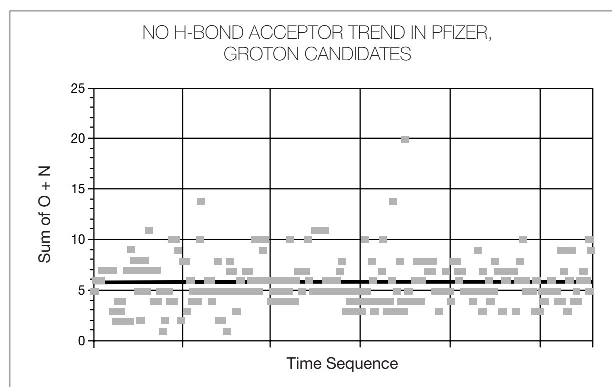


Figure 11.

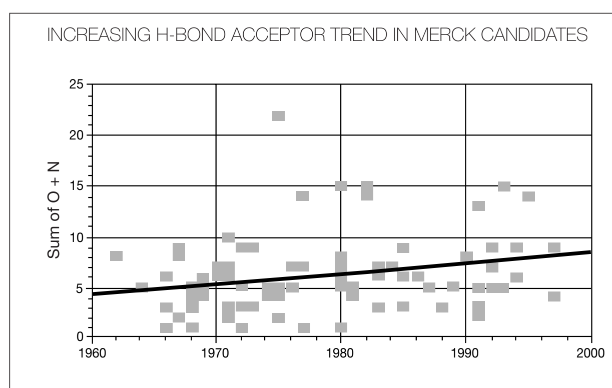


Figure 12.

to molecular weight as a function of time, Pfizer Groton and Merck have very similar patterns of behavior.

With regard to lipophilicity, using the Moriguchi calculation, *Figure 9* shows that over time, between 1965 and 1995, there is lots of scatter but the trend is upward. So in the Pfizer Groton Laboratories, the clinical candidates in more recent times are becoming more lipophilic.

Now what about Merck? *Figure 10* shows that it is very different with the Merck clinical candidates. From about 1965 to 1995 there is no upward trend. The lipophilicity remains about the same and you'll notice that there are very few candidates for the Merck Organization with lipophilicity in the 5 to 6 range.

So now we have a difference. Merck and Pfizer candidates are similar in molecular weight but they are different in respect to lipophilicity. Pfizer Groton candidates are becoming more lipophilic but Merck candidates are not.

With respect to hydrogen bonding (*Figure 11*), I am simply plotting for each candidate how many oxygens and nitrogens there are in the compound. So it's a rough index of hydrogen bonding/bond acceptor ability.

The Pfizer Groton candidates show lots of scatter, but there is no upward rise; there's no trend in recent times for compounds to have greater hydrogen-bonding functionality. Notice there are very few clinical candidates with more than 10 oxygens and nitrogens; most of them have 10 or fewer.

Figure 12, showing the Merck Organization's candidates, is very different. The trend is upwards with the Merck Organization, so with recent time the clinical candidates have more oxygens and nitrogens. Notice, though, that just like Pfizer, there are very few Merck clinical candidates with more than 10 oxygens and nitrogens; most have fewer than 10.

We now have two very successful organizations with very different trends in properties over time. So what is the explanation?

Figure 13 gives my explanation, which I believe is related to how the leads are developed in the two

Lead approach, permeability and solubility	
• Structure Based	• HTS Based
• MWT up - peptidomimetic - fit 3 or more sites	• MWT up - HTS selects for larger size
• H-Bonding up - fit _H bond sites - fit salt bridges	• LogP up - HTS selects for high LogP
• LogP no change - no selection pressure	• H-bonding no change - no selection pressure
• Poor Permeability	• Poor Solubility

Figure 13.

organizations. Let's look at the left-hand column. This is the Merck worldwide organization. Now, I have called it 'structure-based' because actually, between 1965 and 1995, the Merck Organization generated its starting points for programs using every single technique of rational drug design, but not high-throughput screening, not then. Only in the last eight years has Merck been doing high-throughput screening.

So, for example, in structure-based drug design there is a tendency for molecular weight to go up because maybe you are working on peptidomimetic structures, you're trying to fit three or more binding sites. Hydrogen bonding goes up because you are trying to fit hydrogen bonding sites, you're trying to fit salt bridges. But in structure-based drug design, where you're using an X-ray of the target as part of your information, there is no change in lipophilicity in log-P. With this method of rational drug design, there's no selection pressure for log-P to change.

Now, if molecular weight goes up and hydrogen-bonding properties go up, then those two changes very reliably translate into poor intestinal permeability. So an organization that generates its starting points for its programs by every technique of rational drug design except high-throughput screening, will worry more about permeability problems as being related to poor oral absorption.

The right-hand column shows the Pfizer Groton Laboratories. Among all the Pfizer laboratories, those in Groton do more high-throughput screening than any other Pfizer laboratory in the world. Now if you develop your starting points for programs using high-throughput screening, molecular weight goes up because high-throughput screening selects for larger size and lipophilicity, log-P, goes up because high-throughput screening selects for higher log-P.

Let me explain this. What is a high-throughput screen? It is the empirical search for *in-vitro* activity. What you get from high-throughput screening reflects medicinal chemistry principles. What's the most efficient way for a medicinal chemist to improve *in-vitro* activity? It's to add lipophilic groups to a compound, that very reliably improves *in-vitro* activity. Other things being equal, a high-throughput screen will generate leads that are higher in molecular weight and higher in lipophilicity.

But high-throughput screening does not result in any changes in hydrogen bonding because there's no selection pressure. Again, this reflects medicinal chemistry principles. In general, if a medicinal chemist has a starting point, it usually is not very successful to change the polar functionality. Just leave it alone, and you add lipophilic functionality. Now, if molecular weight goes up and if lipophilicity goes up, then those two changes very reliably translate into poor aqueous solubility. So an organization like the Pfizer Groton Laboratories is much more worried about poor aqueous solubility than about poor intestinal permeability.

This is my explanation for you when you go to meetings or you read review articles about poor oral absorption. Sometimes people talk about permeability, sometimes about solubility. It's not really clear why people have such different opinions. I think the emphasis on either permeability or solubility is really related to how the organization develops its starting points.

Now in this next section I'm going to talk about Phase II drug property distributions. I'm going to be talking about the Rule of 5, about improving property profiles, and how Pfizer uses the Rule of 5.

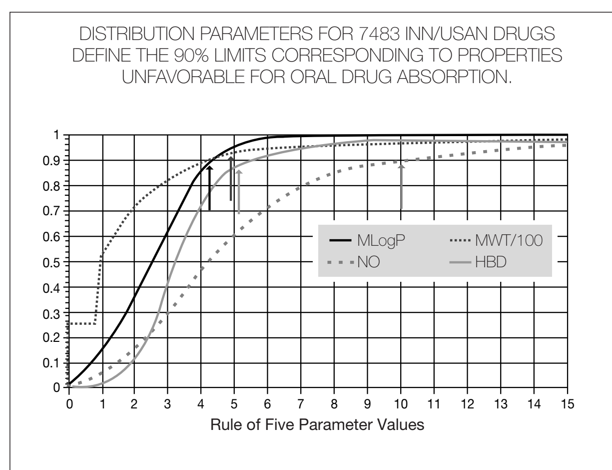


Figure 14.

Figure 14 describes a database-mining exercise, an analysis that I did in early 1995. I was looking at the property distributions for 7,500 Phase II drugs. Actually, I initially did it on 2,500, but I have extrapolated here to 7,500. I looked at four properties – lipophilicity, molecular weight, the number of hydrogen bond acceptors (just from counting the number of nitrogens and oxygens in the compound), and the number of hydrogen bond donors. Now why did I choose these four properties? Because it was very clear from the literature that these properties were very important in oral absorption.

How do you understand this graph? Let's start with the broken-line curve. For these 7,500 compounds, a 0.9 fraction, or 90 percent of these orally active drugs, will have 10 or fewer hydrogen bond acceptors. For the molecular weight, 90 percent of these Phase II drugs have a molecular weight of 500 or less. For lipophilicity, if you use MlogP as the calculation, 90 percent of these Phase II drugs have a value of 4.2 or less. If you use the Clog-P calculation it's 5 or less. For hydrogen bond donors, shown on the line with circles on it, 90 percent of these orally active Phase II drugs have five or fewer hydrogen bond donors.

This led me to come up with something I call the Rule of 5 mnemonic. Mnemonic is an English word which means saying something so it's easy to remember. I was trying to come up with something that would be easy for our medicinal chemists to remember, because they were making compounds that were just very poorly orally absorbed, and I wanted them to remember something.

So I came up with this rule that if you have more than five hydrogen bond donors, the molecular weight is over 500, the Clog-P is over five, and the sum of nitrogens and oxygens, the number of hydrogen bond acceptors, is over 10, you're very likely to get poor oral absorption or permeation. I called it the Rule of 5 because the number 5 comes in several times and I wanted our chemists to remember it.

The way we use this rule is, we made it part of our compound registration system. Our chemists had to draw in the structure when they registered the compound so that it could be tested biologically. If there was a problem – if two or more parameters were outside the desirable range – a computer screen would come up and it would say something like 'You are likely to have poor oral absorption because your molecular weight is 650 and your log-P is 6.' The chemists could still register their compound, but we made sure that they could not bypass this warning screen, so they could not give the excuse that they had never heard about poor physico-chemical properties.

The next thing we did was to load the results of this calculation into our Oracle databases, so that for every compound we knew we had stored the number of hydrogen bond donors, molecular weight, lipophilicity and the number of hydrogen bond acceptors. Why this helped is because our managers, especially our chemistry managers, knew we had a big problem, especially with aqueous solubility, but they didn't know where the problem was coming from. They didn't know who these people were who were making these very large and very lipophilic compounds.

Once we loaded the results in the Oracle database, they could check. They could get computer print-outs and then at meetings they started asking questions such as, what are the properties of your compounds? And the chemists had to answer. They had no excuse, because we made sure that they saw this. We did this in about the middle of 1995 and within six months, at the beginning of 1996, we also set up a very high-capacity solubility screen, to test every compound that the chemists made. Now, the chemists could no longer say, well, there's not enough capacity in the assay to test my compound, because there was.

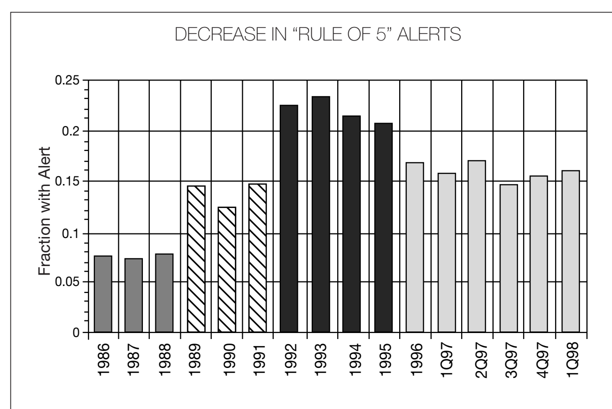


Figure 15.

So, did this work? The answer is yes, it worked extremely well. *Figure 15* plots for a number of years the fraction of the compounds made by the medicinal chemists that had an alert according to the Rule of 5, meaning that there were two parameters outside of the desirable range. The fraction of the alerts is very low in the years before high-throughput screening. The years 1986 to 1988 represent early high-through-

put screening, 1989 to 1991 represent high-throughput screening but before we really had the Rule of 5 or the solubility assay. We started the Rule of 5 in the middle of 1995 and by the beginning of 1996 we were running the solubility screen and, very quickly, the property profiles of the compounds the chemists were making greatly improved. Now, I've stopped this graph in early 1998 but if you were to continue it, it's flat.

What this says is that if you have the property filters in place and you have the associated experimental screens, you really can change the behavior of the kinds of compounds the chemists are making. So you can get the considerable advantages of high-throughput screening without these very bad property profiles. Will we ever get back to the very desirable 1988 level of properties? I don't think so. That's because the targets have changed. The targets are nowadays more complex.

So how does Pfizer use the Rule of 5? Well, I've told you that we use it as an on-line alert at compound registration and we use it as a filter for our high-throughput screening library. Then, about 18 months after I came up with the Rule of 5, we went in and identified all the compounds in our high-throughput screening file in the Groton Laboratories that broke the Rule of 5, with two parameters out of the range – and we stopped screening those compounds.

The compounds were still in the file, we just didn't screen them any more. The argument was, why work with those compounds? Even if they come out active *in-vitro* in screens, they're too difficult to turn into orally active compounds. We use the Rule of 5 as a filter for purchased compounds; we don't buy any compounds if they break the Rule of 5.

We use it as a criteria for focused library synthesis. Like many companies in combinatorial chemistry nowadays, we are making more libraries but with smaller numbers of compounds. We're using the Rule of 5 as part of the guideline, part of the library design process. And we use the Rule of 5 as a guideline for quality clinical candidates. We have a whole long list of requirements for our clinical candidates, one of which is the question, does the compound break the Rule of 5?

Now it's sometimes allright to break the Rule of 5, but if you do, then the people who are proposing the candidate have to have detailed experimental proof of why it is OK, why it will not cause problems in development. You could, for example, break the Rule of 5 if you have a very potent compound or a highly permeable compound, but you have to explain why that's OK.

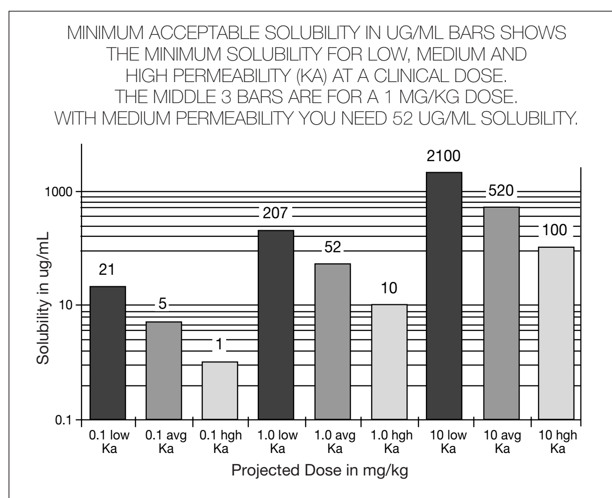


Figure 16.

Now I'm going to talk about some specifics on aqueous solubility. I'm going to talk about presenting data to chemists. What is acceptable solubility? What's the relationship between solubility, potency and permeability? I'm going to be talking about solubility by types of compound, to give you a realistic expectation of the kind of solubility you can expect in Phase II compounds, in commercial or academic compounds, and in the kind of compounds that come from medicinal chemistry. Then I'm going to introduce the concepts of lipophilicity and crystal packing as causes of poor aqueous solubility.

Figure 16 is one that we show to our medicinal chemists. The chemists ask us, how much solubility do I need in my compound? This is our answer.

Let me explain it by looking at the three middle bars, depicting a one milligram per kilogram dose. If the clinical potency of the compound is thought to be about one milligram per kilogram (though perhaps a dose of 100 mgs may be a little higher), and if the permeability (Ka) is average, then the chemist needs 52 micrograms per milliliter minimum thermodynamic solubility. If the permeability is in the upper-tenth percentile, then you maybe only need 10 micrograms per milliliter. And if you are in the bottom-tenth percentile in permeability – for example, you might have that in a peptidomimetic – then you need several hundred micrograms per milliliter solubility.

Now, chemists who are making insoluble compounds see this data, and chemists are always looking for explanations, excuses so that their compounds look better. So they look at the bars to the left-hand side and they say: 'Aha, if my potency was much better, if a

dose were very low, if I had a tenth of a milligram per kilogram *in-vivo* potency, and if I had very good permeability, then maybe one microgram per milliliter solubility would be acceptable.' And then we have to explain to our chemists that it's extremely hard to find these highly *in-vivo* potent compounds with low dose.

I estimate that among current clinical candidates these low-dose compounds are probably not more than 10 percent, they're probably less than 10 percent of clinical candidates. It seems it's mostly a matter of good luck to find these compounds; it does not seem as if there's currently any rational way to discover these compounds. So most of the time, 80 percent of the time, chemists have to live with these one milligram per kilogram candidate compounds, or about 100 milligram, maybe 200, total-dose compounds.

Now there's another point with this *Figure*, and I'm really glad that Professor Sugiyama showed some of his slides. Professor Sugiyama showed equations, right? Two or three years before I made up this *Figure* our pharmaceutical sciences people were talking to our chemists and, just like Professor Sugiyama, they showed equations. I don't know about chemists in Japan, but American chemists hate mathematical equations. As soon as the pharmaceutical sciences people showed an equation, the chemists would look around and not pay any attention at all.

All I did was I took the equations in a pharmaceutical sciences paper and converted them into a graph. The characteristics of chemists are that they are superb in pattern recognition, and a graph is a pattern, so they understand this, a chemistry structure is a pattern… So the message here is, if you want to talk

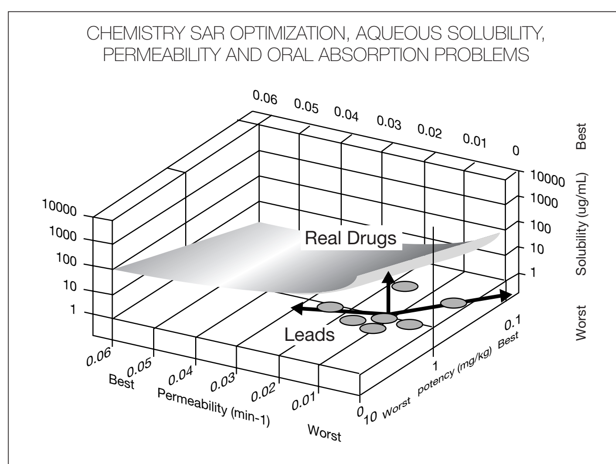


Figure 17.

to chemists, it's not enough to give the correct message. You must talk in a language and a way that the chemists really understand.

Now, *Figure 17* is a graph that we also show to our chemists and it illustrates the relationships between the three properties that are important for early oral absorption that the chemists can control through the chemistry structure. They are: permeability from worst to best; *in-vitro* potency from worst to best, and solubility from worst to best. The square-shaped interior surface defines the region of space above which real orally active drugs lie, and the circles are the starting points, the leads. It's the chemist's job, through changing the structure, to get above this surface to obtain an orally active compound.

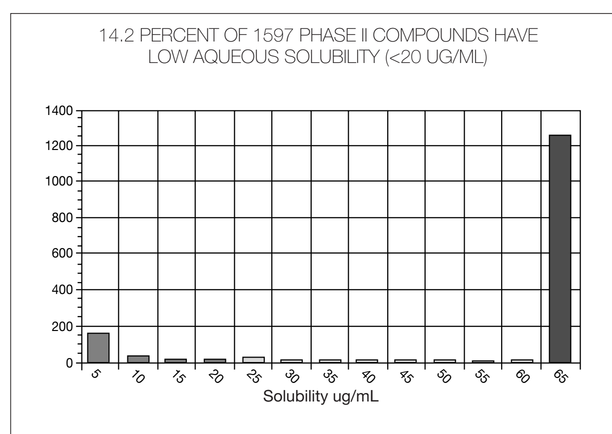


Figure 18.

Now the problem is, if the chemist just moves along the *in-vitro* potency arrow lying on the base, it's usually quite possible to get compounds that are highly active *in-vitro*, and maybe go from micromolar to nanomolar. But in the process you very often do not improve solubility, nor do you improve permeability. So you end up in the bottom right-hand corner. You have a very active *in-vitro* compound. You can get a publication in a medicinal chemistry journal. But that compound will never make any money for your company, and it will never help sick people, because it's not orally active.

Now I'm going to introduce the concept of the kind of solubilities you can expect for different collections of compounds. I was actually able to experimentally test almost 1,600 Phase II compounds in our experimental assays (*Figure 18*). Here compounds that had 20 micrograms per ml or lower solubility, would be considered to have poor solubility. About 14 percent

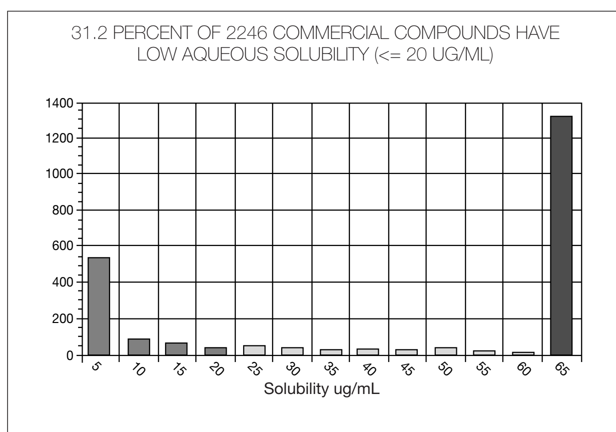


Figure 19.

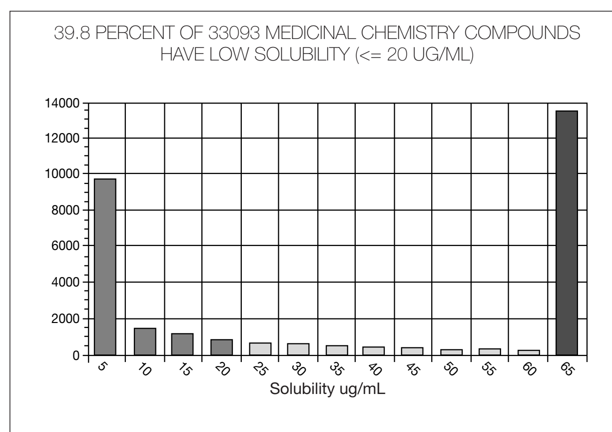


Figure 20.

of these Phase II compounds had poor solubility. I think this is absolutely the best one could ever get, sort of a gold standard among compounds: 14 percent poor solubility.

What about compounds that came from commercial, from academic sources? I was able to test about 2,200 of these compounds experimentally (*Figure 19*), and now the poor solubility rate is much higher, it's about 31 percent. These commercial compounds were the ones that went into the high-throughput screens, and the actives from those screens were the starting points for our medicinal chemistry. So what did medicinal chemists do to the solubility, with these kinds of starting points?

Well, the solubility got worse. Now, around 40 percent of the compounds had very poor solubility (*Figure 20*). This level of solubility in general would not result in any *in-vivo* activity in a biology assay and would result in little or no systemic exposure in a drug metabolism assay. So the chemists have made the compounds less soluble. That makes a great deal of sense because, when chemists increase *in-vitro* activity, as I told you, they typically add to molecular weight, they add to lipophilicity, and that makes the compounds less soluble.

The other point here is that I think the medicinal chemists in Groton are very good chemists, so this 40 percent poor solubility is a very realistic number. It's probably the best you will ever achieve nowadays. If you have good organization, good planning, then maybe you can keep the poor solubility at 40 percent. Now that sounds like a lot, and it is, but it's still possible to get quality clinical candidates when chemists are making 40 percent poorly soluble compounds. But if you are not very, very careful, this per-

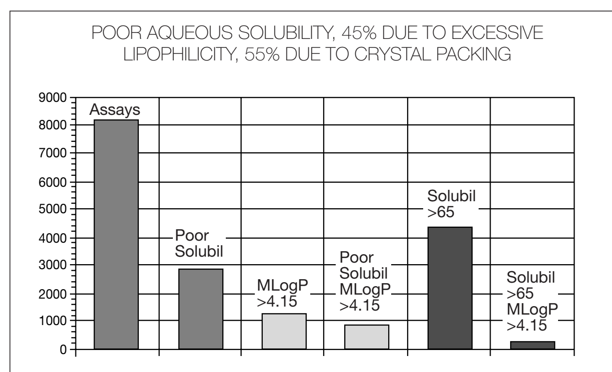


Figure 21.

centage of poor solubility easily can go up to 50 or 60 percent or higher.

Figure 21 is a very busy and complicated *Figure*, so I will give you the message just from the title. As of very recently, the Pfizer Groton Laboratories screen-tested more than 60,000 compounds for aqueous solubility. What we have found very consistently is that in about 45 percent of the compounds that are experimentally poorly soluble, we can detect a problem of excessive lipophilicity. In fact, we found that if the calculated log-P of the compound is above the Rule of 5 limits, above 5, about 75 percent of those compounds will be experimentally insoluble, poorly soluble.

But in about 55 percent of the compounds that are experimentally poorly soluble, the computer does not detect any kind of a problem. These compounds do not break the Rule of 5, and so the poor solubility must be due to a crystal packing issue. What this points out is that it's extremely important to have an experimental solubility screen, because currently

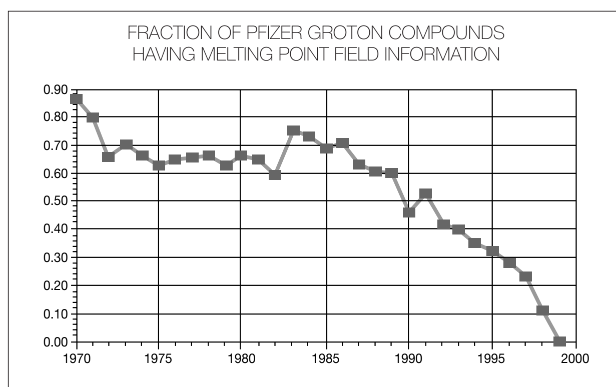


Figure 22.

there is no computer program that will reliably predict poorly soluble compounds due to crystal packing.

Now I'm going to go into a new section and talk about how compounds differ in aqueous and DMSO (dimethylsulfoxide) solubility based on crystalline form. Let me explain. There was a German gentleman named Ostwald who came up with something called the Rule of Stages in about 1880. It says that the sequence of compound batch isolation proceeds towards the thermodynamically most stable form.

What that means is that when chemists first isolate a compound and they don't know much about the isolation method, it's very common that the first material isolated is amorphous; that's the highest energy solid form. As they learn more about the isolation conditions they gradually begin to isolate crystalline materials, initially the highest energy polymorph, and finally ending up with the lowest energy polymorph. The significance of that is that, uniformly, the amorphous materials in the highest energy form are the most soluble, both in water and in DMSO.

Now I'm going to show you that amorphous compounds are becoming extremely common. I'm going to talk about changes in compounds' solid-state properties. I'm going to be talking about changes in purification methods. I'm going to tell you that in many organizations, melting points have disappeared, and that predominantly the industry is now working on amorphous compounds.

In early discovery changes are occurring that affect compound purity. There is a great deal of pressure on chemistry to increase output, to make more compounds. The managers tell the chemists, make more compounds. How are the chemists going to do it? Well, one of the ways is to stop carrying out steps in the chemistry process that take a lot of time.

First, crystallizing a compound to a sharp melting point takes a lot of time, and you don't need a melting point to know that you have made the correct compound because an NMR (nuclear magnetic resonance) spectrum or a mass spectrum will tell you that. Secondly, in combinatorial chemistry, combinatorial compounds are now being purified by automated procedures, by automated HPLC (high performance liquid chromatography), so nobody crystallizes combinatorial compounds. These are the current, across-the-industry purity criteria for combinatorial compounds purified by automated procedures.

This level of purity is lower than when chemists crystallize compounds, because crystallization is a very good way of removing impurities. So the consequence of this is that nowadays compounds appear more soluble than before because they're predominantly isolated in the amorphous state, and that's the state that's more soluble, and also because they contain more impurities and those impurities typically enhance solubility.

Well, *Figure 22* is my proof, at least within the Groton Laboratories, that melting points have disappeared. It shows all of the compounds synthesized by traditional medicinal chemistry from 1970 to the year 2000. In 1970, 90 percent, or a 0.9 fraction of the compounds our medicinal chemists made had a melting point. Then from about 1995, melting points disappeared. In the year 2000, zero percent, zero fraction melting points. Actually, it wasn't zero, we had 18 compounds with a melting point.

So what happened in the year 2000? Our pharmaceutical sciences people started a class to teach our new chemists how to crystallize compounds. We taught the chemists about the importance of solid-state properties, about solid forms. Why was that?

The reason was that it's perfectly acceptable to work with amorphous compounds in early discovery but when you get close to the late discovery/development interface, and especially if you're interested in oral absorption, you must compare crystalline materials. We had to get the chemists to change their chemistry, change how they isolated the compounds, so that in the late stages they would start to really make crystalline compounds. Since they didn't know how to do this, we had to train them. This is just the newer chemists, the older chemists knew how to do it.

What are the consequences of amorphous compounds? Amorphous aqueous solubility is always higher than when the compound is crystalline, which again reiterates the point. The crystalline state is very important in late discovery because you oftentimes

can get poorer oral absorption when a compound is crystallized.

We actually had one clinical candidate in the Groton Labs that we chose when it was amorphous, and then it was crystallized. The solubility dropped by over a factor of 100 and it caused all kinds of problems. We were able to rescue that compound with formulation technology but it was very, very difficult. That's the kind of situation you would really like to avoid.

Now, amorphous compound solubility in DMSO is just like it is in water; it is always higher than when the compound is crystalline. What that means is that most of these amorphous compounds dissolve relatively easily in DMSO. In my laboratory, we dissolved over 40,000 compounds in DMSO at quite high concentration, 60 millimolar and in general we had very little trouble dissolving the compounds.

So we were able to dissolve the compounds in DMSO. But you are more likely to get errors in the screening data because, if the compounds precipitate from DMSO solution then the screening concentration may be much, much less than you think it is. And if the compounds stay in DMSO then you are much more likely to test insoluble compounds. So by testing DMSO solutions you end up with many more insoluble compounds as leads, but if you had started by testing powdered crystalline compounds you'd never have been able to get them in solution, and therefore you'd never have detected the activity in an assay.

Now this is something I believe very strongly. At the Groton Laboratories we maintain two kinds of solubility assays: one intended for very early discovery, one intended for late discovery and the development interface.

The early discovery assays start from the DMSO stock solution; we actually use a light-scattering endpoint. This kind of assay always overestimates the solubility versus the thermodynamic and it's relevant to early discovery *in-vivo* SAR (structure activity relationships). We use this kind of assay very early in discovery when the chemists are changing their structure and they're just trying to get a little bit of activity *in-vivo*, a little bit of systemic exposure.

But when you come close to the development interface then you have to do a proper thermodynamic solubility assay. We've actually built a robot which will speed that process up, but we demand that the chemists give us crystalline materials because if you screen amorphous materials in a thermodynamic solubility assay you could be completely wrong. The thermodynamic assay has an equilibrium endpoint. It's essential at the clinical candidate stage and we tell our chemists that the distinction between these two types of solubility assays is absolutely critical. The light scattering assay is intended for early discovery and must never be used late in discovery, when the thermodynamic assay should be used instead.

Compound solubility in water and DMSO is really determined by two factors: solvation energy and crystal disruption. For any one compound there's a sort of continuum in the importance of each. Maybe for one compound, solvation energy is more important, for another it could be crystal disruption. For the low melting point, large lipophilic compound, solvation is more important, and for the high melting point hydrophilic compound, crystal disruption is more important.

For solubility in water and DMSO, if you have a large, lipophilic compound and it's maybe not very soluble in water, then DMSO greatly helps the solubility. But a very crystalline compound may show no computational problem, no Rule of 5 violation. It's insoluble in water. It might have a high melting point – and that would be a clue to this kind of a problem, if you had melting points. Probably you have strong intermolecular crystal lattice interactions. For that kind of a compound, DMSO just does not help improve solubility. So if a compound is insoluble in water because of this crystallinity issue, then ultimately it is very likely the compound will come out as a crystallized precipitate from DMSO.

Suppose you are an end user, a person who uses compounds dissolved in DMSO, and the compound disappears from the DMSO solution. You might say, well, I don't care what the explanation is. But you really should care because the explanation is important in understanding what to do to solve the problem.

If the problem for the compound disappearing from DMSO is a chemistry problem, a chemical integrity problem, meaning that the compound is changing its structure, it's going to something else, then it's very clear what you need to do. You need to keep the solution of compound in DMSO cold and frozen, you need to avoid oxygen and you need to keep it dry. Why do you need to keep it dry? Because hydrolytic instability requires water. If there's no water in DMSO you cannot get hydrolysis.

If the problem though is precipitation crystallization, if that's what's removing the compound from DMSO, then keeping it cold and frozen is the absolute worst choice possible. If you think about it, how does the chemist crystallize compounds? He / she takes so-

mething in solution and cools it down. You want to avoid that, and you want to avoid freeze/thaw cycles.

What we found in our laboratory is that there is a timing factor in compound DMSO solubility. First of all, once a compound crystallizes from DMSO, very often it will not easily re-dissolve, and people don't understand that. But what's happening is that when the compound is first dissolved in DMSO it is amorphous, so it goes in at high solution. But when it crystallizes out, it's coming out as crystalline material which is inherently less soluble and therefore you will probably have a lot of problems re-dissolving that compound in DMSO.

We also noticed a narrow working time-window for keeping most compounds dissolved in DMSO. What we found out is that generally there was no problem in keeping compounds in solution for the first day or two. But past that time, compounds started to precipitate. This explains why compounds are active when solutions in DMSO are freshly made from powders, but not when compounds are stored for a long period of time in DMSO. They have probably precipitated and, again, freeze/thaw cycles increase the probability of crystallization.

Here is some very practical advice we give to the end user of compounds in DMSO. *Figure 23* is a phase diagram between liquid DMSO and solid DMSO. The X-axis shows how much water there is in the DMSO; at the extreme right of the diagram is very dry DMSO, no water. The melting point of dry DMSO is 18 degrees Centigrade. So the scientists think they can stop crystallization by putting the compounds dissolved in DMSO into the refrigerator, which is usually at about 4 or 5 degrees Centigrade, and the DMSO will be frozen, and you cannot get precipitation if the DMSO is frozen.

That argument is incorrect. The reason is that the melting point of DMSO is incredibly dependent on the amount of water in DMSO. If you just have 9 percent water in DMSO, the melting point goes down from 18 degrees to 4 degrees Centigrade. And 4 to 5 degrees Centigrade is the temperature of the non-freezer part of a laboratory refrigerator. So scientists are storing samples in DMSO, they're still liquid, they are very cold, these are ideal conditions for crystallization.

How easy is it to get 9 percent water? Incredibly easy. If you get the compounds in DMSO from the central organization that produces them, you probably have 5 percent water in them. Just open the samples, especially in humid weather as you have here (in Tokyo) at this time of year, and you easily have 9 percent or more. So the typical biology procedure of storing samples in DMSO in the non-freezer part of a lab refrigerator is very bad practice.

This advice is for short-term storage, from a few days to a few weeks. Do not store samples in DMSO in a refrigerator. Keep them in a chemistry glass dessicator at room temperature, keep dry nitrogen or argon over the samples, maybe throw a towel over the dessicator to keep light out, just keep them at room temperature. This is what you should do for short-term storage.

Now I am in my last section, talking about solubility and combinatorial chemistry. I'm going to tell you that among combinatorial libraries, solubility is a major problem but permeability is seldom a problem.

Figure 24 is my illustration of this point. It shows a collection of about 48,000 compounds from a very reliable source of combinatorial compounds. On this CD-Rom there were 30 chemical sub-series and I worked out the average calculated solubility for each

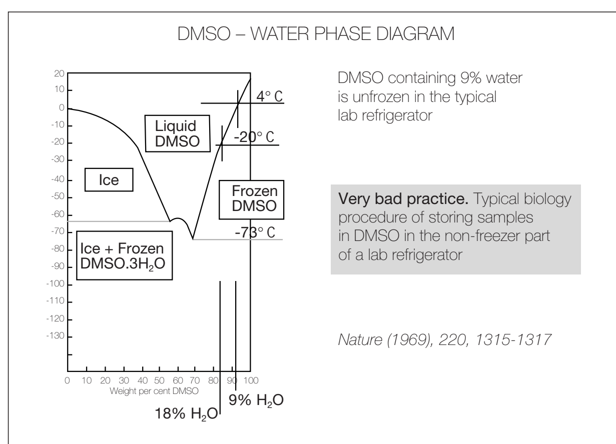


Figure 23.

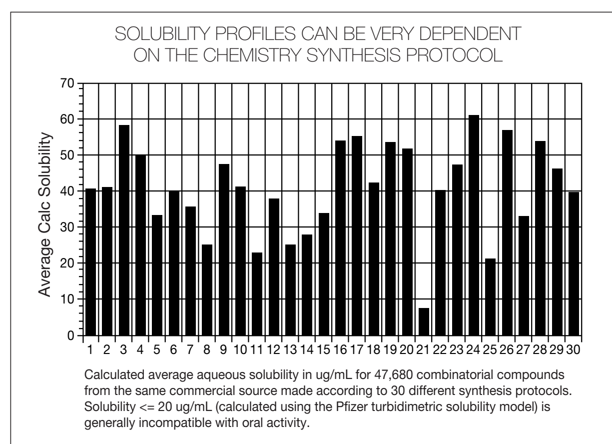


Figure 24.

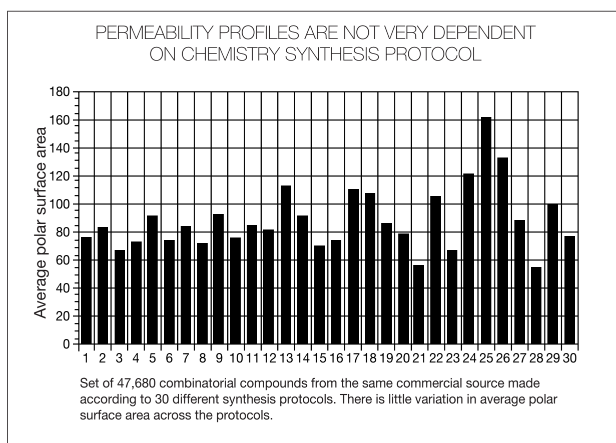


Figure 25.

of these sub-series. Now in this calculation less than 20 ug/ml (micrograms per ml) represents bad solubility, anything higher is approaching towards acceptable. The point here is that there's a lot of variation. This is the hallmark, the characteristic of combinatorial libraries: aqueous solubility is very dependent on the chemical series, it varies a lot across chemical series. Now, what about permeability?

Figure 25 is very different. For each of these 30 chemical series, I've calculated the average polar surface area. If you have a polar surface area of 150 square angstroms or more, you likely are going to have passive intestinal permeability problems. Here, only a single series, series 25 maybe has a problem, everything else is OK. Again, that's the characteristic of combinatorial libraries: you generally do not have a permeability problem.

Why is that? It's entirely a chemistry issue. In combinatorial chemistry it's extremely easy to make large, lipophilic compounds. It's difficult to make hydrophilic polar compounds. The automated purification procedures like reverse-phase HPLC that you use in combinatorial libraries work much better for the large, lipophilic compounds, but they work much more poorly for the hydrophilic compounds. So it's entirely a chemistry issue.

Figure 26 gives another way of illustrating it. Each of the squares is a collection of compounds and, for each of those collections, I've calculated what percent of the compounds have a high polar surface area greater than 150 square angstrom. So if you move out towards the right, on average those compounds will be less permeable.

I've also done a calculation for each library collection of average poor solubility. To the lower left-hand side is where 7,500 Phase II drugs lie; here are 330 drugs with human fraction absorbed. This region of chemistry space is what I call traditional drug space. This is the region of space occupied by nice, well-behaved compounds. If you are working with compounds in this region of space you probably are not going to be very interested in predictive models or higher-throughput experimental screens – for example, for solubility and permeability – because you don't need them.

Where do combinatorial libraries lie? They lie in the region outside the traditional drug space, very consistently. I think the realistic goal for combinatorial libraries is to have about 40 percent poor solubility, just like I showed you in the medicinal chemistry in earlier examples. But if you do not have good library design, if your chemistry is bad, then poor solubility can just go way up. It's very, very easy to get 50 to 60 percent poor solubility in badly-designed, badly-made combinatorial libraries. Actually, in the worst permeability region to the right, among many combinatorial libraries I was only able to find three that had a permeability problem. It's almost impossible to find combinatorial compounds that have problems with permeability.

So here's my summary. I think it's very important to use the appropriate aqueous solubility assay. We use a kinetic assay in early discovery and at that stage it's perfectly OK, in fact it's probably efficient, to work with amorphous compounds. In late discovery we use a thermodynamic assay, and we test crystalline compounds only. We tell our chemists, and we teach them, to worry about crystallinity in late discovery. Chemists need to know something about solid-state properties, that the crystalline state is important to aqueous solubility.

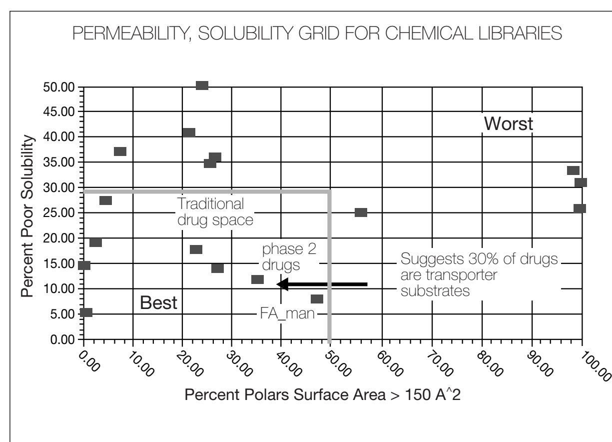


Figure 26.

The need for collaboration between chemists and pharmaceutical scientists is also a very important point. I'm glad that Professor Yamashita showed the slide from Professor Ronald Borchardt presented earlier this year at an ADMET conference in Whippany, NJ USA because collaboration between chemists and pharmaceutical scientists and drug metabolism scientists is absolutely essential.

End users of compounds in DMSO need to be alert for compound precipitation from DMSO. A very practical message: if you are storing compounds in DMSO for short periods of time, a few days to a few weeks, do not store those DMSO compound stocks in the refrigerator.

And the final point is, I really believe that poor aqueous solubility is here to stay. I speak at many, many meetings. I have not seen anything that would tell me that the solubility problem is going to improve. In fact, I think it's very realistic to say that about 40 percent poor aqueous solubility is probably the best you can expect. You have to be really careful it doesn't get much worse, because it's incredibly easy to get 50 to 60 percent – even worse than that – poor aqueous solubility.

Lastly, I'd like to thank the Pfizer Organization for their support of me in my post-retirement activities, and I'd like to thank you for your attention.

Professor Yuichi Sugiyama, University of Tokyo: Thank you, Chris, for your very exciting talk. It's nearly lunchtime now but I would like to take a few questions from the floor, so please ask any questions. Japanese is also acceptable as we also have translators.

Ibuke, Fujisawa Pharmaceuticals, Japan: You made a number of interesting points. One was that Merck uses a structure-based approach and Pfizer a high-throughput approach, and there are different issues associated with each. But which strategy was better at obtaining new product output?

Dr. Christopher A. Lipinski, Pfizer Inc., USA: Which strategy is better for new product output? Is it everything except high-throughput screening, or is it high-throughput screening? I have to be honest here. The Pfizer Laboratories in Sandwich in the United Kingdom generate their starting points almost exactly like the Merck Organization and in the 1990s all the drugs from Pfizer came from the English laboratories. They did not come from the Pfizer Laboratories in Groton, Connecticut, so you could probably draw a conclusion from that.

Ibuke, Fujisawa Pharmaceuticals, Japan: The reason I asked this question is for pharmaceutical scientists. Poor solubility is manageable in our view, through various solid dispersion methods or so-called non-aqueous systems, using solvents and dissolving those. On the other hand, poor permeability is intrinsic to the nature of the compound itself, and so we cannot overcome this issue. So, for me, poor permeability is a larger challenge. That was my impression. Do you have any comments on this?

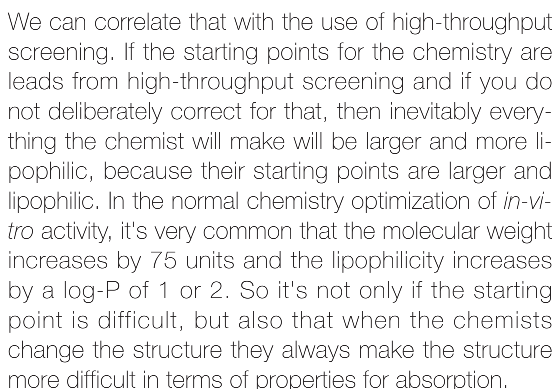
Dr. Christopher A. Lipinski, Pfizer Inc., USA: What can you do about poor permeability? Let's focus on chemistry. There actually is something you can do about it in chemistry but there's a problem in that you can't computationally predict it. The literature is very, very clear. If you can form an intramolecular hydrogen bond within a compound, that will improve permeability by at least a factor of 10, maybe 20 or 30. So for those kinds of compounds that are conformationally flexible, that can form an intramolecular hydrogen bond, it's maybe a good idea to have an experimental assay that will pick up that property; a parallel artificial membrane permeability assay, for example.

Now the problem from the library design perspective is that there is no computational program that can predict very well, either for a single compound or for many compounds, whether in terms of energy a compound forms an intramolecular hydrogen bond. And in many of the meetings at which I speak, I tell people I hope that there are people in the audience who develop software. I tell them that this is a big opportunity because if an organization had a software method for predicting an intramolecular hydrogen bond, then that would be a great advantage.

Now once you get away from intramolecular hydrogen bonding, then the questioner is completely correct; there is nothing you can do in formulation. The only thing you can do is change the chemical structure, make a prodrug. So if you have to deal with a problem in terms of poor oral absorption, it's much better to deal with poorly soluble compounds than to deal with poorly permeable compounds because you really don't have any formulation rescue technology there to help you.

Question from the audience: Thank you very much. Can I ask a question in English? *Figure 15* clearly shows that the number of compounds with Rule of 5 alerts decreases with time. Is this because of the introduction of so-called focused library synthesis, or are there other reasons?

Dr. Christopher A. Lipinski, Pfizer Inc., USA: You saw that over the years chemists were making larger and more lipophilic, less soluble compounds.



We can correlate that with the use of high-throughput screening. If the starting points for the chemistry are leads from high-throughput screening and if you do not deliberately correct for that, then inevitably everything the chemist will make will be larger and more lipophilic, because their starting points are larger and lipophilic. In the normal chemistry optimization of *in-vitro* activity, it's very common that the molecular weight increases by 75 units and the lipophilicity increases by a log-P of 1 or 2. So it's not only if the starting point is difficult, but also that when the chemists change the structure they always make the structure more difficult in terms of properties for absorption.

Question from the audience: I have another related question. Actually, the percentage of alerts is something like 20 percent, right? My question is about the strictness of the Rule of 5. For example, if you increase the strictness of each rule then, of course, the percentage of compounds with alerts will increase, and then you can decrease the high-throughput screening effort. Therefore my point is that the strictness and the effectiveness of the Rule of 5 always depends on the money allocated to high-throughput screening. When you initially proposed the Rule of 5, how did you determine the strictness for each parameter?

Dr. Christopher A. Lipinski, Pfizer Inc., USA: I did it based on the 90th percentile, and I chose those values because I wanted to be extremely conservative. I was trying to convince chemists to change their behavior and it would not have been a good idea to come up with rules where there were very many exceptions, so I made the rules very strict. So, if you break two parameters in the Rule of 5 there's a very high probability that you will have a great deal of difficulty obtaining an orally active compound.

Now for the first part of your question. There is a difference in the viewpoint about the Rule of 5 across different Pfizer laboratories. At our English laboratories which, like Merck, do very little high-throughput screening, they say it's bad to have just one parameter wrong.

At the Groton Laboratories, that does a lot of high-throughput screening, we allow two parameters; we say it gets bad if two parameters are in the wrong range. I can also tell you that for very well-designed combinatorial libraries, we are able to hold the number of compounds with two parameters in the wrong range to below 1 percent, but for one parameter out of range it's around 20 percent.

Professor Yuichi Sugiyama, University of Tokyo: Thank you. It is now time to close the morning session, so thank you again for your great contribution to this symposium.

Novel approaches for oral delivery of poorly soluble drugs

Dr. Hirokazu OKAMOTO

Novel approaches for oral delivery of poorly soluble drugs

Dr. Hirokazu Okamoto

Associate Professor, Meijo University, Nagoya, Japan.

Noriko Yamanouchi, Capsugel Japan: Gentlemen, we would like to begin the afternoon session. Dr. Ibuki will chair Session II-a, Invited Lectures.

Chair: Dr. Rinta Ibuki, Fujisawa Pharmaceutical Co., Ltd, Japan : I am Ibuki from Fujisawa Pharmaceuticals. Section II-a will include three presentations, talking about various approaches to poorly soluble drugs. The speakers are all very experienced experts in this field. Rather than give a formal lecture they want to be provocative and stimulate discussion. They will each give a short presentation so that we can have a longer discussion period.

The first speaker is Dr. Hirokazu Okamoto from Meijo University. Dr. Okamoto, as you know, has worked in both industry and academia. He is very experienced and I am sure we will learn a lot from him.

Dr. Hirokazu Okamoto, Associate Professor, Meijo University, Nagoya, Japan: Thank you very much, Dr. Ibuki, for the introduction, and thank you for giving me this opportunity. I would like to thank the organisers and everyone involved. I will be talking about novel approaches for oral delivery of poorly soluble drugs and from the title you can see it is rather abstract.

I'll be talking about solubility and absorption improvement for poorly soluble drugs and will present a number of methods. Dr. Yamashita said that we need deep and wide knowledge, but I won't go into details today. I won't go into the deep issues, but I'd like to talk about the wider issues.

Dr. Lipinksi mentioned HTS (high-throughput screening) – in other words, combinatorial screening programs. He said that 40% of the compounds screened are poorly soluble in water, and talked about how to develop them into oral active products.

The issues associated with poorly soluble drugs include poor bioavailability; highly variable bioavailability between patients; highly variable bioavailability in the fed or fasted states, and a slow onset of action.

Flux (J) is calculated as shown in *Figure 1* and can usually be obtained by multiplying permeability (P) by the drug concentration at the absorption site (C) and R. But, as Dr. Sugiyama mentioned, with recent compounds we now know that an increasing number go through first-pass metabolism, or efflux by a transporter such as P-glycoprotein; they are fluxed out, thrown out in other words. So flux would be represented by the multiplication of these three factors, P, C and R.

Theoretical consideration for improved absorption of poorly soluble drugs

$J = P \times C \times R$

P: intrinsic permeability coefficient (R=1)

C: drug concentration at the absorption site

R: parameter between 0 to 1 representing the extent of the absorbed drug avoiding the first-pass metabolism and/or efflux by a transporter such as p-glycoprotein

Figure 1.

Possible methods to improve the oral bioavailability of poorly soluble drugs			
	Intrinsic P	**C**	**R**
Chemical modification of drug molecules			
Prodrug	±	↑	±
Salt selection	±	↑	±
Physical modification of bulk drugs			
Polymorph selection	±	↑	±
Particle size reduction	±	↑	±
Formulational modification of drug products			
Co-solvent	↓	↑	± or ↑
Emulsion	± or ↑	↑	± or ↑
Complexation	± or ↑	↑	± or ↑
Solid dispersion	±	↑	± or ↑

Figure 2.

Figure 2 summarizes the possible methods available that can enhance the oral bioavailability of such poorly soluble drugs. One method is the chemical modification of drug molecules by using prodrugs or salt formation. For the physical modification of bulk drugs, we have polymorphism selection and particle size reduction. With modification of formulation or drug products, we have co-solvents, emulsions, complexation – cyclodextrins, for example are used in complexation – and also solid dispersion. So for each of these, by increasing the solubility, the dissolution rate can be improved. In addition, with formulation modification the permeability can be improved or, in some cases, efflux and metabolism can be restricted and this will improve the oral absorption of poorly soluble drugs.

I would now like to talk about prodrugs and particle size reduction, co-solvents, emulsion and solid dispersion. These techniques will each be explained in turn.

First of all, concerning prodrugs. In general, we are targeting poorly soluble drugs and so they need to be water-soluble. But if they are hydrophilic then their permeability tends to be reduced or sacrificed. So how can we increase absorption, or the water solubility of the drug, while maintaining a high level of permeability? That is our challenge.

Figure 3 shows one of the strategies to cope with that issue. The prodrug itself has hydrophilicity, but in the small intestine it passes through the membrane round the brush border and is metabolized by the brush border enzyme (BBE). Then it changes to the parent drug and solubility is improved. Now, when the drug passes through the membrane, the permeability remains at a high level, and so this prodrug strategy can improve solubility as well as maintain permeability.

The prodrugs shown in *Figure 4* were designed according to this strategy. Cam 4451 was a parent drug with low aqueous solubility. Leucine, dimethylglycine or phosphate ester was used for developing the prodrug. The original solubility is less than 2 micrograms per milliliter and the solubility of each prodrug is 0.1, 3 and more than 61 milligrams per milliliter, and so solubility is significantly improved.

The table in *Figure 4* contains some numbers. The half-life in rat intestine perfusate is shown within the third column. The next column is the half-life in brush border membrane (BBM) homogenate that includes the BBM enzymes. The selectivity ratio is in the most right column. The larger this number is, the more stable the prodrug is within the GI tract; it is also metabo-

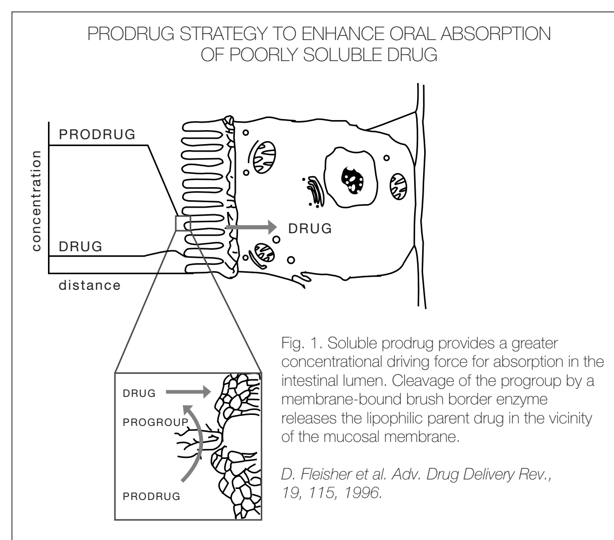


Figure 3.

DESIGN OF WATER-SOLUBLE PRODRUGS TARGETED TO INTESTINAL BBM ENZYMES

Table 1. Reconversion Half-Lives and Selectivity Ratio of Prodrugs

Prodrug	Pro-group	$t_{1/2}$ in perfusate	$t_{1/2}$ in BBM (min)	Selectivity ratio
Cam-4580	Leucine	5.34 ± 0.98	148 ± 38.4	0.036
Cam-4562	Dimethylglycine	17.3 ± 5.3	70.5 ± 6.5	0.245
Cam-5223	Phosphate	5.62 ± 2.5	0.0668 ± 0.0062	84

Note: Selectivity ratio = $t_{1/2}$ in perfusate / $t_{1/2}$ in BBM.

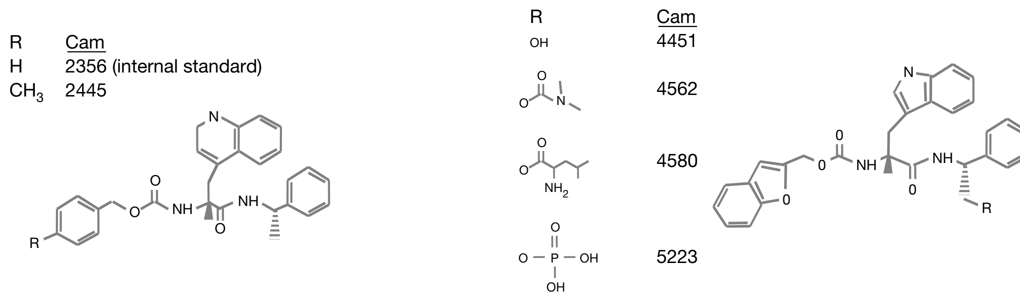


Fig. 1. Chemical structures of Cam-2445, Cam-4451, prodrugs, and internal standard

O. H. Chan et al. Pharm. Res., 15, 1012, 1998.

Figure 4.

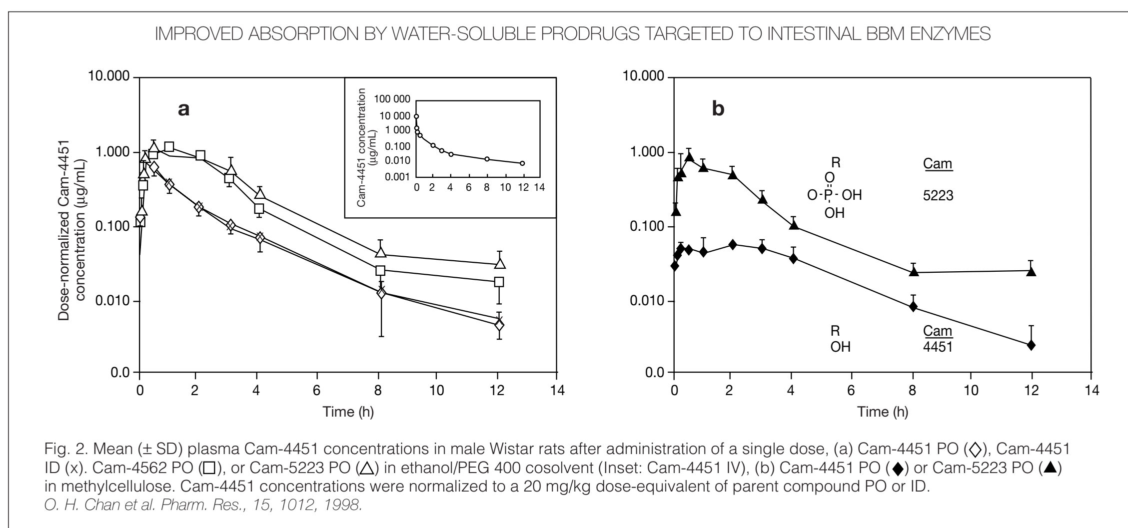


Fig. 2. Mean (± SD) plasma Cam-4451 concentrations in male Wistar rats after administration of a single dose, (a) Cam-4451 PO (◇), Cam-4451 ID (x). Cam-4562 PO (□), or Cam-5223 PO (△) in ethanol/PEG 400 cosolvent (Inset: Cam-4451 IV), (b) Cam-4451 PO (◆) or Cam-5223 PO (▲) in methylcellulose. Cam-4451 concentrations were normalized to a 20 mg/kg dose-equivalent of parent compound PO or ID.
O. H. Chan et al. Pharm. Res., 15, 1012, 1998.

Figure 5.

lized close to the membrane. So in these prodrugs the phosphate ester has high solubility, plus it is selectively hydrolyzed at the brush border membrane.

Turning now to focus on the right-hand side, graph b in *Figure 5*. This indicates the prodrug with the phosphate ester and the parent drug. After administering it to the rat, the phosphate ester concentration was 10 times higher. So the solubility and bioavailability of a poorly soluble drug can be improved with a prodrug.

Going on to the next topic, *Figure 6* is about particle size reduction strategy. Reducing particle size has been a conventional method and the dissolution rate can be calculated according to the formula shown in the slide. By reducing the particle size the surface area will increase and so the dissolution rate is accelerated. Another advantage of doing this is that several micrometers at nano level means a higher saturated solution and better solubility. Solubility is shown by the upward-facing arrows in the *Figure*, and the dissolution rate increases with smaller particle size, as you can see.

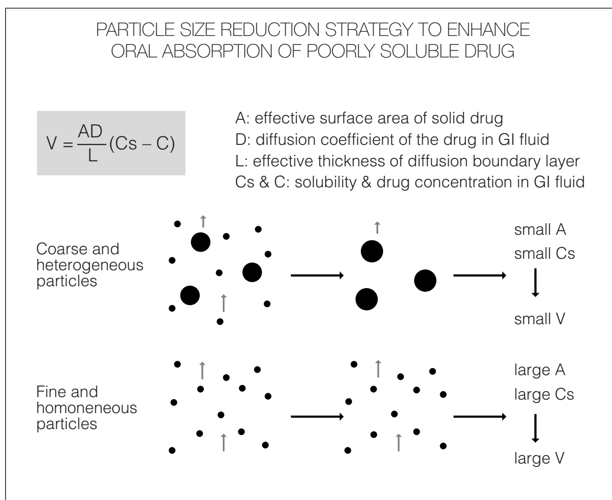


Figure 6.

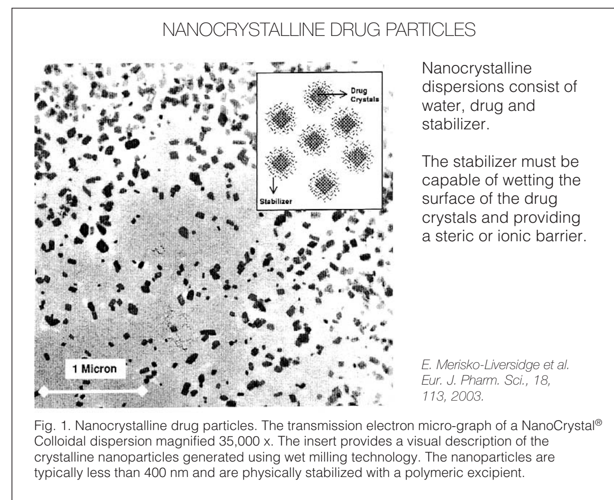


Figure 7.

It is necessary to have homogeneous particles. If you have a mixture of large and small particles, the smaller ones have high solubility and the dissolved drug is used for growth of the larger particles, so this is not a good strategy. Instead, the drug should be made up of small, fine and homogeneous particles.

Figure 7 is an example of nanocrystalline drug particles. To disperse them evenly you need to add a number of stabilizers. Stabilizers are usually additives such as the cellulose type of polymers, polyvinylalcohol and PVP.

Figure 8 shows the apparatus used for the reduction of particle size. The drug and the stabilizer are incorporated in a suspension and this is put into the media milling machine. The media and the polymer are mixed together at high speed and a sheer force is used for reducing the particle size. After 30 to 60 minutes of processing it is brought down to nano-order size. The smaller the size of the particle, the higher the plasma concentration. With a reduced particle size, the absorption rate goes up. Not only that, but there are a number of other absorption advantages; the effect of food is not as large as before, for example.

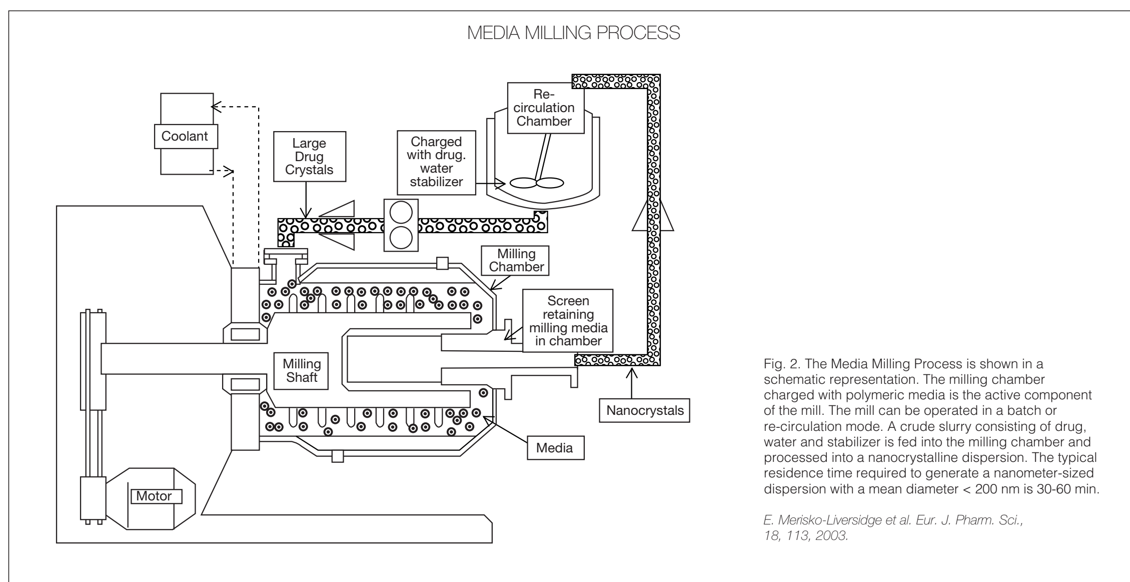


Figure 8.

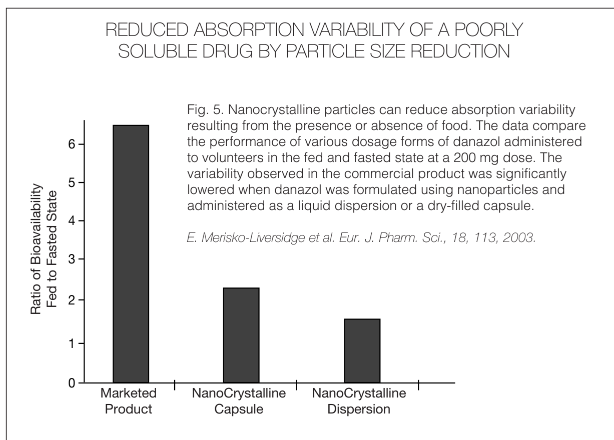


Figure 9.

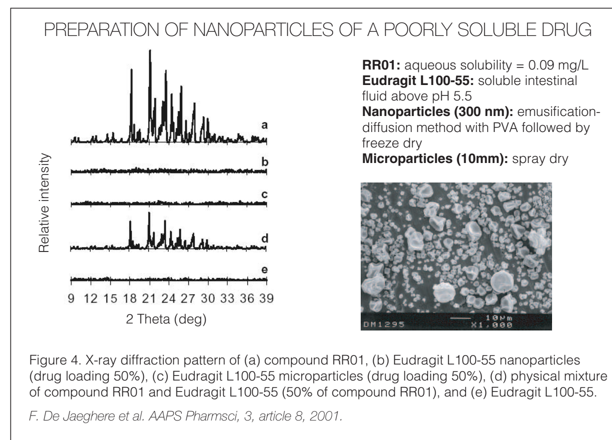
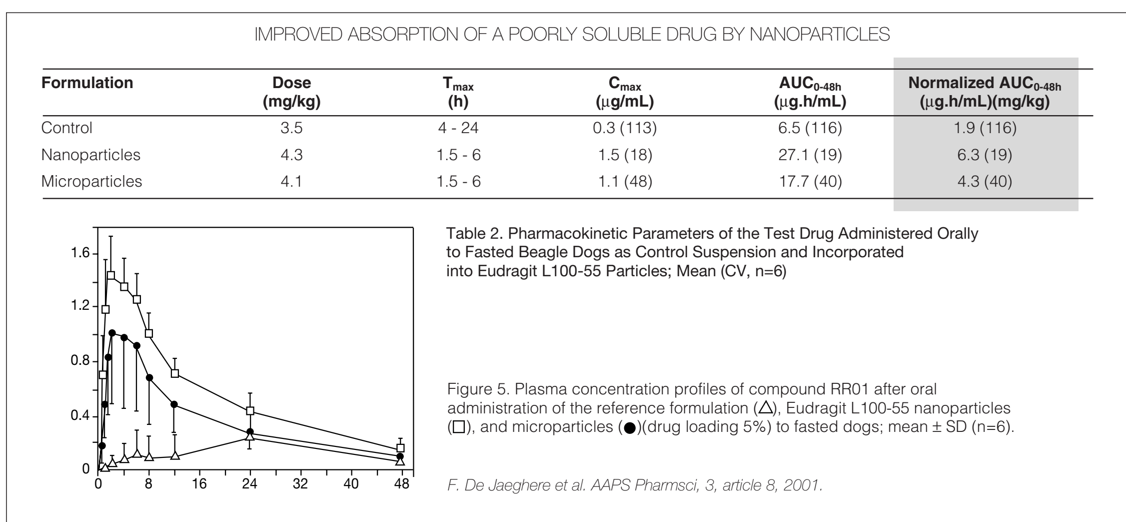


Figure 10.

Figure 9 shows danazol, a poorly soluble drugs. With the marketed product, post-prandial absorption – the bioavailability – is six times higher than pre-prandial. This is because bile salts improve the absorption. But if you make a nano crystalline, the solubility is higher from the start, so the role of bile salts in improving solubility is reduced. As a result, the bioavailability before and after food is not so variable; it is close to a ratio of 1.

I have talked about the drug substance being reduced in size, but now let me talk about the actual formulation being reduced. In *Figure 10* they used a nanoparticles with an enteral-coated additive. The particle size is 300 nanometers and the X-ray diffraction data b means that it has become amorphous. Spray-dried microparticles 10 micrometers in size were used as a control, and you can see that X-ray diffraction c is also amorphous.

The blood concentration data is shown in *Figure 11*. The pattern of plasma concentration is as shown. The pharmacokinetic parameters are summarized in the top chart. If you compare the dose-normalized AUC (the area under the curve) the control, which is the conventional formulation, is 1.9, and in comparison the nanoparticles AUC have risen to 6.3. With the spray-dried microparticles it is a little lower than that. So by reducing the particle size to 300 nanometers you can increase the solubility and bioavailability as well. Compared to the control and microparticles the variability of AUC is smaller and so precision in the dosing can be achieved by the nanoparticles.



IMPROVED ABSORPTION OF A POORLY SOLUBLE DRUG BY NANOPARTICLES

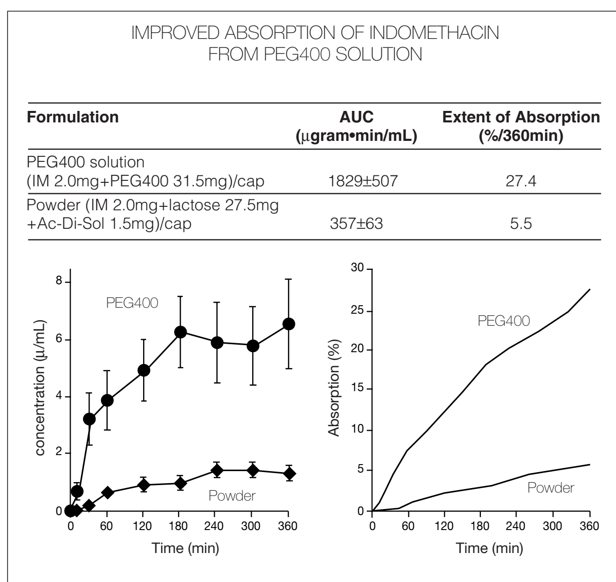
Formulation	Dose (mg/kg)	T_{max} (h)	C_{max} (μg/mL)	AUC_{0-48h} (μg.h/mL)	Normalized AUC_{0-48h} (μg.h/mL)(mg/kg)
Control	3.5	4 - 24	0.3 (113)	6.5 (116)	1.9 (116)
Nanoparticles	4.3	1.5 - 6	1.5 (18)	27.1 (19)	6.3 (19)
Microparticles	4.1	1.5 - 6	1.1 (48)	17.7 (40)	4.3 (40)

Table 2. Pharmacokinetic Parameters of the Test Drug Administered Orally to Fasted Beagle Dogs as Control Suspension and Incorporated into Eudragit L100-55 Particles; Mean (CV, n=6)

Figure 5. Plasma concentration profiles of compound RR01 after oral administration of the reference formulation (△), Eudragit L100-55 nanoparticles (□), and microparticles (●)(drug loading 5%) to fasted dogs; mean ± SD (n=6).

F. De Jaeghere et al. AAPS Pharmsci, 3, article 8, 2001.

Figure 11.


IMPROVED ABSORPTION OF INDOMETHACIN FROM PEG400 SOLUTION

Formulation	AUC (μgram•min/mL)	Extent of Absorption (%/360min)
PEG400 solution (IM 2.0mg+PEG400 31.5mg)/cap	1829±507	27.4
Powder (IM 2.0mg+lactose 27.5mg +Ac-Di-Sol 1.5mg)/cap	357±63	5.5

Figure 12.

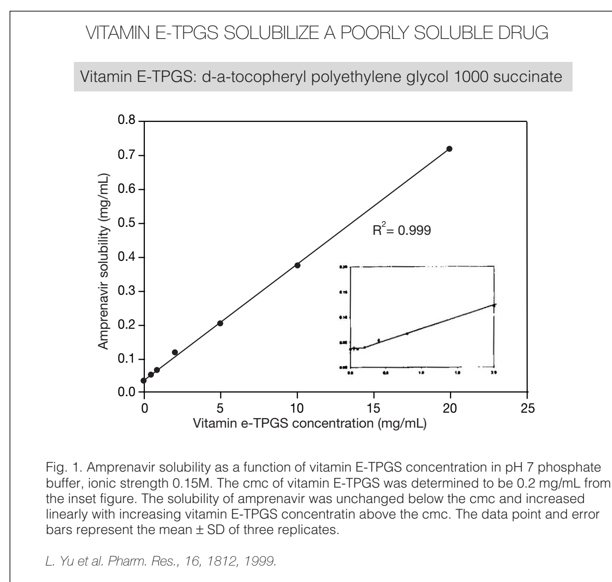


Fig. 1. Amprenavir solubility as a function of vitamin E-TPGS concentration in pH 7 phosphate buffer, ionic strength 0.15M. The cmc of vitamin E-TPGS was determined to be 0.2 mg/mL from the inset figure. The solubility of amprenavir was unchanged below the cmc and increased linearly with increasing vitamin E-TPGS concentratin above the cmc. The data point and error bars represent the mean ± SD of three replicates.

L. Yu et al. Pharm. Res., 16, 1812, 1999.

Figure 14.

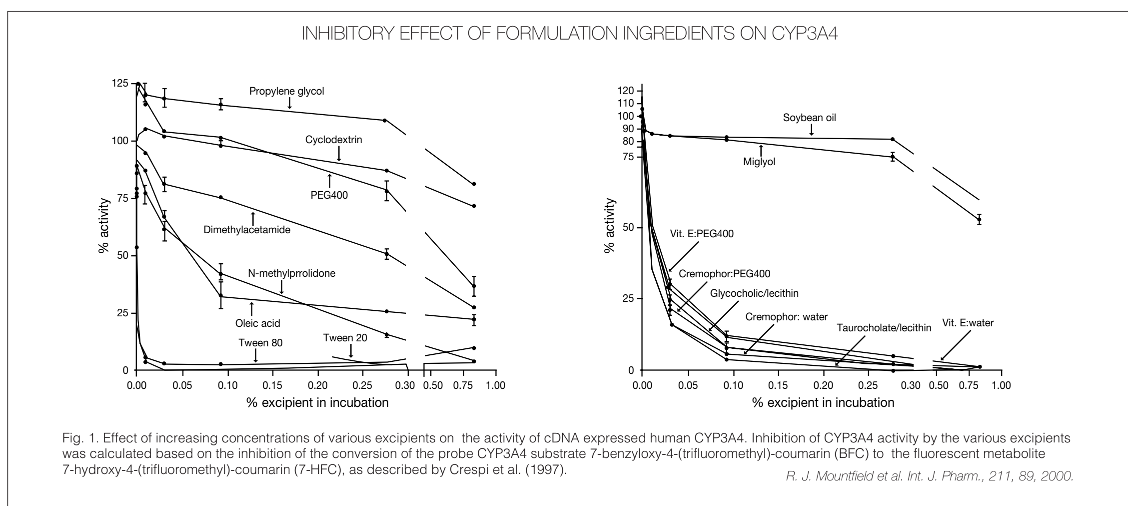


Fig. 1. Effect of increasing concentrations of various excipients on the activity of cDNA expressed human CYP3A4. Inhibition of CYP3A4 activity by the various excipients was calculated based on the inhibition of the conversion of the probe CYP3A4 substrate 7-benzyloxy-4-(trifluoromethyl)-coumarin (BFC) to the fluorescent metabolite 7-hydroxy-4-(trifluoromethyl)-coumarin (7-HFC), as described by Crespi et al. (1997).

R. J. Mountfield et al. Int. J. Pharm., 211, 89, 2000.

Figure 13.

Those are the advantages of micronization and now I would like to talk about the use of co-solvents. In our view, five of the merits of using co-solvents are: increased drug solubility in co-solvent/GI fluid mixture; increased permeability; decreased metabolism and/or efflux transport; fine particles if precipitation occurs, and micelle formation with surfactants. There may be some other advantages, too.

Figure 12 is the only example of work that comes out of our laboratory. We administered indomethacin to rat intestine and noted the absorption. We used two capsule forms, one including PEG 400 solution, and the other containing indomethacin powder with some additives. The results show that with the solution concentration goes up significantly, and the absorption obtained is also higher, as calculated by the deconvolution method. So by using PEG the result is that we can get better absorption at a faster pace.

There is a further advantage to using co-solvent – and not just as a solution that enhances absorption. Another reason is that, depending on the compound, the co-solvent itself may have an inhibitory effect on the metabolism.

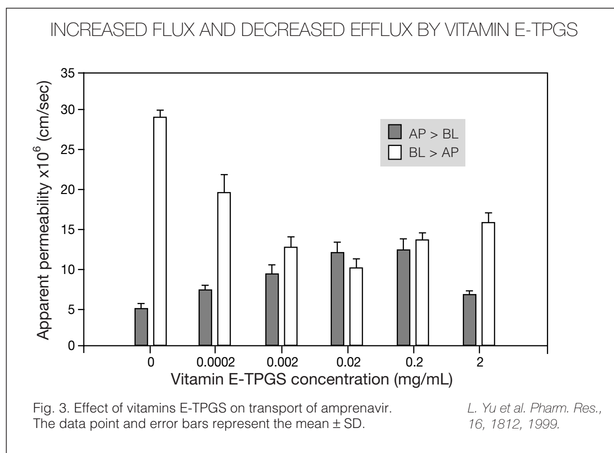
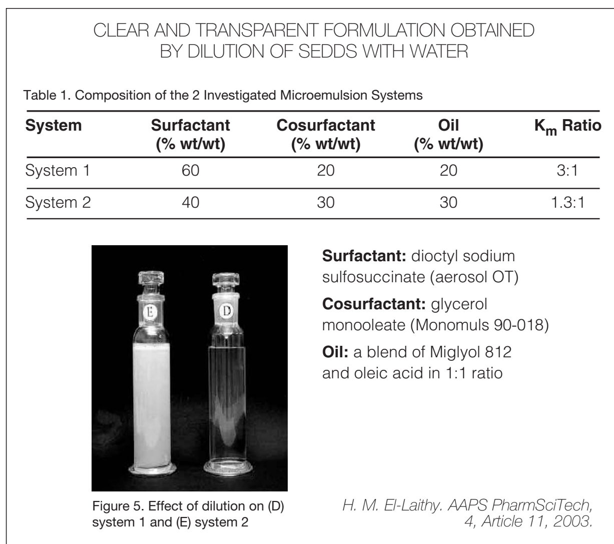


Fig. 3. Effect of vitamins E-TPGS on transport of amprenavir. The data point and error bars represent the mean ± SD.

L. Yu et al. Pharm. Res., 16, 1812, 1999.

Figure 15.



CLEAR AND TRANSPARENT FORMULATION OBTAINED BY DILUTION OF SEDDS WITH WATER

Table 1. Composition of the 2 Investigated Microemulsion Systems

System	Surfactant (% wt/wt)	Cosurfactant (% wt/wt)	Oil (% wt/wt)	K_m Ratio
System 1	60	20	20	3:1
System 2	40	30	30	1.3:1

Surfactant: dioctyl sodium sulfosuccinate (aerosol OT)

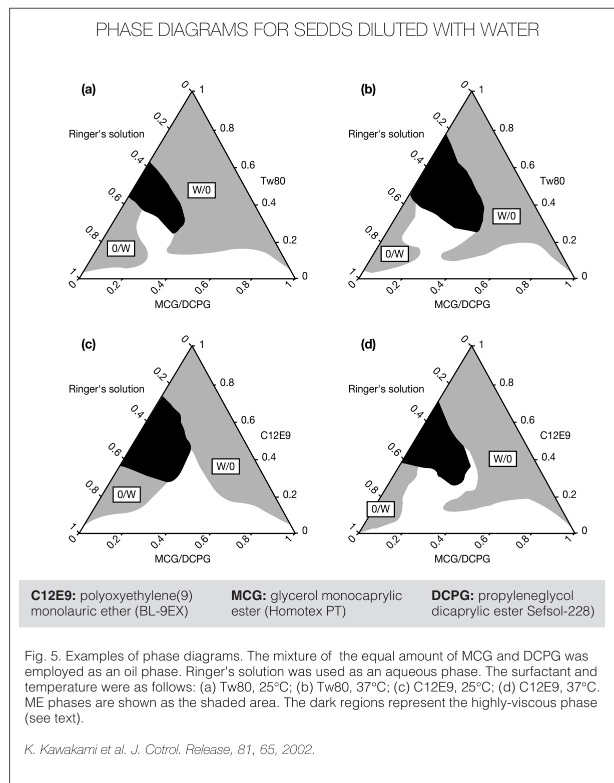
Cosurfactant: glycerol monooleate (Monomuls 90-018)

Oil: a blend of Miglyol 812 and oleic acid in 1:1 ratio

Figure 5. Effect of dilution on (D) system 1 and (E) system 2

H. M. El-Laithy. AAPS PharmSciTech, 4, Article 11, 2003.

Figure 16.



C12E9: polyoxyethylene(9) monolauric ether (BL-9EX)

MCG: glycerol monocaprylic ester (Homotex PT)

DCPG: propyleneglycol dicaprylic ester Sefsol-228)

Fig. 5. Examples of phase diagrams. The mixture of the equal amount of MCG and DCPG was employed as an oil phase. Ringer's solution was used as an aqueous phase. The surfactant and temperature were as follows: (a) Tw80, 25°C; (b) Tw80, 37°C; (c) C12E9, 25°C; (d) C12E9, 37°C. ME phases are shown as the shaded area. The dark regions represent the highly-viscous phase (see text).

K. Kawakami et al. J. Cotrol. Release, 81, 65, 2002.

Figure 17.

As was mentioned by Dr. Sugiyama this morning, with CYP3A4 there are various inhibitory effects and they have studied those. In *Figure 13* the horizontal axis shows the excipient concentration in incubation and the inhibitory activity is shown on the vertical axis. From this you can see that maybe Tween 80, oleic acid, or by using various additives, CYP3A4 activity is inhibited. That's what this diagram shows.

Figure 14 is another example, using vitamin E-TPGS. It was used for the formulation of amprenavir (Agenerase). Dissolution of the drug is dependent on the concentration of vitamin E-TPGS, and this would improve the solubility. On the Y-axis of the inset graph, first there is a horizontal line and then a slight upwards bend; this is where the vitamin E-TPGS concentration reached its CMC. In this case it's at 0.2 mg per milliliter.

Figure 15 is a CACO-2 cell culture that was used to demonstrate flux and efflux from the apical to the basal and from the basal to the apical. What kind of absorption was seen? The horizontal line shows the vitamin E-TPGS concentration; as the concentration level goes up, so the flux direction rises. But once it goes above the CMC then micellar uptake takes place, and so the apparent permeability is reduced. On the other hand, vitamin E-TPGS inhibits flux from the basal to the apical, and as the drug is a P-glycoprotein substrate, vitamin E-TPGS is also inhibiting its activity. So using this kind of co-solvent in a formulation not only solubilizes the drug, it also improves membrane absorption permeability by reducing efflux.

And now turning to another subject, to emulsion strategy. As for the emulsions themselves, several other speakers will be touching on this, so due to time constraints I would like to skip some of the explanations. One type that is currently hotly debated is SEDDS, or self-emulsifying drug delivery systems. SEDDS is an emulsion system that uses oils, surfac-

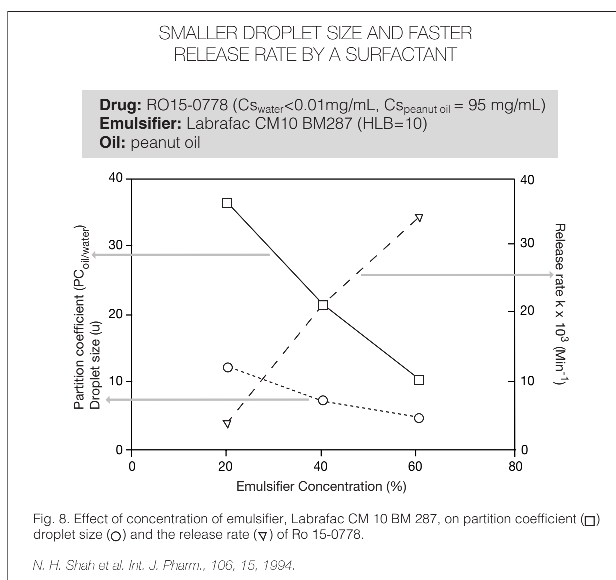


Fig. 8. Effect of concentration of emulsifier, Labrafac CM 10 BM 287, on partition coefficient (□) droplet size (○) and the release rate (▽) of Ro 15-0778.

N. H. Shah et al. Int. J. Pharm., 106, 15, 1994.

Figure 18.

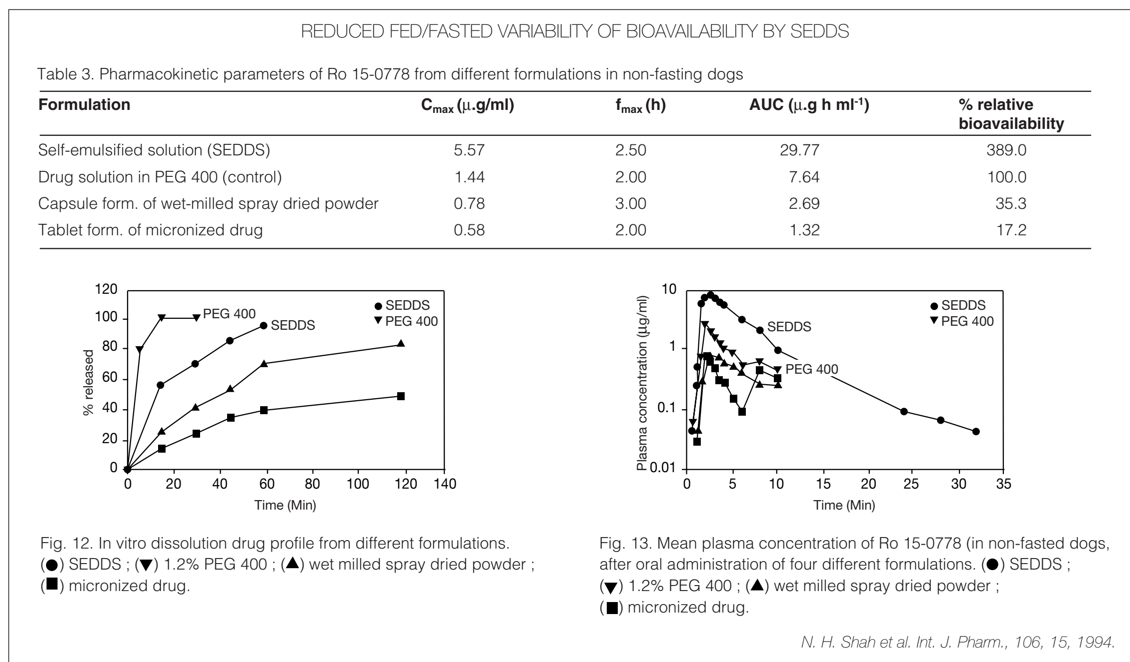
tants and drugs, and by administering it in the gastrointestinal tract, O/W (oil in water) emulsion will be spontaneously generated.

Figure 16 is a comparison of two formulations. The one at the top, system 1, is the SEDDS; system 2, is without SEDDS. If we add water then (D) shows the result for system 1. In (D) we get a very clear and transparent formulation, whereas in (E), which is without SEDDS, it is white and you get a homogeneous layer. What the diagrams in *Figure 17* are showing is the relationship between oils, surfactants and the GI fluid, and the kind of water emulsion that can be formed.

Figure 18 shows the relationship between droplet size and the release rate. As the concentration level goes up, the droplet size goes down and the release rate is also improved. So, with SEDDS, it's better to use smaller particles in order to obtain higher absorption.

Figure 19 is an *in-vitro* dissolution test and an *in-vivo* absorption test using an SEDDS solution, with drug in a PEG 400 solution as control. In the dissolution test, SEDDS had a worse score than the PEG solution, but after it was administered to a dog, SEDDS performed better than the PEG. Dissolution was not good, but absorption was higher with SEDDS. Maybe some recrystallization takes place in the PEG solution, maybe that's the reason why absorption might be more difficult, whereas the SEDDS creates a clearer solution.

In *Figure 20* the three bottom formulations in the *Table* are SEDDS-related formulations, along with an MC (methylcellulose) suspension. Chart (a), normal, indicates the fed state and chart (b) the fasted state.



REDUCED FED/FASTED VARIABILITY OF BIOAVAILABILITY BY SEDDS

Table 3. Pharmacokinetic parameters of Ro 15-0778 from different formulations in non-fasting dogs

Formulation	C_{max} (μ.g/ml)	f_{max} (h)	AUC (μ.g h ml^{-1})	% relative bioavailability
Self-emulsified solution (SEDDS)	5.57	2.50	29.77	389.0
Drug solution in PEG 400 (control)	1.44	2.00	7.64	100.0
Capsule form. of wet-milled spray dried powder	0.78	3.00	2.69	35.3
Tablet form. of micronized drug	0.58	2.00	1.32	17.2

Fig. 12. In vitro dissolution drug profile from different formulations. (●) SEDDS ; (▼) 1.2% PEG 400 ; (▲) wet milled spray dried powder ; (■) micronized drug.

Fig. 13. Mean plasma concentration of Ro 15-0778 (in non-fasted dogs, after oral administration of four different formulations. (●) SEDDS ; (▼) 1.2% PEG 400 ; (▲) wet milled spray dried powder ; (■) micronized drug.

N. H. Shah et al. Int. J. Pharm., 106, 15, 1994.

Figure 19.

REDUCED FED/FASTED VARIABILITY OF BIOAVAILABILITY BY SEDDS

Pharmacokinetic parameters of oral administration study of nitrendipine

Formulation	T_{max} (h)		C_{max} (μ.g/ml)		AUC (μ.g h/ml)		AUC ratio
	Normal	Fasted	Normal	Fasted	Normal	Fasted	(normal/fasted)
MC suspension	8.0±0.0	1.5±0.5	0.23±0.03	0.04±0.00	1.03	0.05	21.4
Oil solution	4.0±0.0	3.5±1.7	0.53±0.09	0.30±0.09	2.55	1.71	1.50
Tw80 ME	1.3±0.7	1.3±0.7	0.36±0.00	0.59±0.12	2.09	2.50	0.84
C12E9 ME	4.3±3.8	3.0±1.0	0.22±0.19	0.08±0.00	0.58	0.47	1.25
HCO60 ME	≥8.0	>7.0	≥1.45	≥1.44	7.70	6.43	1.20

T_{max} : Time to reach maximum drug concentration (average ±S.E.), **C_{max}** : maximum drug concentration (average ± S.E.),
AUC: area under on line curve from 0 to 8 h calculated by the trapezoidal method.

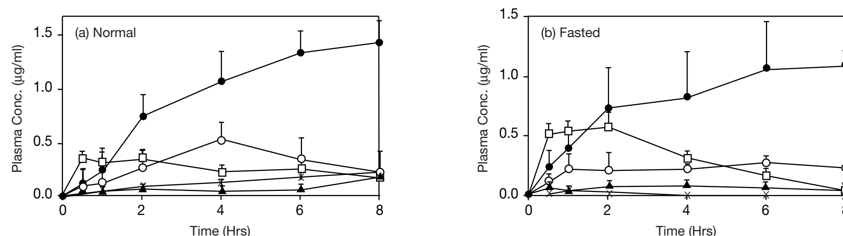


Fig. 2. Plasma concentration profiles after oral administration of nitrendipine formulations to rats under (a) normal and (b) fasted condition. Each data point is an averaged value, and the error bars represent the standard errors. Formulation type: (X) MC suspension, (O) oil solution, (□) Tw80 ME, (▲) C12E9 ME, (●) HCO60 ME. The formulation prescriptions are shown in Table 1.

K. Kawakami et al. J. Cotrol. Release, 81, 75, 2002.

Figure 20.

In the fasted state, absorption from the MC suspension is very poor; the difference in the fed state is about 20-fold. But with the microemulsion system, absorption is very good in both cases, whether it's a fed or fasted situation.

Figure 21 shows two well-known cyclosporine formulations, Sandimmune and Neoral. By using a range of cyclosporine dosages it was possible to calculate the AUC ratio against the dose. With Neoral, the AUC against dose is constant and there is linearity, whereas with Sandimmune there is no constancy, no proportion can be seen, there is no linearity. As a result, Neoral with a smaller droplet size allows more precise administration.

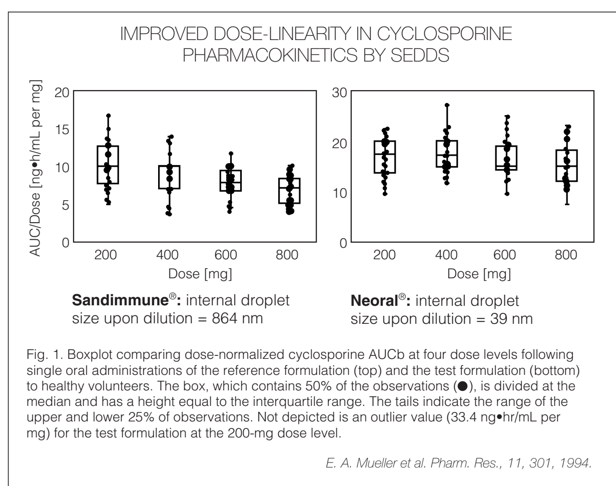


Fig. 1. Boxplot comparing dose-normalized cyclosporine AUCb at four dose levels following single oral administrations of the reference formulation (top) and the test formulation (bottom) to healthy volunteers. The box, which contains 50% of the observations (●), is divided at the median and has a height equal to the interquartile range. The tails indicate the range of the upper and lower 25% of observations. Not depicted is an outlier value (33.4 ng•hr/mL per mg) for the test formulation at the 200-mg dose level.

E. A. Mueller et al. Pharm. Res., 11, 301, 1994.

Figure 21.

The final example I would like to discuss is the solid dispersion system (*Figure 22*). This is a three-component solid dispersion system using YM022, TC-5E and HCO-60, and it's been formulated by spray drying technique. The drug becomes a solid dispersion system and, if it is dispersed in water, then the particle size is about 160 nanometers and we get a colloid type of particle. I won't go into details due to lack of time, but the drug and HCO-60 interact with each other, whereas TC-5E would act as the framework to form that colloid.

Figure 23 shows YMO22 formulations that were administered to dogs. The concentration level is very high when drug is administered as solid dispersion system (SD5), and also when it's in suspension (SD5W). Compared to the physical mixtures (APM) or the drug alone (AP), absorption is highly improved when solid dispersion system (SD5) are used. The formulation is dispersed in the intestine where it forms a colloid, and absorption is improved.

DESIGN OF A 3-COMPONENT SOLID DISPERSION SYSTEM

	SD5
YM022: aqueous solubility <1mg/mL	10
TC-5E: hydroxypropyl-methylcellulose 2910	35
HCO-60: polyoxyethylene hydrogeneated caster oil 60	5

Table 3. Composition of Fractionned Colloidal Particles Formed from SD5

Centrifugal condition (rpm)	Total weight Weight of YM022	Composition ratio[(a)] YM022:TC-5E:HCO-60
5000	1.9	1.0: 0.5 :0.3
10000	1.5	1.0: 0.2 :0.3
50000	1.6	1.0: 0.1 :0.3

(a) determined from IR spectra (I_{1077}/I_{1097}).

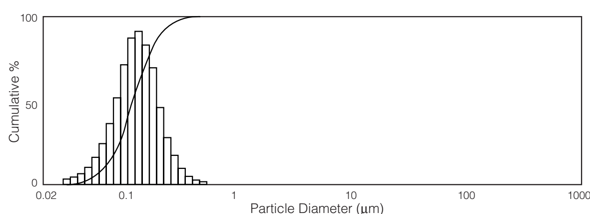
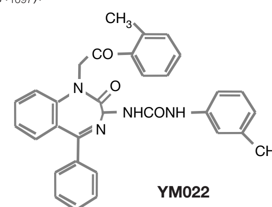


Fig. 4. Particle Size Distribution Curves of Various Solid Dispersions in Water after Stirring for 30 min.



K. Yano et al. Chem. Pharm. Bull., 45, 1339, 1997.

Figure 22.

If you look back at *Figure 2*, it will remind you of the possible ways to improve oral bioavailability. One of the directions we are moving towards is nanotechnology. For example, particle size reduction, emulsion, solid dispersion, those are the new technologies that are now being exploited. Very small, controlled and uniform particles represent the kind of means by which solubility and absorption can be improved. It's likely that in the future a lot of our researchers will be working in this area. Thank you very much, this concludes my presentation.

IMPROVED ABSORPTION OF A POORLY SOLUBLE DRUG BY SOLID DISPERSION SYSTEMS

	YM022	TC-5E	HCO-60	
○ SD5	10	35	5	CH_2Cl_2/MeOH, spray dry
● SD5W	10	35	5	Aqueous suspension of SD5
□ SD5	10	35	0	CH_2Cl_2/MeOH, spray dry
△ AP	10	0	0	CH_2Cl_2, spray dry
▲ APM	10	35	5	Mixture of AP, TC-5E & HCO-60

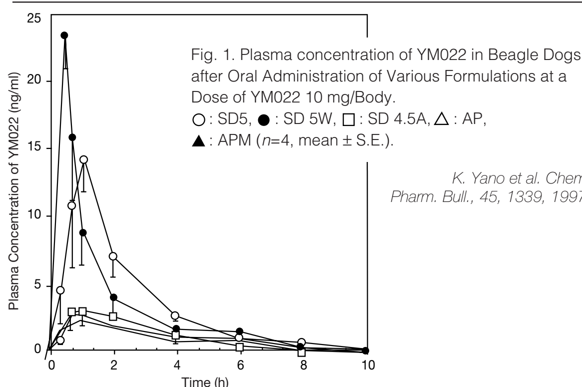


Fig. 1. Plasma concentration of YM022 in Beagle Dogs after Oral Administration of Various Formulations at a Dose of YM022 10 mg/Body.
○ : SD5, ● : SD 5W, □ : SD 4.5A, △ : AP, ▲ : APM (*n*=4, mean ± S.E.).

K. Yano et al. Chem. Pharm. Bull., 45, 1339, 1997.

Figure 23.

Chair: Dr. Rinta Ibuki, Fujisawa Pharmaceutical Co., Ltd, Japan: Thank you very much, Dr. Okamoto. You covered a number of approaches. I would like to invite questions or comments from the floor. Please state your name and affiliation.

Dr. Akira Yamamoto, Professor, Kyoto Pharmaceutical University, Kyoto, Japan: I am from Kyoto Pharmaceutical University. Thank you very much for your talk. We are looking at the effects of various additives, and what you said about P-glycoprotein was very interesting. You also mentioned the co-solvent effect on amprenavir HIV protease inhibitor, which I found very interesting.

With regard to the vitamin E derivative, TPGS, if you increase its concentration the efflux of amprenavir decreases and so P-glycoprotein must be involved. I understood that. But in *Figure 13*,you said that CYP3A4 activity is also inhibited by excipients. In other words, amprenavir HIV protease inhibitor probably is a substrate of CYP3A4, so the effect is not present in this case. But this is only according to the literature, and according to the literature on P-glycoprotein, the effect of the additive on P-glycoprotein is the only focus; they didn't study the effect of CYP3A4. However, if it is serving as a substrate, CYP3A4 would be inhibited and permeability would be improved. In the *in-vivo* situation, that is a possibility.

Also, with your indomethacin example, you used a PEG in the formulation. In the case of indomethacin it

does not become a substrate for p-glycoprotein and it will not become a substrate for CYP3A4 either. That is the only improvement of this ability.

Dr. Hirokazu Okamoto, Associate Professor, Meijo University, Nagoya, Japan: Using PEG means it is a dissolved situation. It is diluted in water and, of course, some precipitation will occur. But with the addition of water the precipitate probably is a finer particle compared to the actual drug, the original drug. So even if there is precipitation the particle size is reduced, and the absorbability is improved.

Dr. Shinzi Yamashita, Professor, Setsunan University, Hirakata, Japan: You presented various types of methodologies. I listened to this and I am now wondering, which is the best? Surely it has to do with the drug itself? This morning Dr. Lipinski spoke about poor solubility. But when people speak about poor solubility in this general way they could be referring to the molecule itself, or to its lipophilicity, or to the fact that it's poorly soluble. It could be that crystallization leads to poor solubility. I think there could be a number of quite different reasons.

When we talk about poor solubility itself, one methodology may be appropriate for highly lipophilic products, but it may not be applicable to the crystal type, where another methodology may be better. And in the crystalline system, yet another methodology may be better. We speak of a poorly soluble substance as a drug, but it may be indicating two different things and if you could give me some kind of suggestion in that regard…

Dr. Hirokazu Okamoto, Associate Professor, Meijo University, Nagoya, Japan: This is my personal view. Micronization is the first option for all compounds in drug development. You don't add anything, and if it increases the absorption level, that's desirable. Apart from that, at what phase in development would we look into the matter of poor solubility? I think it has to do with the stages of development. At the initial stage we would be looking at the salt selection and a prodrug, but in the latter stages of development, some kind of additive may be required. In that case you would have to look into it from the formulation point of view, the dosage point of view.

Chair: Dr. Rinta Ibuki, Fujisawa Pharmaceutical Co., Ltd, Japan: Now, the final question.

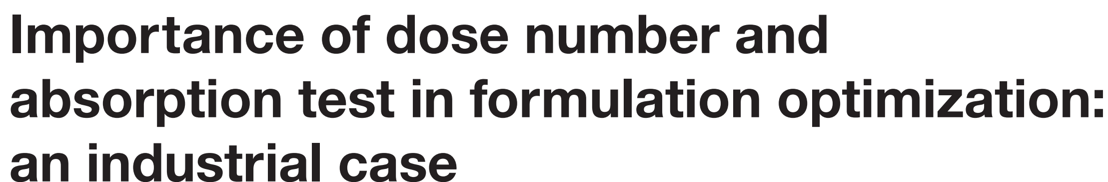
Dr. Yuichi Sugiyama, Professor, University of Tokyo, Tokyo, Japan: In the very first part of your presentation you talked about prodrugs. Together with the * company we are developing a prodrug, and the challenge always is whether cleavage should occur in the GI lumen or at the membrane. There is no significance or value in cleavage in the lumen. But according to your presentation today you can have cleavage at the brush border membrane and I think it is good to compare cleavage there with cleavage at the lumen. One point I want to ask you is, when you use an ester with the brush border membrane, where is the active site? I think the surface is on the lumenal side. So even if you use BBM, it's not inside the membrane and if it's water-soluble to start with, then the absorbability that enables it to pass through will not be improved. It's not limited to esters, but the brush border membrane topology of that cleavage enzyme is important. Do you have any data on that?

Dr. Hirokazu Okamoto, Associate Professor, Meijo University, Nagoya, Japan: Well, which direction is it facing? I don't have much data but what I have presented today is that the enzyme is bound to the BBM, the prodrug dissolves and comes close to the BBM, and then it is changed to the parent drug and goes into the membrane. But when it goes into the membrane, depending on where the active site is, it has a totally different meaning, and that data is very important in this type of meeting. It is a focused strategy, but at the micro level the mechanism has not been announced or published yet.

Chair: Dr. Rinta Ibuki, Fujisawa Pharmaceutical Co., Ltd, Japan: Thank you, I would now like to end this session.

Importance of dose number and absorption test in formulation optimization: an industrial case

Dr. Hiroshi KIKUCHI



Importance of dose number and absorption test in formulation optimization: an industrial case

Dr. Hiroshi Kikuchi

Principal Investigator, Daiichi Pharmaceutical Co. Ltd, Japan

Chair: Dr. Rinta Ibuki, Fujisawa Pharmaceutical Co., Ltd, Japan: The next speaker is Dr. Hiroshi Kikuchi of Daiichi Pharmaceutical Co. Ltd. As you may know, Dr. Kikuchi is the company's principal investigator, and just as professionally active outside the company as within it. I believe that his talk will be both stimulating and interesting.

Dr. Hiroshi Kikuchi, Principal Investigator, Daiichi Pharmaceutical Co. Ltd, Japan: Thank you very much for your introduction. I would like to thank Professor Yamashita, the organizer, and also Dr. Amidon for inviting me as a speaker, and I would like to thank Capsugel for this opportunity. I feel quite nervous because liposomes or gene delivery are the topics I am used to speaking about, and on this auspicious occasion I am speaking about the importance of dose number and the absorption test for the first time.

Ten years ago I stayed at Dr. Amidon's laboratory in the University of Michigan for one year to study oral absorption, and since coming back to Japan I have been involved in the oral formulation project in addition to the DDS project (liposomes, gene delivery, etc.). Though the research results of our oral formulation project have not yet been in open domain, today I can disclose one of the results of our project to a limited degree. I have also been involved in Dr. Yamashita's research for the past decade, but although it has produced a great deal of information, this cannot be published or put into open domain. After my today's presentation Dr. Yamashita will be able to refer to the results of clinical trial which will be disclosed here. Dr. Yamashita's research as to clinical prediction is linked to these results of clinical trial. I will only be talking about some preliminary data, for the benefit of our colleagues in the industry. I am sorry that I cannot disclose the names of the new chemical entities or give their molecular weight and so forth.

Let me start. Our company operates in the worldwide market, so development is usually started overseas. Five years ago, however, we started to handle the drug discovery process in Japan. We determined the dose range for a product I shall call D-compound at 100 mg, given as two 50 mg tablets. The CV (coefficient of variance) value of the area under the curve (AUC) was very good, so the dose was raised to 200 mg and we still had no problem with bioavailability. However, we found out that the dosing regimen was different overseas.

Overseas, the dosing regimen was 500 mg (as two 250 mg tablets), a very large dose compared to the Japanese therapeutic dose, although the formulation was pretty much the same. But when we started a pilot study in the UK population, we began to have problems; we began to see lower absorption and a higher CV (*Figure 1*). Given that the site was in a different country, we thought the analysis methodology might have been different. We measured the Japanese data, while the overseas clinical research organization measured the UK data, so there might have been a difference in the methodology. But we found that we had a major problem at 400 mg: the mean bioavailability was 71 percent, and the CV was 49 percent. In a parallel development we had an overseas dose of 250 mg, and when this was given, as with the 500 mg dose, absorption was reduced to 67 percent and the CV also went 38 percent.

Absorption of D-Compound in Clinical Trial

Product used	Dose	Site	BA		
			mean (%)	CV (%)	N
Tablets 50 mg*	100 mg	Japan	95	20	6
	200 mg	Japan	89	22	6
	100 mg	UK	88	29	12
	200 mg	UK	78	33	12
	400 mg	UK	71	49	12
Tablets 250 mg*	500 mg	UK	67	38	24
Solution, po	400 mg	UK	86	21	12
IV	400 mg	UK	100	24	24

* Similar components were used in both formulations.

Figure 1.

As you may know, the US Food and Drug Administration (FDA) would like to maintain the CV below 30 percent, although it is not a written requirement – I even checked yesterday that these are the requirements in the UK or at the FDA, but I could not find out the written requirement. Anyway, we were told by the overseas department that we had to reformulate the product for overseas markets. It might only be an internal rule at the FDA that the CV has to be below 30 percent, but we made it our target, along with a target of 80 percent absolute bioavailability (BA). Of course, 90 percent would have been better but since 80 percent was the absorption achieved in Japan we targeted the same BA for the overseas market. To do that, a new formulation was needed and as the clinical trials were already in progress, the UK site asked to have the study drug within one year.

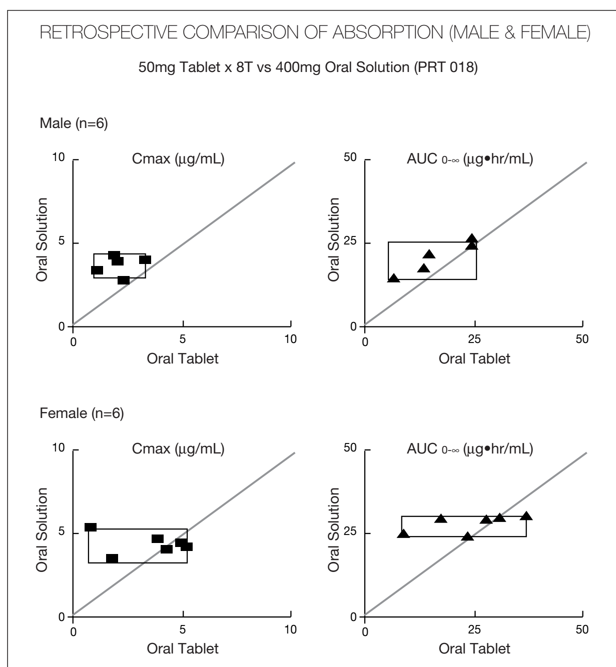


Figure 2.

Why did we have these variations in the UK? We did an analysis, but ultimately we do not know. We found a number of phenomena, but none of them was definitive.

There was a gender difference, for one thing. *Figure 2* shows the absorption (Cmax and AUC) after administration of the oral solution or the tablet to the same person (male and female). In males, the oral solution showed almost no variation in absorption and the tablet also showed a small variation. But in the females, although the solution did not show any variation, there was quite wide variation with tablet administration. We hypothesized that there may be some female-specific reasons for this variation; there are various stomach shapes, for instance, such as a J or an L shape. That was our hypothesis, but we were unable to reach a conclusion.

We determined pH solubility profile as shown in *Figure 3*. This 100 mg administration was the dose that was used in the clinical trial in Japan. According to the calculation of the dose number, or the dose divided by the solubility, divided by 250 ml, the dose number was 0.4 at pH5 and 4.4 at pH7 (below 5 in the all pH region). So it was confirmed that there was no problem in solubility of this drug from the view point of dose number.

However, when the dose number was calculated for the 500 mg dose, the value was 22 at neutral pH and so we were faced with a major problem in terms of the

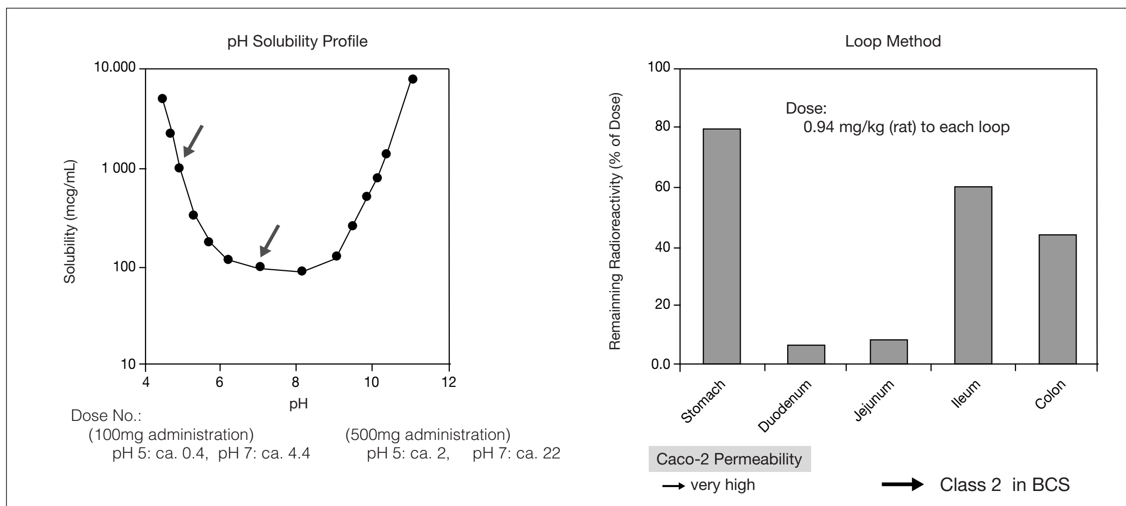


Figure 3.

solubility. Yet all the tablets we were testing were based on a similar formulation. Though we knew 'dose number' at that time, but honestly speaking we did not regard it as important. But looking at the result of the clinical trial we recognized the real importance of dose number – because we failed.

Figure 3 also shows that the permeability of this compound is good from the loop method and Caco-2 permeability test. As a result, we classified this compound as a Class II drug in BCS. As a Class II drug has low solubility, so when we formulate such compounds we have to look at the dissolution rate. However, the 500 mg

New Oral Solid Dosage Formulation

Ingredient	Component	Form. A mg/tablet	Form. B mg/tablet	Form. C mg/capsule
Active ingredient	D-Compound (as Anhydrate)	266.5 (250)	266.5 (250)	266.5 (250)
Diluent	AAAAAA	aa	---	---
Diluent	BBBBBB	bb	---	---
Disintegrant	CCCCCC	c1	c2	---
Binder	DDDDDD	d1	d2	---
pH adjustor	Organic acid	x1	x2	x3
pH adjustor	Sodium bicarbonate	---	y2	---
Wetting agent	Polysorbate 80	---	---	z3
Glidant	EEEEEE	---	---	ee
Lubricant	FFFFFF	f1	f2	f3
Lubricant	GGGGGG	g1	g2	---
Coating agent	HHHHHH	h1	h2	---
Solvent	Water, purified *	q.s.	q.s.	q.s.
Solvent	Dehydrated ethanol *	q.s.	q.s.	---
	Total	536 pale yellow to yellow size: 11.1 mm	478 film-coated tablet size: 10.1 mm	320 white opaque size: no. 0

* removed during the manufacturing process

- Amount of organic acid for improvement of solubility.
- Effervescent ingredient (organic acid and sodium bicarbonate); totally 25% in Form. B core tablet.
- Polysorbate 80 is added as a wetting agent in Form. C.

Figure 4.

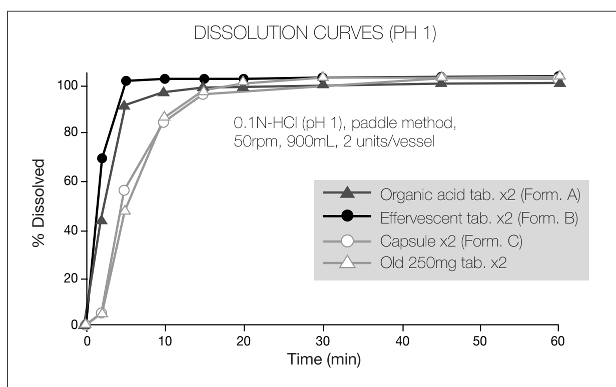


Figure 5.

dose was prepared using the original formulation, and that caused the problem in the clinical setting.

To find out the reason, we began with the hypothesis that the high variance of AUC/Cmax might be caused by lack of solubility in the digestive tract, and/or by rapid movement through the digestive tract, so preventing disintegration or dissolution from taking place. In our reformulation strategy we aimed to increase the dissolution rate and, if possible, the absorption level. The concept was to produce a drug where rapid disintegration and dissolution was independent of pH and of transit time in the digestive tract.

We looked at nine points in our approach to reformulation. But we had to place this study drug in clinical trial within a year, so time was limited. We thought that changing the disintegrants and perhaps adding organic acids might be a good way of improving disintegration/dissolution. The second point involved using effervescents to produce quick disintegration within the stomach, and the third was to use a hard capsule formulation; compared to a tablet, it would have been possible to achieve faster dissolution and disintegration.

The fourth way was to add surfactants to improve wetting. In the worst-case scenario, maybe we would turn to the fifth way and make an oral aqueous formulation. Well, it is not possible in Japan. Western authorities may accept this dosage form, but for us the options were soft capsule formulations containing liquid/dispersion, or to make amorphous forms with polymers, or – as the eighth choice – to grind the particle size to micrometers.

The ninth approach was to make a change in the chemical form of the drug. However, if the chemical form was changed to another one, we had to go back to the pre-clinical stage. So the ninth approach was judged impractical, and we relied on the first eight.

Figure 4 is a sort of conclusion to all this. Organic acids were added to the formulation of two tablet forms (Form A and Form B), and sodium bicarbonate was also added to Form B for effervescent effect. Organic acid and wetting agent (surfactant) were added to the capsules (Form C). We screened them with several tens of formulations and these were the promising candidates. Let me skip the details.

Dissolution was more or less adequate with all the various types of solutions. One example is shown in *Figure 5*, which gives the result of a dissolution study run at pH 1, using the old 250 mg tablet, an organic acid tablet (Form A), an effervescent tablet (Form B), or a capsule (Form C). All these formulations had no problem at pH 1.

But at pH 5 you can see some difference in the old formulation, as shown at the bottom of *Figure 6*. Only 30 percent was dissolved after an hour. Both the modified formulations, the organic acid (Form A) and the effervescent tablet (Form B), showed a high dissolution rate, while the capsule was just medium. With a neutral pH (pH6.8) the old tablet had the lowest dissolution curve. As opposed to this, the improved formulation tablets had faster dissolution, with the capsule coming somewhat below the tablets.

As already mentioned, using an oral aqueous formulation, or a soft capsule formulation containing liquid/dispersion, or making amorphous forms with polymers, or micro-grinding were also considered as candidate approaches. But the conclusion was that they were not desirable.

The D-compound is quite acid in an oral aqueous solution, so dissolution itself is not a problem, but it tastes very bitter. I tried it myself, but as soon as the solution hit the tongue it felt as if my tongue was burning, so it was not possible to use it as a product.

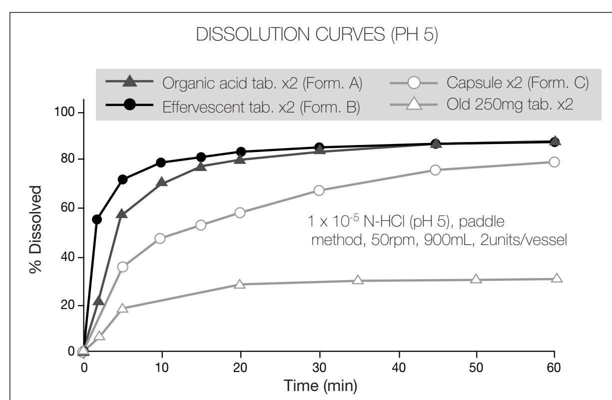


Figure 6.

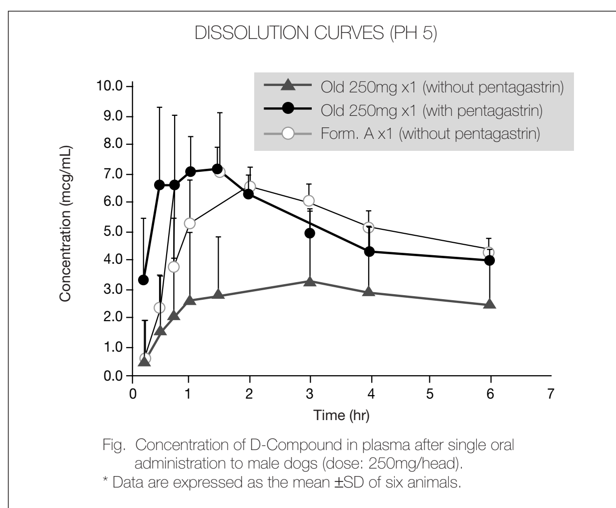


Fig. Concentration of D-Compound in plasma after single oral administration to male dogs (dose: 250mg/head).
* Data are expressed as the mean ±SD of six animals.

Figure 7.

We also have to ensure a certain dose, and we found with the soft capsule that the concentration was too high to be in a stable state as a solution/dispersion form. We would have had to use a large number of capsules, or a large-sized capsule. We would also have had to outsource capsule manufacturing, and the cost would have been higher. Today, we can fill a solution/dispersion form in the hard capsules on our filling machines so we would be able to use a hard capsule. But five years ago, Capsugel's products (Capsule Liquid Filling & Sealing Machine; CFS 1000) were not available, and because of higher cost and other issues we gave up on the soft capsule idea.

The amorphous form with organic polymers did not improve the dissolution. As for the amorphous form, we had already pulverized the particles down to 10-20 micrometers. We also tried to grind particles into even more minute sizes, but there was no improvement.

To summarize our reformulation results. Adding organic acids, adding effervescence, and using organic acids and surfactant in hard capsules all improved dissolution and solubility. These three approaches were selected as candidates. With the others – an oral solution, soft capsule forms, making amorphous forms with organic polymers, and grinding the particles to mincrometer order – we felt that there was no chance of bringing them into the clinical trial environment, so we gave up on them.

We conducted a comparison study on dogs, using the old 250 mg tablets with and without pentagastrin, and formulation A, the organic acid reformulation, without pentagastrin (*Figure 7*). As you know, the pH of the dog's stomach is higher than that of the human's. So if we could tilt the gastric environment to an acid environment with pentagastrin in dogs, we could make an acidic pH environment similar to human's stomach, and that would lead to higher oral absorption of this D-compound. Even with the higher pH in dogs, the reformulated

Pharmacokinetic parameters of D-Compound in plasma after single oral administration to male dogs (dose: 250mg/head)

Sample	$AUC_{0\text{-}6h}$ (µg x hr/mL)	Cmax (µg/mL)	Tmax (hr)
Old Tab. 250mg x1 (without pentagastrin)	15.62 ± 11.31 (CV 72.4%)	3.72 ± 2.58 (CV 69.3%)	1.63 ± 1.07
Old Tab. 250mg x1 (with pentagastrin)	30.76 ± 3.70 (CV 12.0%)	7.96 ± 1.43 (CV 18.0%)	1.04 ± 0.51
Form. A 250mg x1 (without pentagastrin)	29.87 ± 3.90 (CV 13.1%)	7.31 ± 1.86 (CV 25.5%)	1.58 ± 0.20

* Data are expressed as the mean ±SD of six animals.

Figure 8.

Pharmacokinetic parameters of D-Compound in plasma after single oral administration to male dogs (dose: 250mg/head)

Sample	$AUC_{0\text{-}6h}$ (µg x hr/mL)	Cmax (µg/mL)	Tmax (hr)
Form. A 250mg x1 (without pentagastrin)	29.87 ± 3.90 (CV 13.1%)	7.31 ± 1.86 (CV 25.5%)	1.58 ± 0.20
Form. B 250mg x1 (without pentagastrin)	28.85 ± 1.26 (CV 4.4%)	7.25 ± 0.61 (CV 8.4%)	3.17 ± 0.75
Form. C 250mg x1 (without pentagastrin)	20.77 ± 6.34 (CV 30.5%)	5.27 ± 1.61 (CV 30.6%)	1.42 ± 0.58

* Data are expressed as the mean ±SD of six animals.

Figure 9.

Product used	Dose	Site	mean (%)	BA CV (%)	n
Form. A (Tablet) 250 mg	500 mg	UK	104	**19**	9
Form. C (Capsule) 250 mg	500 mg	UK	108	**9**	9
Solution, po	500 mg	UK	93	8	9
IV	500 mg	UK	100	10	9
cf: Old Tablets 250 mg	500 mg	UK	67	**38**	24

Absorption of D-Compound in Human PK Study

Figure 10.

drug without pentagastrin showed very good oral absorption.

Figure 8 shows the pharmacokinetic (PK) parameters. This is the same data as in the previous *Figure*, the old tablets with and without pentagastrin, and formulation A without pentagastrin. The comparison shows that the AUC and the Cmax for formulation A (without pentagastrin) was almost twice as compared with those of the old tablets (without pentagastrin), and the CV values were decreased very much.

We then tested the effervescent tablets and the capsules without pentagastrin (*Figure 9*). For formulation A and the effervescent tablets (formulation B) the AUCs were almost 30 µg·hr/mL in dogs, and the capsules were 21µg·hr/mL. The CV for the capsule (formulation C) was very high in the dog – 30 percent – whereas the CVs for the tablets were lower. Normally, I think the capsule candidate would have been dropped at this point, before moving on to clinical studies. But because of manufacturing difficulties with formulation B, the effervescent tablet, it was dropped instead and we decided to continue carrying out clinical studies with formulations A and C.

Please keep this in mind. Under normal circumstances, when you have these outcomes from a dog study you would choose only formulation A, the organic acid reformulation, with high absorption and a low CV, because the capsule (formulation C) had a higher CV and lower absorption. Pharmaceutical scientists, you may think that we should be making predictions based on animal studies. But if you select only formulation A, and conduct clinical studies only on formulation A, you will never know whether formulation A was the best formulation.

That is why we consider the next part of our project as very important. Usually it is not possible, but we were fortunately able to study both formulation A and formulation C in human trials, and we obtained very interesting results.

Figure 10 shows the results from actual clinical studies, using tablets (formulation A) and capsules (formulation C). With the old tablets, absorption was 67 percent and the CV was 38. But with the formulation A tablets there was almost 100 percent absorption and the CV was 19 percent, much lower than the target of 30 percent. As for the capsule, with dogs we had a poor figure but in humans we had even better results than with the tablets. So in the end, in the clinical study we decided to use capsules, as in formulation C. I have not been able to spend enough time on this *Figure* in the interests of time, but I would like to return to this issue in the panel discussion.

So what are my conclusions? In the case of the 100 mg tablet dosage, we had a very good outcome in Japan. But when we took it to the UK and gave it as a 500 mg dose, absorption was poor and there was larger variance. With a little self-reflection, we now realize that we were not wise in using a similar formulation for the 100 mg, despite the dose number being high. However, we paid a lot of attention to dissolution and solubility, and we used organic acids and surfactants and this led to better clinical results.

Because of this experience we feel that we have to pay attention to the dose number and the Biopharmaceutical Classification System (BCS) in formulation studies, especially for Class II drugs. When I studied at the University of Michigan 10 years ago, Professor Amidon was speaking about BCS in his lectures. At that time, I was wondering why it was important to classify drugs into four categories and I did not pay very much attention. But as I gained more experience I came to realize the importance of the BCS classification, especially with Class II drugs.

I was with a development team for a few years and that is when I did the work I have been talking about today. We had to complete the project within a year and we actually completed it in nine months. There were 26 people working with me and half of them were more or less dedicated to this project. We worked into the

early hours, to complete the development and start the clinical trials. I would also like to thank the manufacturers.

Normally, we conduct screening with animal studies and we can only use the best candidate in human trials. But we do not know whether that best candidate out of an animal study is the best candidate for humans. We will never know the truth, because we can only use one formulation and we have to make a prediction of how effective the drug is in humans. We therefore have to accumulate data to make better predictions. We were lucky enough to be able to use several candidates in humans and, interestingly, we were able to show that a candidate that did not do so well in animals did better in humans.

Chair: Dr. Rinta Ibuki, Fujisawa Pharmaceutical Co., Ltd, Japan: Thank you very much on behalf of Capsugel. It has really been a wonderful talk. I would like to open the floor for discussion.

Question from the audience: Thank you very much for the interesting talk. I'm really envious that you could run two formulations in a clinical study. On behalf of all my industry colleagues I would ask you about the discrepancy between dogs and humans. How do you explain this difference?

Dr. Hiroshi Kikuchi, Principal Investigator, Daiichi Pharmaceutical Co. Ltd, Japan: Well, after this project I went back to the discovery team and what I told them was, we have this clinical data and we have some data in dogs, but we need to test it in other animal models like monkeys and other species. The reason why we used dogs is that we cannot administer the study drug formulation to monkeys because of the size of tablets and capsules. But with the clinical outcome we attained I thought it was necessary for us to go back again to animal tests. By doing that I believed our predictability accuracy would be improved.

Dr. Shinji Yamashita also helped us to look into dissolution in the GI tract, and we used simulation models. We really wanted to bring about better predictability of the best candidates by using those data for the benefit of the whole industry and the therapeutic setting, so that this might be used as the starting point to link it to human data.

Chair: Dr. Rinta Ibuki, Fujisawa Pharmaceutical Co., Ltd, Japan: I have a question for you. You are saying that the FDA has an internal rule that the CV should remain within 30 percent. Could you comment on that, please?

Dr. Hiroshi Kikuchi, Principal Investigator, Daiichi Pharmaceutical Co. Ltd, Japan: I am not aware of any internal insight or guidance regarding the coefficient variance on bioavailablity or bioequivalence. Obviously, higher bioavailability leads to a low coefficient variance, and usually this is more likely to be successful in the clinic. But it does not necessarily mean that a high CV cannot be approved. In our case the overseas department demanded that the coefficient variance had to be less than 30 percent because it was not easy for patients to use this drug.

Dr. Lawrence X. Yu, Food and Drug Administration, USA: I think the best example is Phosmax from Merck. The bioavailability is 1 percent, the CV I don't know, it's a couple of hundred percent or something like that. But the drug's still approved and still you try to protect human health. So I think we should look at the overall picture based on safety and efficacy, not so much at a specific rigid rule based on specific numbers such as the coefficient valence of bioavailability.

Chair: Dr. Rinta Ibuki, Fujisawa Pharmaceutical Co., Ltd, Japan: Thank you very much. Let me move on to the third speaker. Dr. Akira Kusai of Sankyo Company Ltd will talk on How to handle insoluble APIs: our experience at Sankyo.

How to handle practically insoluble APIs: our experience at Sankyo

Dr. Akira KUSAI

How to handle practically insoluble APIs: our experience at Sankyo

Dr. Akira KUSAI, Director, Pharmaceutical Development Laboratories

Sankyo Company Ltd

Chair: Dr. Rinta Ibuki, Fujisawa Pharmaceutical Co., Ltd, Japan: After Dr. Kusai's presentation there will be a discussion with all three speakers. Dr. Kusai, please, are you ready?

Dr. Akira Kusai: Thank you very much for inviting me today. As you can see, the word 'practically' is added to the title announced before. In principle I would like to deal with the issue of practically insoluble pharmaceutical active ingredients: how we at Sankyo have handled them in the past and today.

I would also like to mention several topics to activate our discussion later.

Dr. Okamoto has already discussed a number of ways to improve dissolution behavior of APIs; salt formation, solvation and surface active agents, which have been used for several decades. As he mentioned, cyclodextrin derivatives of enhanced aqueous solubility have been available recently. Then this approach is currently increased. The dispersion in oily base is another method, and I believe Dr. Benameur will discuss this point at his presentation lator. More and more often these days, the dispersion in oily base is filled into capsules. However depending on the regulations in each country, some types of oily base can be permitted to be used while others cannot, or they are restricted in their use. So we have to be very careful in selecting the oily base.

Micronizing APIs to nano-size is good approach, which has been getting much attention, and I will discuss this in detail later. The increase in surface area may increase the dissolution rate, but the dispersed state of the nanoparticles has to be maintained after formulation process and during storage. It may coagulate during storage. To keep the particles from coagulation, various additives are incorporated. However, this may result in high viscos dispersion. The recent meeting of the Society for powder technology, Japan focused on this area. They say the 100-nanometer level was achieved, but this is actually not nano level but submicron level. Co-grinding or mix grinding is another approach, where the carrier and the drug are mixed and ground together. As long as it is conducted at labo-scale, everything goes well. But taking the powder out, powder adhesion to the equipment, productivity and how to handle the generated heat become issues, when it is conducted at the larger scale, pilot sale and production scale. Solid dispersion is another existing technology, and today I am going to focus on it as an example.

With respect to nanoparticles, Dr. Ibuki, who is serving as Chairman today, presented why nanonization is so effective at the 19th Symposium on Particulate Preparations and Designs at fall in 2002. The reasons he stated are: improvement of the dissolution rate, reduction in crystallinity, supersatulation and the mobility inhibition in the GI tract, i.e. longer retention time. Further the direct contact with the GI tract is also included. The factors are related to each other in a complex manner and interact with each other to cause the effect. *Figure 1* is kindly offered from Dr. Naito, Professor at the Joining and Welding Research Institute, Osaka University. It is very interesting, and illustrates how many molecules are on the side, how many on the surface, the total number of molecules and the ra-

Percent of Molecules on Surface vs Particle Size

No. of Molecules on a Side	No. of Molecules on Surface	Total No. of Molecules	Surface/ Total (%)	Particle Size
2	8	8	100	
4	56	64	87.5	
10	488	1,000	48.8	2 nm
100	58,800	1×10^6	5.9	20 nm
1,000	6×10^6	1×10^9	0.6	200 nm
10,000	6×10^8	1×10^{12}	0.06	2 μm
100,000	6×10^{10}	1×10^{15}	0.006	20 μm
1,000,000	6×10^{12}	1×10^{18}	0.0006	200 μm

Prof. M. Naito, Joining & Welding Research Institute, Osaka University

Figure 1.

tio of the number of molecules on the surface against them. If particle size is reduced by one-tenth, then naturally the total number of molecules per particle will be reduced by three orders of magnitude, and the ones that are on the surface will be reduced by two orders of magnitude. Then the ratio of surface against total is increased by 10 times. With particles of 200 nanometers or less, this ratio becomes important, for molecules on the surface are obviously magnificated. If you micronize or create particles of 200 nanometers or less, they are expected to exhibit very interesting properties. As Dr Okamoto mentioned, the nanocrystals still stay at several hundred nanometers. New types of technology and equipment are then required to reduce them by one digit with well controlled manner. A lot of subjects may be overcome. But it could lead us to the new world of quite interesting phenomena, as we can easily imagine from these data.

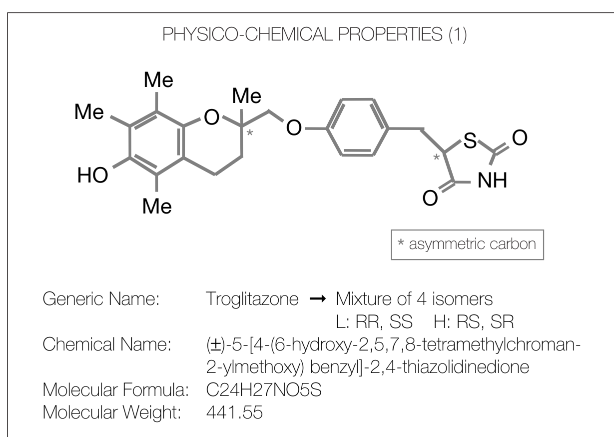


Figure 2.

Now I would like to focus on the solid dispersion and present on troglitazone as a case study. Our company developed it, and marketed for a couple of years. But due to the unexpected adverse event , it is withdrawn from the market. Some of the data will be presented to facilitate discussion later.

Figures 2 and *3* shows the physico-chemical properties of troglitazone. It is basically within the range that Dr. Lipinski described, but aqueous solubility is very low, at microgram/mL level with difficulty of numerical expression. Under the physiological conditions it does not practically dissolve, although its solubility increases a little under the alkaline condition. We cannot achieve the required level of dissolution under

Physico-chemical Properties (2)

Property		Value
Appearance	White to yellowish white crystalline powder	
Melting point	around 175°C	
Dissociation constant	around 6.1 (pKa1), around 12.0 (pKa2)	
Partition coefficient (log PC)	2.7 (n-octanol / pH7 phosphate buffer)	
Solubility	pH 1.2 (JP1)	< 0.01μg/mL
	pH 6.8 (JP2)	< 0.01μg/mL
	pH 7.0	0.4 μg/mL
	pH 8.0	0.9 μg/mL
	pH 10.1	29.0 μg/mL
	ethanol	5.3 mg/mL
	ethyl ether	8.3 mg/mL
	acetonitrile	9.5 mg/mL
	acetone	105 mg/mL

Figure 3.

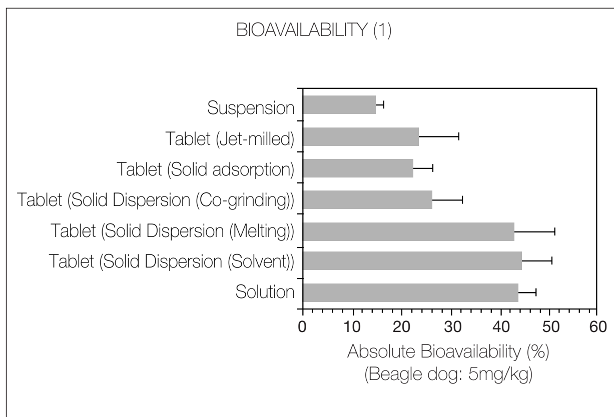


Figure 4.

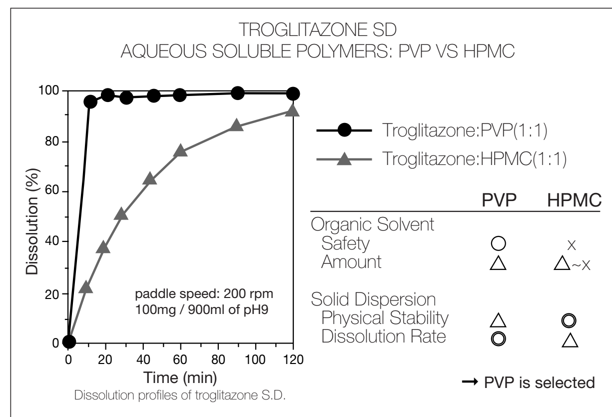


Figure 5.

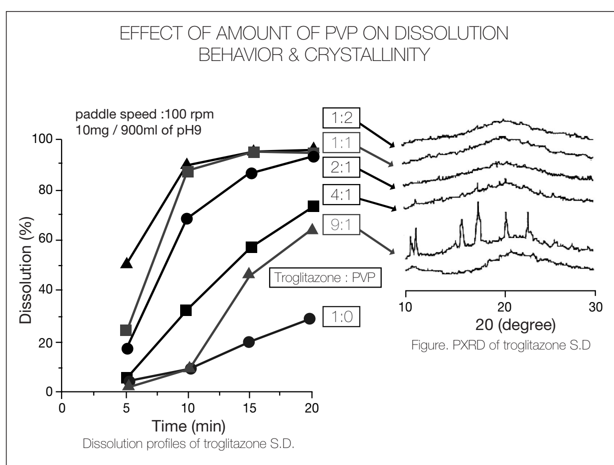


Figure 6.

the physiological pH within the GI. We are able to achieve order of milligram/mL as solubility in organic solvents.

So we compared a number of approaches how much extent they can improve the bioavailability. *Figure 4* gives those data obtained with beagle dogs; the oral suspension, the tablet made of jet-milled troglitazone at the order of several microns as particle size, the tablet prepared by solid-adsorption method, and tablets made of solid-dispersion prepared by a co-grinding method, a melting method and a solvent method. Melting was performed by heating. The solvent method was conducted by dissolving troglytazone with excipients in organic solvent and followed by evaporating the solvent to provide the solid mass. The solid dispersions prepared by the melting method and the solvent method exhibit bioavailability equal to that of the solution. If the co-grinding had provided solid dispersion at 0 % crystallinity, it could have shown the comparable level of availability. It is noted that it is very difficult to 100% reduction of crystallinity with the co-grinding method.

Figure 5 summarizes the comparison of two aqueous soluble polymers, PVP and HPMC, on preparing solid-dispersions (SD). We finally selected PVP. Here I would like to explain why we did so. We compared the dissolution profiles of SDs prepared with PVP and HPMC. One factor was what kind of organic solvents can be used; ethanol and acetone can be used for PVP, but chlorine-type organic solvents should be applied for HPMC. So PVP is preferable in terms of safety for environment and workers at operations. It is also taken into consideration how much solvent is required to obtain the solid dispersion, i.e. maximal dissolved amount of solid in solvent. Further, it must have practical physical stability during storage, the amorphous state and the dissolution behavior. As you can see, PVP's dissolution rate is sperior. As HMPC is less wettabe, and then SD with HPMC is inferior in wetting property and dissolution rate to those of PVP. Taking all these factors into consideration, we selected PVP.

Figure 6 displays the effect of troglitazone ratio against PVP on the dissolution behavior and the crystallinity pattern measured with a powder X-ray diffraction (PXRD). The drug substance itself dissolves about 30 percent in 20 minutes, but the dissolution rate is enhanced with PVP. The right-hand side of the *Figure* shows the PXRD crystallinity patterns. There exisits still some crystallinity at the ratio of 4 to 1. At the ratio of 2 to 1, no crystallinity is observed at all.

Figure 7 demonsrates the effect of initial crystallinity on physical stability. We stored samples of different level of initial crystallinity at accelerated condition.

Effect of Amount of Initial Crystallinity on Physical Stability

Initial	60°C			40°C/75%RH				Crystallinity
	2W	4W	6W	1M	2M	4M	6M	Change
~0%	~0%*	~0%	~0%	~0%	~0%	~0%	~0%	→
17%	19%	14%	21%	15%	19%	16%	15%	→
48%	67%	85%	89%	53%	75%	83%	N.T.	↑

** Apparent crystallinity (measured by PXRD)*

Test tablets were stored in glass bottle with desiccant.

Figure 7.

Samples at 0% and 17% of initial crystallinity did not change their initial crystallinity. That at 48% ended up at increased crystallinity after storage. Thus it is evident that the initial crystallinity is very important.

Figure 8 shows the electron probe micro-analysis of troglitazone physical mixture (PM) and SD, which indicates how the sulfur distributes in the mass. You can see the heterogeneous distribution of sulfur in the left-hand photo (SD). However, the homogeneous distribution is observed in the right-hand photo (PM).

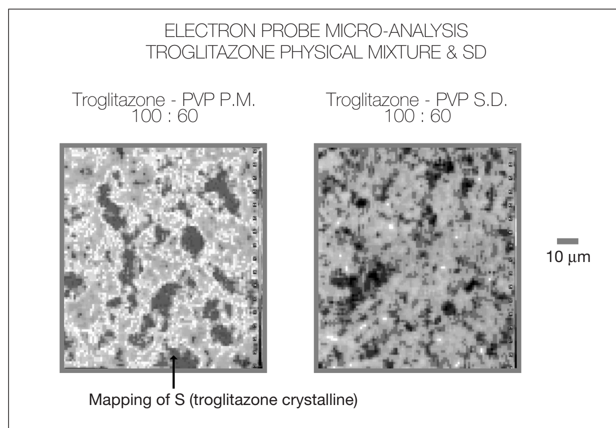


Figure 8.

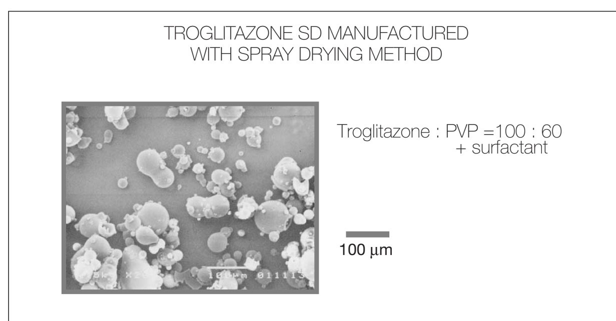


Figure 9.

When solid dispersion is manufactured with the solvent method, there are a number of options on equipments to be used. The spray dryer is one candidate, and the others are the vacuum drying system, the fluidized bed granulator and the vacuum granulator. To select the most favorable equipment, certain measures were estimated. First is the safety issue; what kind of safeguard against explosion do they have? Second is whether they require a carrier. Further measures are the residual solvent level, the density and the flowability of the SD. Those are the measures to determine the equipment. When the solvent is sprayed onto the carrier, we found it is rather difficult to control and reduce the residual solvent level. That's the reason why we chose the spray-dryer as an equipment.

Figure 9 displays the appearance of SD prepared with the spray-drying method. They are fairly large spherical particles. *Figure 10* compares the dissolution amount of troglitazone itself and its SD at 20 min in the various dissolution media. It is clear that dissolution property is markedly improved with SD.

Troglitazone SD
Dissolution Behavior

Comparison between dissolved amount of Troglitazone from Troglitazone and Troglitazone S.D.

pH	Dissolved amount*	
	Solid Dispersion**	Troglitazone
1.2(JP1)	3 µg/ml	< 1 µg/ml
6.8(JP2)	28 µg/ml	< 1 µg/ml
8.0	112 µg/ml	6 µg/ml
8.5	392 µg/ml	13 µg/ml
9.0	881 µg/ml	41 µg/ml
9.7	1678 µg/ml	151 µg/ml

* after shaking for 20min at 37°C

** Troglitazone : PVP =100 : 60 + surfactant

Figure 10.

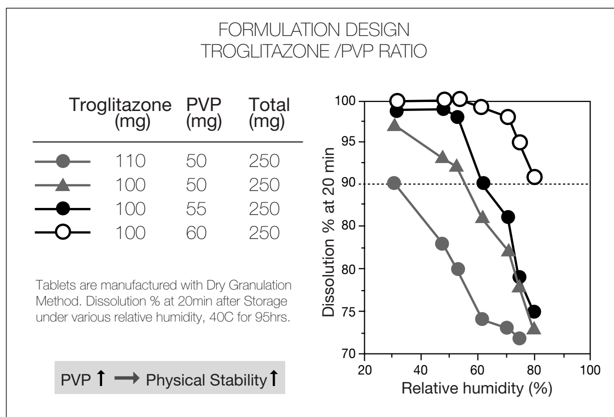


Figure 11.

Figure 11 illustrates the effect of PVP ratio against troglitazone. I'd first like to compare the three sets of figures at the bottom in the left-hand box with 100 mg troglitazone, where the total tablet weight is fixed at 250 mg. On the right-hand side the dissolution percentages at 20 minutes against various relative humidity as storage condition have been plotted, where the dotted line suggests the temporal target of dissolution level after storage. You can see that physical stability against moisture increased with the amount of PVP. If you increase the amount of troglitazone to 110 mg for the hypothetical worst case of its purity at 90% with keeping the amount of PVP at 50 mg, it becomes rather sensitive to moisture. This demonstrates that the amount of PVP is very important; the physical stability increases with PVP ratio.

Next, we have to optimize thr amount of the diluent and disintegrant (*Figure 12*). The experimental conditions are the same as for the previous *Figure*. As you can see, if the diluent weight is kept constant at 23 mg, the physical stability increased with the amount of disintegrant. When the total tablet weight is fixed at 250 mg, the stability increased with the amount of disintegrant. The higher the total weight, the better is the physical stability. It is a matter of course, but the tablet size should be appropriate for human oral administration.

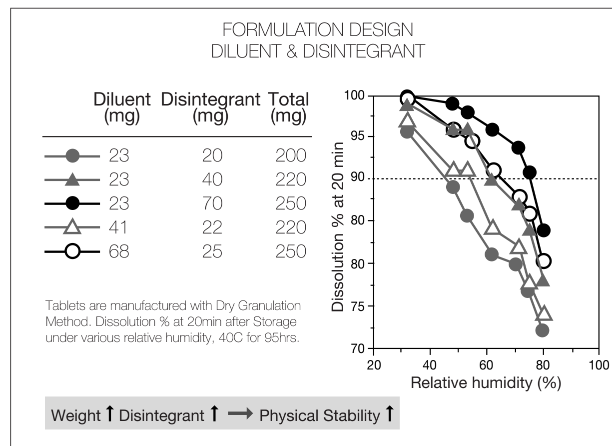
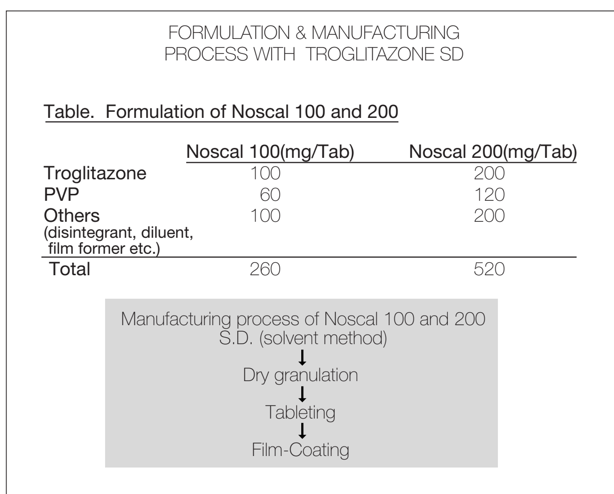


Figure 12.



FORMULATION & MANUFACTURING
PROCESS WITH TROGLITAZONE SD

Table. Formulation of Noscal 100 and 200

	Noscal 100(mg/Tab)	Noscal 200(mg/Tab)
Troglitazone	100	200
PVP	60	120
Others (disintegrant, diluent, film former etc.)	100	200
Total	260	520

Manufacturing process of Noscal 100 and 200
S.D. (solvent method)
↓
Dry granulation
↓
Tableting
↓
Film-Coating

Figure 13.

Figure 13 summarizes the formulation and manufacturing process. Noscal 100, the tablet of 100 mg strength of troglitazone is formulated with 60 mg of PVP and other excipients such as disintegrant, diluent and film former to make 260 mg as total weight. With respect to the process, the specific volume of SD prepared with the spray-drying method is too large to directly compress into tablets. Then the dry granulation process was introduced to provide denser mass, followed by tableting and film coating.

Figure 14 demonstrates the dissolution profiles of the tablet prepared with SD and control tablets. We basically used the same excipients for the control tablets. However, in order to obtain the same disintegration time, the amount of disintegrant is increased with reduction of the amount of diluent. It is evident that the dissolution % is improved by five-fold with the SD method.

Finally, *Figure 15* summarizes the bioavailability data after oral administration to beagle dogs, which is improved by about three-fold with the SD method.

Now I'd like to close my presentation. Thank you very much for your attention.

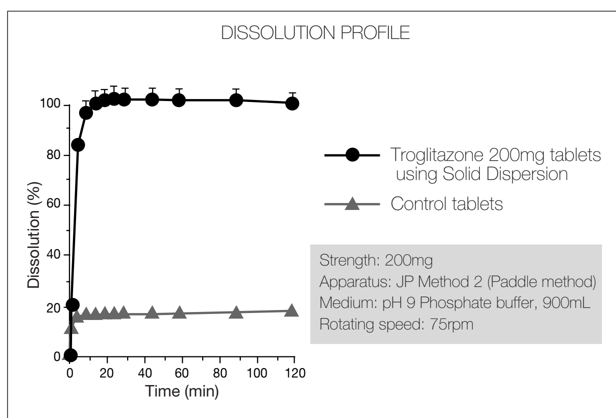
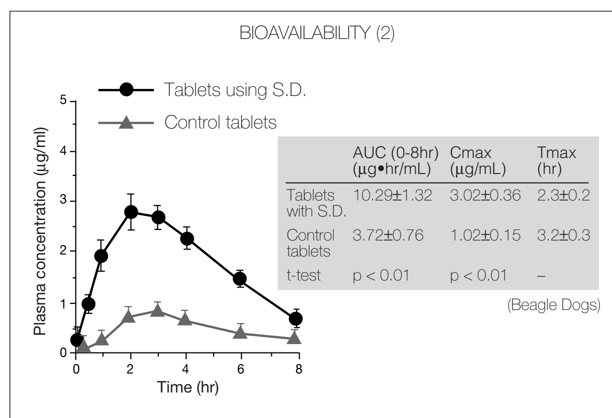


Figure 14.



	AUC (0-8hr) (µg•hr/mL)	Cmax (µg/mL)	Tmax (hr)
Tablets with S.D.	10.29±1.32	3.02±0.36	2.3±0.2
Control tablets	3.72±0.76	1.02±0.15	3.2±0.3
t-test	$p < 0.01$	$p < 0.01$	–

Figure 15.

Chair: Dr. Rinta Ibuki, Fujisawa Pharmaceutical Co., Ltd, Japan: Thank you very much, Dr. Kusai, for your presentation. You spoke in particular about solid dispersion, and thank you for the thorough information you gave us. Any questions or comments on this presentation, any discussion points to raise, please? We have 10 minutes left, so we have time for discussion with all three lecturers. So if you have questions for any of them...

Dr. Akira Kusai, Sankyo Co. Ltd, Japan: Dr. Yamashita suggested earlier that one to one appropriate method may exist to each API candidate. With respect to the poorly soluble APIs, we hope they are excluded at the screening steps. When we formulation scientists are involved to handle them, it is better to try a number of possible approaches such as solid dispersion, co-grinding, and various types of oily based dispersion instead of anticipating the most appropriate method, for we do not have the precise measures to assume. We have to be careful on the chemical and physical stability during storage even if the formulation exhibits good properties immediately after preparation. If that is the case, we have to give up.

As Dr. Kikuchi mentioned, a simple formulation is better. That is ideal, but we have to choose the realistic approach from the various options. I think this is how we are managing it these days.

Dr. Shinji Yamashita, Professor, School of Pharmacy, Setsunan University: One further comment that's relevant to this point: we apply solid dispersion and micronization method and we can expect to improve dissolution. But if we ignore the long-term stability issue and administer that type of product, we may find there is no efficacy. There are many such examples.

So, depending upon the compound, a formulation strategy may work, or it may not. The question is, how do we select from the options? We did make mistakes in the past. You at Sankyo, for example, tried with troglitazone to get succeeded. I think the one you described was a very good exsample. We actually want to know the case of failure. How many mistakes have you made along the way in terms of formulation decisions?

Dr. Akira Kusai, Sankyo Co. Ltd, Japan: Well, in several cases we tried hard to improve the dissolution behavior, but found that it did not improve absorption. During applying various methods to improve dissolution, we faced with a number of issues. For example, we had to drop many substances where formulation was not developed smoothly and the physico-chemical stability was unfeasible. In a number of cases, the dissolution behavior was improved but bioavailability was not.

And, as Dr. Kikuchi said, we conduct a lot of experiments using dogs before clinical stage, but are these results truly held even in humans? Complex formulation and complicated manufacturing process are now developed to make dissolution behavior improved. We will conduct the human clinical study with the drug products. However the formulation and manufacturing process has never been justified by comparing with the conventional dosage forms clinically. How can we make it? We have to accumulate such data in order to understand the relationship between dosage forms and clinical PK data and make them use to promptly optimize the following formulation development study on poor soluble APIs with less effort.

I'm not sure this answer is appropriate to your question, but this is my opinion. If any other manufac-

turers here would like to give us some comments, I would be pleased to hear from them.

Dr. Soon-ih Kim, Ono Pharmaceutical Co. Ltd, Osaka, Japan: The work conducted by Dr. Kusai is very interesting. It focuses on what the industry is actually concerned with today. I am going to make a presentation on the selection of the compound later. Can we apply these technologies at the selection stage of candidates? Once you have got into a certain phase, then there is left little freedom to pick options and methodologies up. Now, it's better to make dosage forms with a conventional formulation: you will get their better stability. But when you have to improve bioavailability, the drug should be provided at an amorphous or liquid state. I think it is worth conducting that at the stage of screening API candidates instead of doing at the formulation laboratories.

Dr. Akira Kusai, Sankyo Co. Ltd, Japan: To be very frank with you, I think it would be desirable if drug substances were selected automatically based on their physical and chemical properties before applying those methodologies at the formulation stage. That's the area Dr. Kim is working today with sufficient experience at the formulation field. You can choose the most promising candidate before formulation stage. You need to get a certain amount of API candidates to ensure that you can select the most preferable one among them. Also, as Dr. Lipinski mentioned, at the initial stage there's the issue of crystallinity. So it's a bit difficult to answer your question directly.

Dr. Shinji Yamashita, Professor, School of Pharmacy, Setsunan University: Listening to your comments from the chemist's point of view, what kind of improvement can the chemist expect at early formulation stage? Dr. Lipinski said that a difficult API is going to be two or three times more expensive, and require two or three times the effort – I imagine it as three times. If that kind of cost is going to be incurred, then surely improvements should be made at formulation stage. If it's going to be expensive, very challenging and also time-consuming, would you give up? Is this kind of technology going to be useful at early stage or not? I think the final decision would be very much dependent on the applicability of the technology at the early stages.

Chair: Dr. Rinta Ibuki, Fujisawa Pharmaceutical Co., Ltd, Japan: Perhaps Dr. Lipinski could also comment on this point, please? Maybe first from Dr. Kusai?

Dr. Akira Kusai, Sankyo Co. Ltd, Japan: Traditionally, we would try to develop dosage forms whatever compounds have been selected as API candidates. That was the case in the past. However, as Dr. Lipinski said, with poorly soluble APIs, they require a lot of time to develop and also there exsist some inherent risks. Are we ready to take those risks or not? It's true that we can discuss the risks when we pick the API candidates up to be developed. These days we may contribute somehow to the decision-making steps. I don't think it is too much to say, but in order to keep the schedule, it's better to avoid developing a candidate if some of these properties are very difficult to handle during formulation.

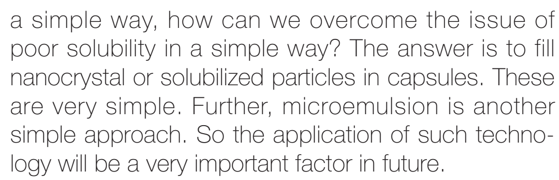
On the other hand, if a candidate that might become as a blockbuster, whether to go or no go is a management decision. And if some kind of improvement can be attained, then we should do our best to develop it. I think we need to take all those factors into consideration at well-balanced manner. I don't know if this could respond well to your question, but the final decision whether or not to move on with an API candidate must be discussed within the organization.

Dr. Christopher A. Lipinski, Pfizer Inc., USA: There was a definite viewpoint – at least at the Pfizer Groton Laboratories – and that is that at my lab and at other labs like ours, we actually completely discouraged any early formulation work in discovery. The reason was that we did not want to do anything that would hinder our chemists from changing the structure. We say it's the chemist's job to try to improve the structure, and to get better biological behavior earlier with early formulation work just slows down the process. The chemist doesn't make the changes.

In fact, sometimes we even had to rescue it with formulation technology. We actually had to try to discourage the formulation scientists from presenting exciting information in internal poster sessions. We were afraid that if our chemists saw examples of success stories then they would say, OK, there was no need for them to change the chemical structure, the pharmaceutical scientist could eventually solve the problem, and we did not want that message to get across. We wanted the chemist to focus on trying to solve the problem in chemistry, early on.

Chair: Dr. Rinta Ibuki, Fujisawa Pharmaceutical Co., Ltd, Japan: Thank you very much, Dr. Kusai, for your presentation.

Can I have one more minute? There were three presentations from three speakers and there are two points I would like to take up at this juncture. Various technologies will be discussed at the early development stage and it will be difficult to introduce new technology at that point. How can we improve this in



a simple way, how can we overcome the issue of poor solubility in a simple way? The answer is to fill nanocrystal or solubilized particles in capsules. These are very simple. Further, microemulsion is another simple approach. So the application of such technology will be a very important factor in future.

My second point is that variability is also an issue, although poor solubility and absolute bioavailability are issues. How do we evaluate and assess that? That is our challenge, and frankly speaking we don't have the answer unless we test it in humans. But how can we collect the PK data from humans, how can we evaluate the PK in humans? Of course there are regulatory issues to overcome, but we would like to make progress here in Japan, so that we can have a system to evaluate the PK in humans.

So, thank you to all of the speakers.

Accelerating Discovery and Development of Poorly Water-soluble Actives by the Aqueous Solubilizing System (ASS)

Dr. Soon-ih KIM

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Ono Pharmaceutical Co. Ltd, Osaka, Japan

Chair: Roland Daumesnil, Capsugel Inc., North Carolina, USA: We are going to continue with the theme of formulation strategy. I think it was extremely interesting and I'm sure we will have a debate on it afterwards during the panel discussion. As well as the presentations on formulation strategy we have had so far, there will be additional ones from the industry and from a development center.

Before we start there is something I would like to talk about. You are here facing a lot of top specialists, people you are probably seeing for the first time and will never see again, like Chris Lipinski. Don't be shy. Ask questions. There are no stupid questions. If you want to clarify something, if there is something you do not understand, ask a question. Do you think I know everything? I don't know everything, just like everybody else, so sometimes you need to ask many questions. Also, we would be so pleased if you would ask questions before the professorial machita, all the speakers, can get their oar in. They are here for the purpose of talking, and Sugiyama loves to be the first Beat them, be the first, and I will help you.

Our first speaker is Dr. Soon-ih Kim. I'm not even going to introduce him, he's extremely well-known in Japan. He has been involved in a lot of formulation, including the Capsugel expert system for capsules. For a few years he has focused his activities on specific kinds of formulation for poorly soluble actives, and I'm sure we will learn something through his presentation. Soon-ih, please, your turn.

Dr. Soon-ih Kim, Ono Pharmaceutical Co. Ltd, Osaka, Japan: Thank you very much, Chairman, for your introduction, and I would like to thank Dr. Yamashita and the staff of Capsugel for the opportunity to speak at this symposium.

Well, as you may know, at the discovery stage of the drug development program there are many compounds that are poorly water-soluble. As a result, that affects research efficiency. All pharmaceutical companies have new chemical entities which, even though they are insoluble in water, have been screened as promising candidates because pharmacological potency takes priority. Yet, when such candidates enter the preclinical and clinical stages, they definitely fail and slow down development due to biopharmaceutical properties (for example, they are poorly water-soluble). That happens in our company, too.

So, what is the level of solubility that does not cause failure or slow down discovery research? And how should we solve insolubility problems? Those are the issues that I would like to discuss with you. It's my contribution to the symposium. Let me start, then.

I believe that you are familiar with the pie chart shown in *Figure 1*, which analyzes the reasons why compounds fail. Lack of efficacy comes high up at 31 percent, as you can see, while poor biopharmaceutical properties account for 41 percent of the failure rate. Looking at the poor biopharmaceutical properties more closely, they encompass solubility; log D; chemical stability; permeability; metabolism; protein binding; plasma stability; RBC binding, and *in-vivo* bioavailability.

I would now like to focus on the two critical issues of solubility and permeability, and discuss the methods of dealing with them in early-phase development.

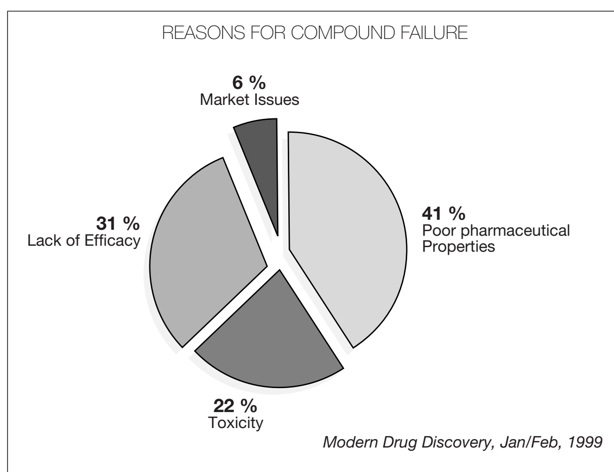


Figure 1.

Recently, there have been trials at early-stage discovery evaluating the performance of biopharmaceutical properties – solubility, permeability, metabolic stability and so forth *(Figure 2)* After selection of candidates, optimization of the bulk substance, due to crystal polymorphism, is also considered to be important. However, due to the fact that there are too many evaluation items and that combinatorial chemistry, which leads to a deluge of promising candidates or compounds, is still advancing, a sophisticated screening flow and associated criteria have yet to be put in place. So even if we have the sophisticated screening flow, if it does not function well, then it is not meaningful. Therefore we have to also address this critical issue.

Eight years ago, Dr. Hashida edited a publication looking at unfavorable properties for oral dosage forms in the Japanese pharmaceutical industry. In descending order of difficulty encountered, they were: poor bioavailability due to low permeability; poor bioavailability due to the first-pass effect; chemical instability; low solubility, and poor productivity due to a high dose.

Well, pharmaceutical scientists are one of the arms involved in the development program, and formulation researchers have more than sufficient knowledge of how to improve solubility. Therefore I believe they can actively contribute to dealing with this issue. We should encourage the involvement of pharmaceutical scientists and formulation specialists from the early discovery stage.

The graph in *Figure 3* illustrates the predicted solubility distribution results from the commercial library run by *in-silico* Our company's library shows a similar distribution, and in a recent project some compounds had a solubility of below 1 microgram per milliliter. I believe Ono Pharmaceuticals is not the only company to experience this type of phenomenon but, in any case, low solubility is the major impediment in the discovery process.

So, as I mentioned earlier, if pharmaceutical scientists helped to solubilize the compounds, that would facilitate their formulation processes in the later stage of development. I believe that this is one of the missions of pharmaceutical scientists or formulation specialists. Since we have such specialists in the audience, I would definitely like to discuss the issue with you in today's symposium, to establish a more efficient discovery process.

For instance, we may come up with some lead compounds through *in-vitro* screening and if they are poorly soluble, an aqueous solubilizing system (ASS) might be employed in order to improve solubility *(Figure 4)* The maximum potential of the compound can be evaluated through *in-vitro* and *in-vivo* screening, and you can narrow down the number of compounds. Then, you can investigate the crystalline optimization for the selected compounds. I believe that this stepped process will turn out to be more efficient than the traditional screening flow. It was recently initiated at Ono in Japan.

This morning, Dr. Yamashita suggested to us that if you look at the permeability of the compound, and if the permeability and absorption are good enough, we can rescue that compound even if it is poorly soluble. As shown in *Figure 4*, even if a compound is poorly soluble we may be able to choose it as a candidate. The degree of solubility allowed would be chosen by pharmaceutical scientists. That is what the medicinal chemists would like to propose to the formulation specialists.

We can cope with poor solubilility by micronization or ASS.

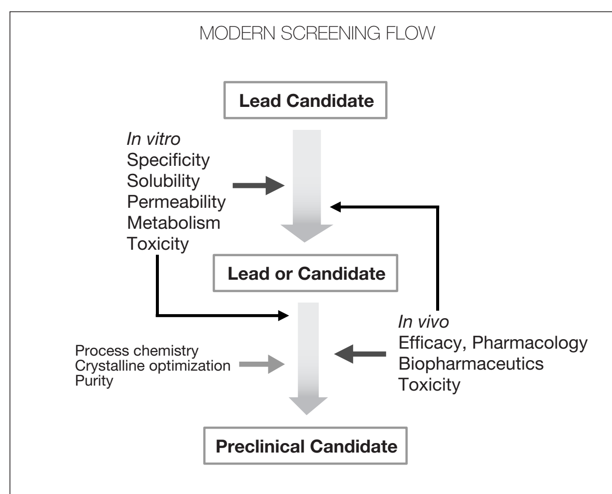


Figure 2.

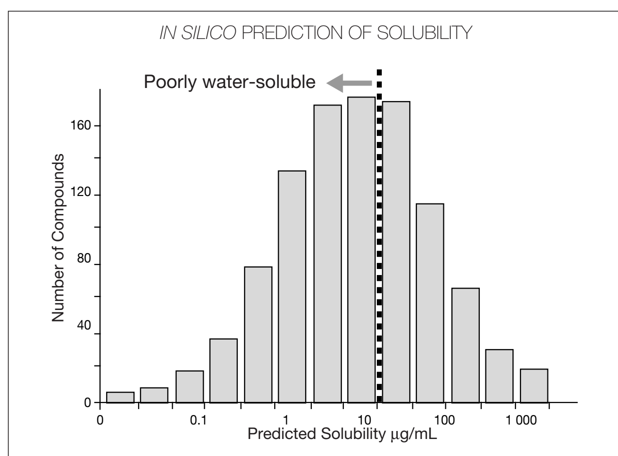


Figure 3.

Based on these considerations, I came up with a portrait of the screening of preclinical candidates. Candidates are classified as BCS Class I and II, or BCS Class II and IV. For those in BCS Class I and II, a standard formulation is suitable, while for BCS Class II and IV it depends on the dosage – let's say a 100 mg dose as a standard dose. But I'm sure you have your own criteria for the dose level at each of your companies. For Class IV, a preformulation with solubilization and micronization, for example, may be the way forward. But if what's thought to be a Class II drug turns out to be a Class IV drug, then the use of a liquid or semi-solid solubilizing formulation may enable it to continue in the development process.

What we have to be concerned about, however – and I would like to come back to this later – is that when it comes to liquid and semi-solid products, we have to test compatibility, chemical stability and *in-vivo* performance Only candidates that pass these tests can be selected as preclinical candidates. That may be a viable approach.

Now I would like to introduce a case study on how this portrait can be applied. Compound X has very low solubility, although its other properties are not so bad. Since we did not have *in-vivo* data at this point, we did not know whether it should be classified as BCS Class II or BCS Class IV. But it was likely to be BCS Class II from a pharmaceutical profile.

I organized a re-arrangement of the biopharmaceutical problems and strategy for Compound X. Oral bioavailability was 3 percent with monkeys and 20 percent with rats, but the toxic dose could not be sufficiently evaluated in rats because Compound X has an adverse action on rats, though not on humans and monkeys. On the other hand, we could not make a confident

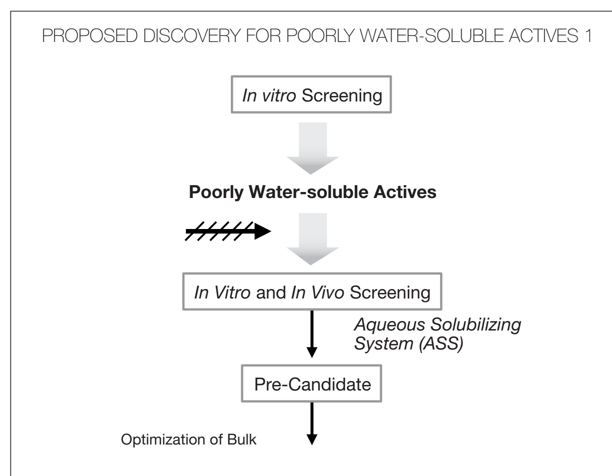


Figure 4.

estimate of safety in monkeys after administration of the suspension. Since the compound did not have very poor permeability and had a low first-pass effect, we decided to use the aqueous solubilizing system (ASS), in order to evaluate its maximum pharmacology and toxicity potential.

The *Table* shows the bioavailability (BA), pharmacology (ED50) and non-observed adverse level (non-toxic dose) of Compound X in suspension and in the ASS system. I cannot go into the details of the pharmacological properties, but in monkeys, no matter how much we increased the suspension dose, the plasma concentration did not increase. Through ASS, the BA was higher by about 10-fold, and the ED 50 was higher by about 10-fold. So it was possible to estimate the non-toxic dose. As a result, Compound X was selected as a preclinical candidate.

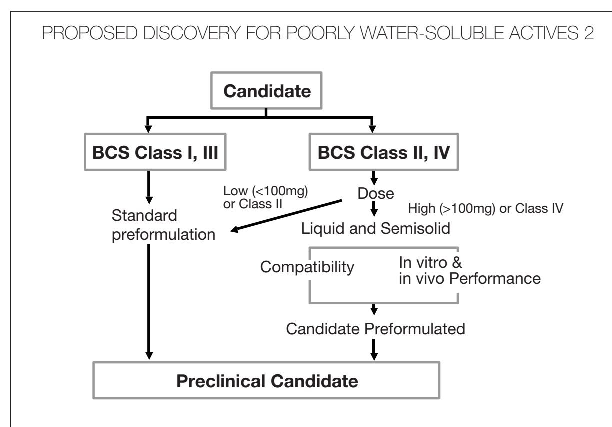


Figure 5.

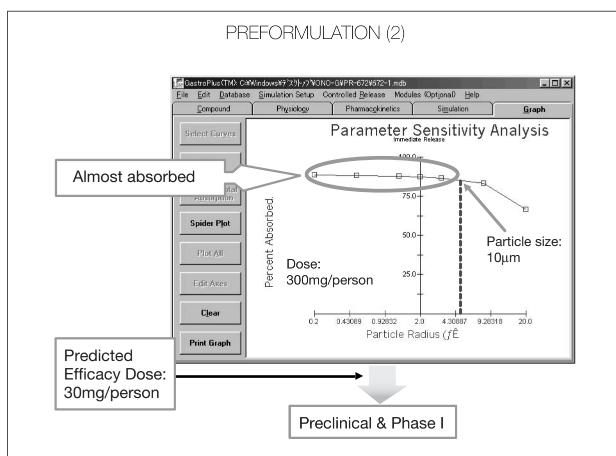


Figure 6.

With Compound X, from early discovery we prepared a liquid or semi-solid formulation and we conducted preformulation studies. The details of the ASS formulation are confidential, but if more than 120 mg/mL dissolves, that should be considered sufficient for encapsulation with hard and soft capsules. As the moderator mentioned, we have the appropriate equipment to conduct filling tests, and we looked at compatibility *(Figure 5)*. The hard capsule filled liquid formulation of Compound X can be prepared by the filling and sealing machine (CFS 1000, produced by Capsugel), and then the capsules can be put through the compatibility test, and *in-vitro* and *in-vivo* profiles.

What was also checked at the same time – and what we had most difficulty with – was analysis. Experts in this area will know how difficult that is. The decision on whether to go on to preclinical or Phase 1 studies had to wait until we had more stability data. As it turned out, we found that the formulation with ASS was unstable for Compound X.

In parallel with liquid formulations, the amorphous state and other possibilities were also examined, as Dr. Kusai suggested. But we found that this compound had to maintain crystallinity to ensure stability, so once again we went back to micronization as a possible formulation strategy.

But how small should we make the particle size? We had to make a prediction. Using commercially available simulation software such as Gastro Plus (produced by Simulation Plus), we conducted an *in-silico* simulation of dissolution and absorption *(Figure 6)* When we ran the simulation, we found that with a dose level of 300 mg per person, the particle diameter size where we had no saturation size problem in our prediction, or no saturation absorption in our prediction, was below 10 microns.

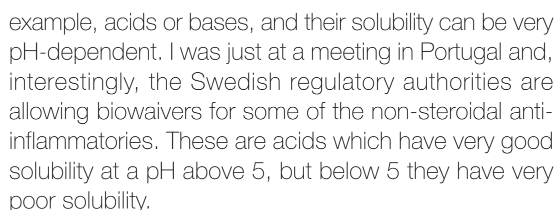
I won't go into the details of the prediction approach, but we are trying out predictions for humans based on *in-vivo* rat PK profiling using ASS. How we make that inference I would like to defer to another occasion, because competitors have introduced a similar product. And because 30 mg per person was predicted as the clinical effective dose, we came to the conclusion that this compound could be selected as a preclinical candidate, and so it was entered into Phase 1 study.

I do not intend to be boastful, but pharmaceutical scientists are able to make a large contribution to the discovery stage. Although we had to wait until the end of our Phase I study to see whether Compound X was truly successful, it means that in the future, when we encounter other poorly-soluble compounds, we would like to take on the challenge.

Finally, with poorly-soluble compounds, their maximum potentials (pharmacology, toxicity, pharmacokinetics) should be evaluated by solubilization from early discovery stage. After evaluation of the potentials, crystalline optimization based on the relationship with solubility and absorption should be conducted on the truly potent compounds These areas are the recent focus in pharmaceutical research. The Japanese industry has to compete against mega-companies and survive in the world. So we have to, I think, focus on pharmaceutical technology such as solubilizing systems, physicochemical technology, drug delivery systems and analytical technologies. These technologies will support Japanese-style discovery, and the Japanese pharmaceutical industry will have to turn to these technologies. Pharmaceutical scientists therefore should be more involved in discovery so that we have truly therapeutic drugs. That is my firm belief. Thank you.

Chair: Roland Daumesnil, Capsugel Inc., North Carolina, USA: Let's try to apply the rule. We have three or four minutes for questions, and I would like someone outside of the usual panel. I give you the problem, I would like to have someone else. James, you are one of our speakers, so you will have the opportunity, don't worry. But I would like to have someone from the floor. I think I am pushing a little, but I would like to change the habit. So rather than have the speakers and the chairs, if someone would like to ask questions before them… No? OK, James comes next.

Professor James Polli, University of Maryland School of Pharmacy, USA: Thank you very much for your presentation. I know you spoke on discovery but I have a question that's maybe applicable to products on the market. We've heard a lot of discussion about definitions for low solubility. Many drugs are ionizable; for



example, acids or bases, and their solubility can be very pH-dependent. I was just at a meeting in Portugal and, interestingly, the Swedish regulatory authorities are allowing biowaivers for some of the non-steroidal anti-inflammatories. These are acids which have very good solubility at a pH above 5, but below 5 they have very poor solubility.

This group of compounds as a whole tends to be very well absorbed in spite of its very low solubility at a lower pH. In some sense they would appear to be low-solubility compounds; however, in other senses they are high-solubility compounds, and *in-vivo* they certainly perform as high-solubility compounds. In your studies, have you identified pH levels that are particularly important for acids or for bases in determining whether or not they function effectively as highly soluble or lowly soluble?

Dr. Soon-ih Kim, Ono Pharmaceutical Co. Ltd, Osaka, Japan: With the ASS system we can prepare a water-soluble formulation irrespective of pH. Our ASS system is pH-independent and poorly soluble compounds could be water-soluble at the whole pH range. I'm not addressing your question, right?

Professor James Polli, University of Maryland School of Pharmacy, USA: No, I don't think so. I must admit, the reason I asked the question is because it's a very difficult one and I have not been able to find anyone in North America that can answer it. It's a very challenging question. But it certainly appears to be the case that many drugs are ionizable and that for certain types of compounds, some regulatory authorities allow biowaivers – for example, with non-steroid anti-inflammatories, which they don't view as bio problems. Why is it that some compounds, even though their entire profile is not highly soluble, why is it that they perform as if they were highly soluble, and can we predict that in advance?

Chair: Roland Daumesnil, Capsugel Inc., North Carolina, USA: I think, James, that that is a very interesting question. I would like to keep it for the panel discussion, because you will have all the speakers and chairs challenging that. Thank you, Dr. Kim, for a nice presentation.

The next speaker is Dr. Hassan Benameur, who is on the staff of Capsugel and is responsible for the development center at Capsugel. Hassan is a very well-known specialist in the formulation of poorly soluble actives using microemulsion and self-emulsifying systems. He has patented a few formulations and I am sure that he will be looking in detail at the pros and cons of these kinds of formulation. Before joining Capsugel, Hassan was responsible for formulation at Gattefossée, a company specializing in lipid-based excipients. Hassan, it's your turn.

Formulation of poorly soluble actives: how to make it an industrial reality

Dr. Hassan BENAMEUR

Formulation of poorly soluble actives: how to make it an industrial reality

Dr. Hassan Benameur, Development Center Director

Capsugel R&D Division Colmar France

Dr. Hassan Benameur, Development Center Director, Capsugel R&D Division Colmar France : Thank you. Yes, we will be focusing on lipid formulation, the lipid-based delivery system. In summary, and extrapolating on from what Dr. Lipinski said this morning, combinatorial chemistry and receptor-based screening lead to two kinds of product: the brick dust and the grease ball. The brick dust is a large, organic and polar molecule with poor solubility and membrane transport properties. The second category, that we call the grease ball, is a lipophilic molecule with poor aqueous solubility and a very high log-P, or partition coefficient, which indicates good permeability.

It's currently impossible today to develop a good formulation approach for the brick dust molecule, while conventional formulations do not work with the grease ball. As a formulator, if chemists ask us which kind of drug we want, or which one is easier to use in formulation, I think all of us would agree that it should be lipophilic one's or the so called grease ball As Professor Yamashita said, scientists today, especially those in pharmaceutical development, have to use what I call internally 'our two feet', to walk a way through the formulation. With these new molecular entities, we have to use the physical factors but also be aware of the biological factors in trying to find the solution (*Figure 1*).

We now know that the Biopharmaceuticals Classification System (BCS), which the US Food and Drug Administration produced as guidance in August 2000, is very important. It means we can classify actives by solubility and permeability and, to be simple, as either low or high. Among the parameters that must be selected at preformulation stage is the dose, defined as high (greater than 200 mg), medium (10 to 200 mg) and low (less than 10 mg). With a lipid-formulation organized system, we normally use a dose of between 10 and 200 mg, which is the classical range for this type of formulation; a low dosage works easily. Particle size is mainly important in terms of the pharmaceutical technology, or production or characterization of the system, and is defined as high (greater than 100 micrometers), medium (1 to 100 micrometers), and low (less than 0.2 to 1 micrometer).

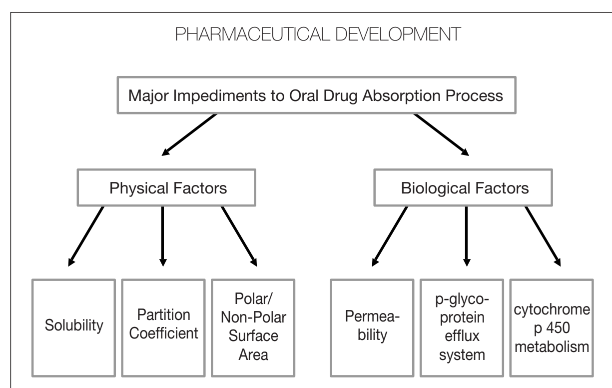


Figure 1.

A high partition coefficient (a log-P greater than 3) is, as I said, the best result at preformulation stage; a medium result is 1 to 3, while a low one is less than zero. We need to aim for a lipophilic formulation, as this will integrate best with a lipid-based formulation and, of

course, we must be aware of the bioavailability parameters: high is greater than 90 percent; medium, 20 to 90 percent, and low, less than 20 percent.

I totally agree with Professor Lipinski that each expert has to do his job. Chemists have to provide the best molecule or the best information they have on new chemical entities (NCEs), and formulation scientists must bring with them the best knowledge they have and use the best tools they can get. So there is a lot of – let's say 'homework' – to do on both sides, to collect all the information, and then the parties need to get together to discuss and exchange information as scientists and to build bridges between disciplines.

So, to summarize several aspects of preformulation studies for NCEs: achieving solid-state stability means that we need to look at the effect of temperature and humidity, as well as the conditions laid down under the International Conference on Harmonization. Then there is the effect of moisture, and the presence of a stable hydrate, as well as possible polymorphs – remember the story of Norvir, where the form that crystallized out from the hard gelatin capsule on storage was a different polymorph, with actives that were less water-soluble. There is also the effect of light.

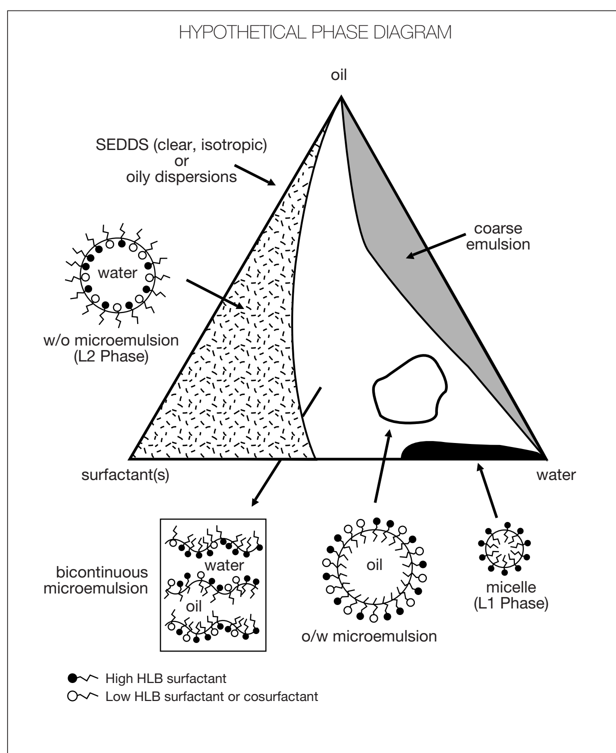


Figure 2.

In terms of the screening methods required, we have to establish the pH solubility profile, the pH stability profile, and also the effect of oxidation. Based on this kind of information we can start choosing the non-aqueous excipients or solvents best suited to a self-emulsifying delivery system (SEDDS) or a self-micronization drug delivery system (SMEDDS). The precise selection will be based on the NCE's solubility and stability.

How we use this information is our commitment to follow a rational approach. Currently, there's a lot of discussion in the literature about how to use SEDDS or related systems (microemulsion, emulsion…). The key point is the know how of the excipients which include their physico chemical characteristics of the excipients and their blending. There are three main categories of excipients that can be blend together: the hydrophilic type, which is PEG (polyethylene glycol), PG (propylene glycol), glycerol and so on; the lipophilic type, comprising oils and MCTs (Medium chain triglycerides), and the amphiphilic surfactant type, the Gelucires, Tweens, Cremophors, and so forth.

Through our choice from this wide range of excipients we can start combining them, in order to select the NCE with the highest solubility and stability. If the drug is stable, we can begin formulation, using phase diagram analysis.

Figure 2 is a hypothetical phase diagram in which you can see that these systems can organize themselves when mixed and dispersed in water. The apex of the triangle shows the oil, while the base represents the surfactant phase, using a surfactant co-surfactant (S/CoS), and the water phase. You can see that the various combinations possible on the surfactant/oil axis produce different structures when diluted with water. It is this kind of rearrangement that we want to develop and to formulate. Through specific combinations you will get different types of arrangement (L1, L2, W/O; O/W…).

Let me run you through the diagram Because the surfactant/oil composition is fixed, by adding a small quantity of water you will get a water-in-oil micro-emulsion. This kind of formulation can be valuable in some cases – let's say, when we want to include a peptide or a hydrophilic system in the capsule – because the continuous phase will be lipophilic. By adding more water we come to a more complex situation, what we call bicontinuous micro-emulsion, which is a mixture of 50% water and 50%oil. If we go on diluting, we will end up with a coarse emulsion which is easy to recognize; it is milky. So you can see that, by blending the excipients, we can get different organizations on the same axis.

The main objective with poorly water-soluble actives is to formulate an oil-in-water micro-emulsion (O/W)

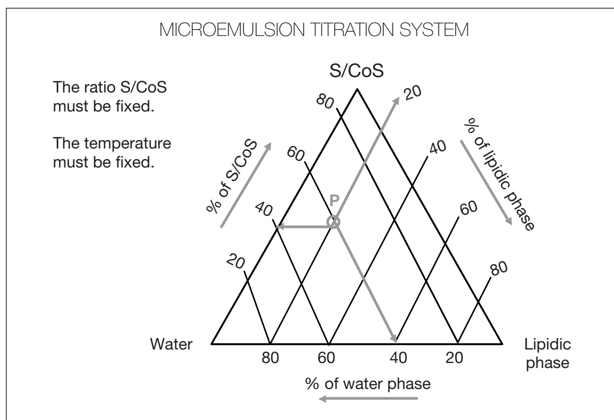


Figure 3.

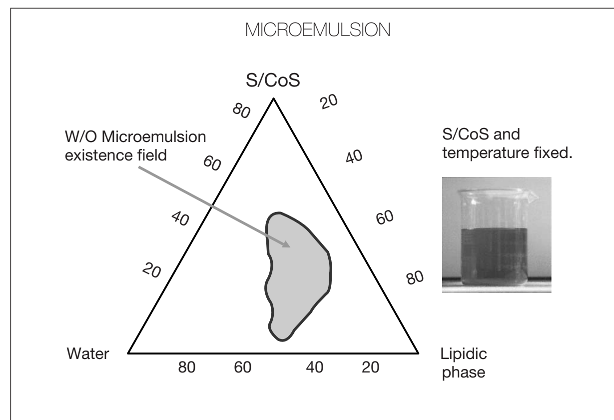


Figure 4.

straight away. The key point is to use the right combination of excipients to avoid getting a different system during the dilution, which will crash out your active This starts by using a clear, isotropic SEDDS system. In some cases an oily dispersion can work, but this is mainly for hydrophilic products because, by definition, water-insoluble products will not be solubilised if they are not solubilised at the beginning (isotropic solution). So there are several ways of doing it.

Chemists have a range of approaches to micro-emulsion, which has been widely used in classical chemistry; the first micro-emulsions were developed in 1954. The approach we employ is based on titration. In other words what we do, as shown in *Figure 3*, is that we fix the composition of the surfactant/co-surfactant – and I will explain the use of this later – with the lipidic phase and we blend them with water to determine the area of the micro-emulsion.

Since solubility is very important, and as the actives have to stay solubilised throughout the GI tract, the excipient used will be non-ionic, because by using a non-ionic excipient we guarantee the solubility. On the other hand, a non-ionic surfactant and co-surfactant come within what we call the phase inversion temperature: it means that their structure will change with the temperature.

I admit it doesn't look obvious. But just to give you the everyday story… We were involved in a study where all the development had been done at 25 degrees Centigrade, which is the lab temperature classically used, but when we repeated the dissolution activity at 37 degrees, everything crashed out. It only happened because we said that as 25 is close to 37, a 12-degree difference would not be a big deal for such a complicated formulation. But it was. So it's very important that this parameter is fixed right from the beginning.

By making different blends, starting from a composition with a low concentration of surfactant/co-surfactant and a high concentration of lipid, we can determine the area of the micro-emulsion. And, as I have shown you in the hypothetical diagram (*Figure 2*), you will get different types of reorganization. But what we are looking for is to define precisely the point where we get the organization that we want to use. In the case of *Figure 4*, which is an example of a water-in-oil micro-emulsion existence field, the point corresponds to the inside contour of the area. This kind of system can be used for peptides that we want to put into hard or soft capsules, because the continuous phase is lipophilic.

From this basis we now have to choose how to optimize the formula. *Figure 5* gives an example of two lipid formulations with the same surfactant/co-surfactant oily phase, and the same quantity of active. What happens when we add water is that one system will give you a micro-emulsion, the other will give you a milky dispersed system. It's clear that these two products will behave

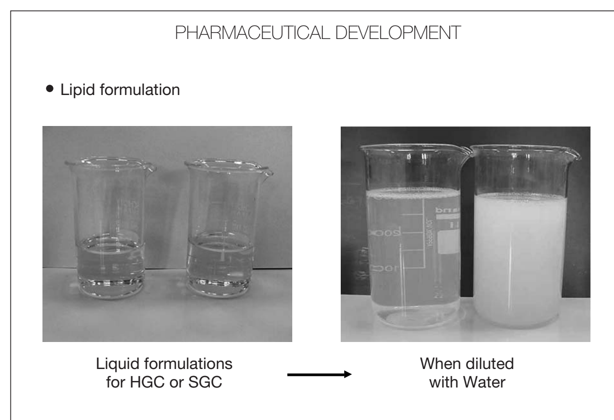


Figure 5.

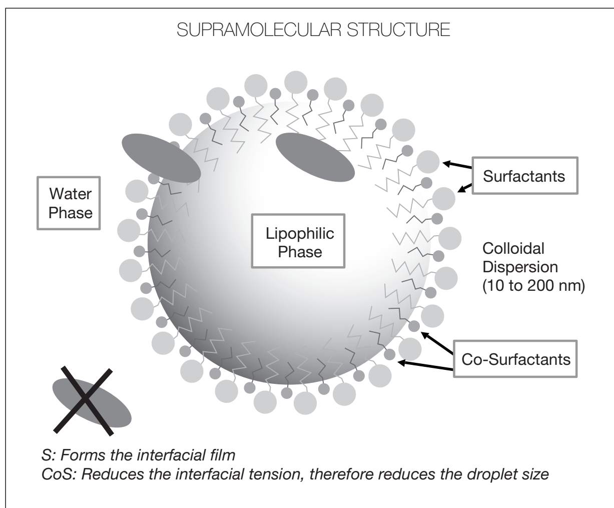


Figure 6.

totally differently from the point of view of absorption. It's well known that a small nano particle, as in clear system, will enhance bioavailability and that, secondly, it will be very stable. The question for the milky system is the active into the organized system. If yes, it is only an increase of particles size which could be optimized during the formulation. If not it is the crash out of the active that causes the milky dispersed system. In this case the formulation will be complex indeed surfactant/co-surfactant ratio should be redefined

So I drew the diagram in *Figure 6*. Normally, with the non-visible system, because you have particles of between 10 and 200 nanometers, it is clear to the naked eye. When you have the water phase and the lipidic phase, which are two immiscible systems, what happens first of all is that the surfactant forms an interfacial film. This is the classic approach formulate an emulsion or colloidal system On the other hand, you will also get a co-surfactant that reduces the interfacial tension, thus reducing the particle size and resulting in a micro-emulsion system. So it is the combination of these three systems – surfactant, lipophilic phase and co-surfactant – that will give you the right combination to do a micro-emulsion.

Now for the active. As I said, with this kind of system the more lipophilic the active, the better it is. This is what formulators like, because then we can build up a system in which the active is in the core of the micro-emulsion. It can also interact with the interface. But what we want to avoid right from the beginning is getting an non isotropic solution during the dilution, because it can crash out the active from the system, and you end up with two co-existing approaches: solid particles and the micro-emulsion.

The way to differentiate them quickly is to put them under a polarized light. This also explains why we like to keep the crystal form with this kind of formulation; because the particle is very small, you can see the crystal even though you cannot see the particle. So a quick screening that we can do during development is to take an isotropic solution and put it under the microscope under a polarized light, to check the absence or otherwise of the crystal. So, again, this is a key aspect We have to know in which power we are, and what all our formulators are. We have to draw the diagram in *Figure 6* for all of the constituents, all of the oily phases, all of the surfactants, and this takes a lot of time and energy.

The way that we do the characterization is to carry out a rapid first screening, by eye. If it's clearly a liquid solution we then check the isotropy under a polarized light microscope, as I have explained. We also check the Newtonian behavior with a rheometer, as well as the thermodynamic stability, using the aging test and centrifugation. Finally, when we have the right formulation, we measure the particle size by photon correlation spectroscopy.

So let's go through the Neoral success story. I'm mainly going to focus on the pharmaceutical aspects, the formulation aspects that make it into a success story.

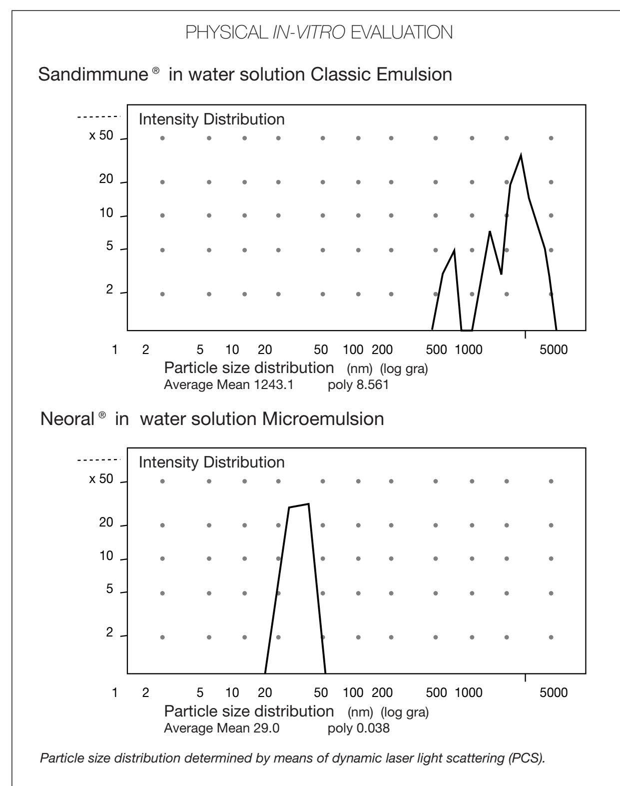
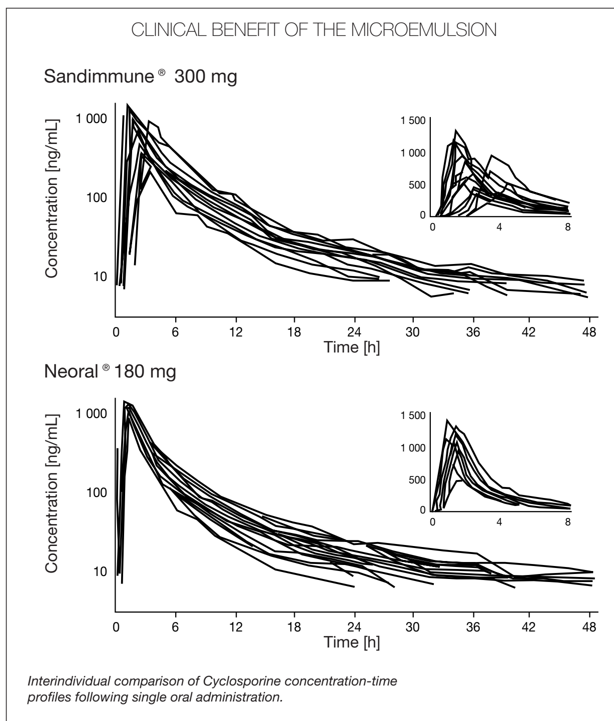


Figure 7.



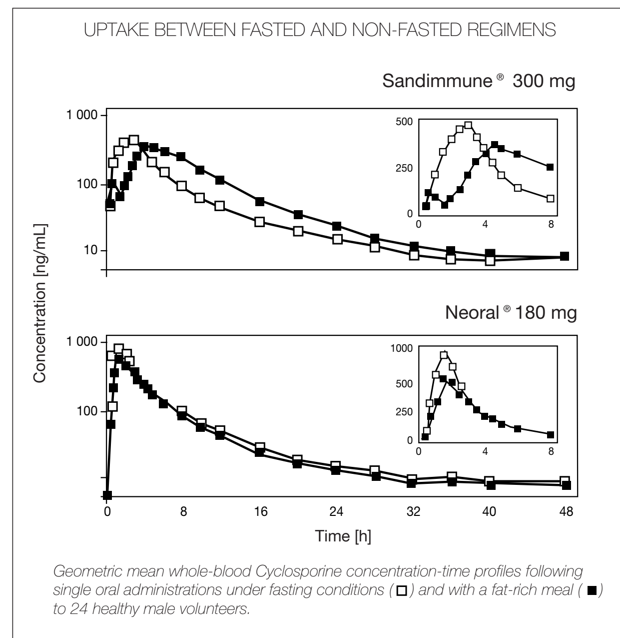
Interindividual comparison of Cyclosporine concentration-time profiles following single oral administration.

Figure 8.

First of all, as I explained earlier, you get two kinds of solution from the physical *in-vitro* evaluation: the classic emulsion and the micro-emulsion (*Figure 7*). You can see the classic emulsion via photon correlation spectroscopy measurement, but the emulsion will have a poly-dispersed index and you will have large particle sizes distribution ranging between 500 and 5,000 nanometers. On the other hand, with a micro-emulsion you will get sizes of an order of magnitude lower (10 times), of between 20 and 50 nanometers, and you have a sharp mono-dispersed system.

What is the *in-vivo* result of this? *Figure 8* shows the biological approach. First of all, the key point to note with this reformulation is that the plasma profile is the same. This was very important as a line extension of the existing Cyclosporine A formulation. Secondly, you can see that the advantage of Neoral as a micro-emulsion is enhanced bioavailability, because its 180 mg dose is now bioequivalent to Sandimmune, 300 mg. But on top of that, if we look at the results of the six-hour blood samples, you can see that there is reduced inter-subject variability with the microemulsion formulation.

When we do such formulations the question that quickly arises is, what happens with this lipidic formulation in a fasted and non-fasted study? *Figure 9* shows that



Geometric mean whole-blood Cyclosporine concentration-time profiles following single oral administrations under fasting conditions (□) and with a fat-rich meal (■) to 24 healthy male volunteers.

Figure 9.

with the emulsion you get a clear differentiation between the fed and non-fasted results. This is mainly due to the fact that because the particles are bigger in the emulsion they interact with the meal, and they are more sensitive to lipid digestion On the other hand, with the small nano particles of the Neoral micro-emulsion the formulation are bioequivalent (same Cmax and Tmax)..

Now to try to answer the question asked before the previous talk. Unfortunately, we cannot use the study we are currently running at the DC as example, so as our model drug, we will use ketoprofen. With ketoprofen, can a micro-emulsion really be the answer? It is listed as a Class 2 molecule under the BCS classification. The solubility in water is extremely low, 51 micrograms per milliliter, and we have a pKa of 4.45, which means that a pH higher than 4.45 will increase its solubility (*Figure 10*). The ketoprofen is well absorbed in the human small intestine at ph above 5.5.

Nevertheless it was a good challenge for us to show that even with this product we can make a formulation to enhance the solubility. So we chose the formulation that's on the market, which is ketoprofen, 50 mg, and made a lipid formulation which was basically a semi-solid. We wanted to make it a semi-solid, rather than the more obvious liquid form, to show that we could enhance the solubility of the product at acidic pH (in the stomach). Formulation, consistent of a blend of excipient a hydrophilic (free PEG), the monostearate of PEG as surfactant, monoditriglyceride as co-surfactant,

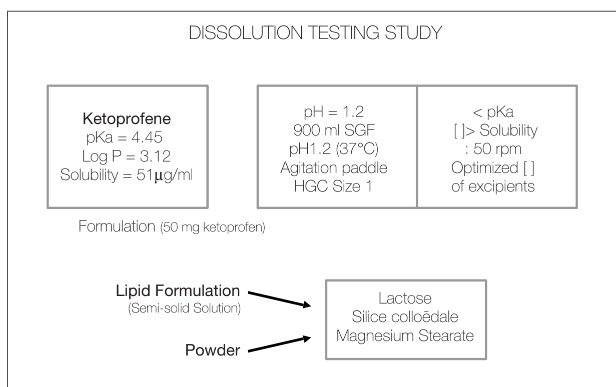


Figure 10.

with the as di and triglycerides as the lipidi. As a reference formulation we used a powder which contain also 50 mg of ketoprofen blended with the right powder excipient lactose, colloidal silicon dioxide and magnesium stearate.

What were our parameters? First of all, we used the pH of simulated gastric fluid without enzymes. That's pH 1.2, which means that the PK was below the PK of ketoprofen, to ensure that it had reduced solubility, and that we really were dealing with a Class 2 molecule (low water solubility high permeability. On the other hand we didn't want to use high speed stirring, so we used a paddle speed of 50 rpm , and we optimized the concentration of the excipient by using a size 1 capsule.

Figure 11 shows the results of the dissolution profile, with the lower chart indicating our reformulation. It's clear that by optimizing a lipid formulation you can solubilise the active, whatever the pH. And this is typically the answer: the system has to be independent of the pH, and this is the way we have proven it by increasing the water solubility of ketoprofen in pH 1.2 by using organized lipid system in our case study water in oil micro-emulsion using non ionic excipients.

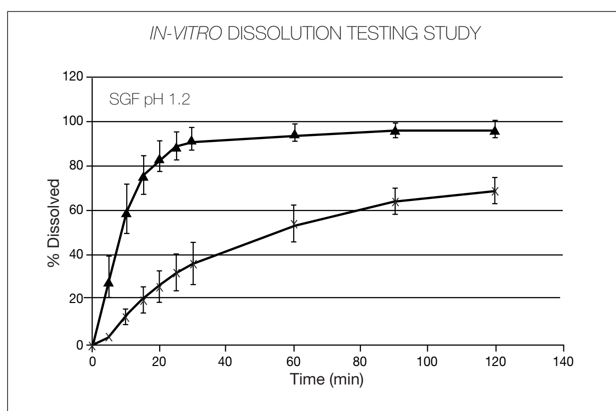


Figure 11.

Let's now try to understand where ketoprofen will be positioned in the micro-emulsion Here, it is very important to remember what we want to achieve as formulators. We can make a very good micro-emulsion, but if the active is no longer released from the system we have fail in our formulation development We want to achieve a stable system that increases solubility, but that will release the active for absorption and thus answering the problem of class II molecules. To achieve this, we set up the study by using placebo microemulsion and ketoprofen formulation in *Figure 12* – we can discuss this in the open panel if you like – and from it we know that the formulation used will make an oil-in-water micro-emulsion. By making measurements of particles size of the microemulsion with and without ketoprofen we obtained clear evidence that the ketoprofen is positioned at the interface and not at the heart of the micro-emulsion. Indeed the particles size distribution increase linearly with the increase of the ketoprofen concentration.

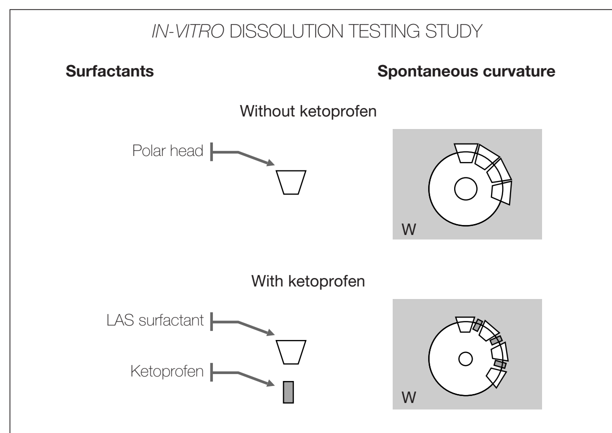


Figure 12.

This was the part that we call the physical factors and characterization.

We still need to answer the biological part of the development. For this we have developed a Caco-2 cell formulation screening. Why? Because it's well-known and its use is well-defined. Maybe it's not the best model today, but it is the standard, and when you are discussing its use with people from other disciplines, they understand what you are talking about. Classically, the main objective is to put the formulation into the culture and see if it can cross the barrier without damage to the system (*Figure 13*).

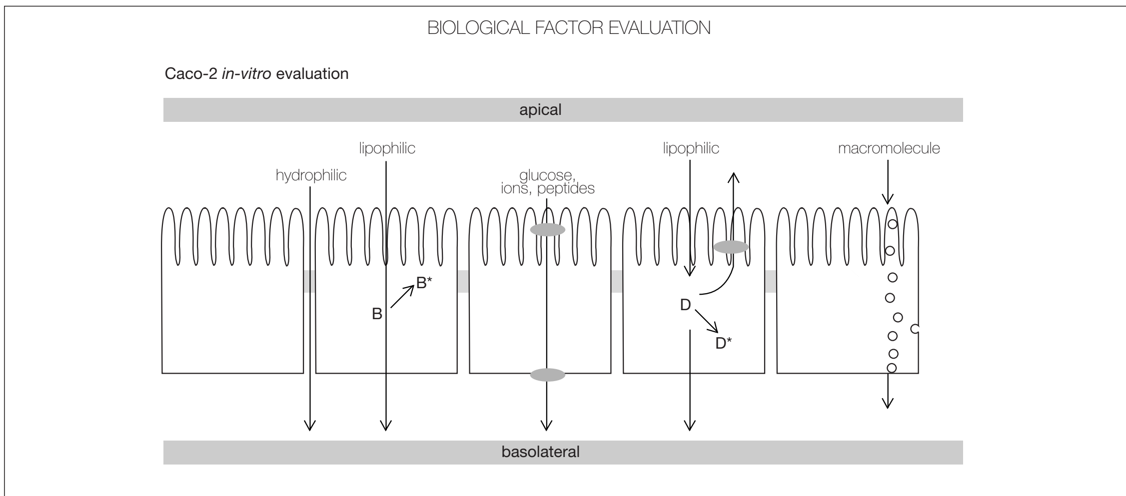


Figure 13.

We have already demonstrated that the product is a Class 2, which means that the solubility is the limiting factor, not the permeability. What we now needed to demonstrate with the formulation, using CACO-2 biological *in-vitro* evaluation, was that the results were in the same area regardless of the approach (*Figure 14*). As reference we used the active solubilised in DMSO, and different formulations: we usually like to compare a liquid and a semi-solid against this reference. The results show that this kind of formulation gives the clear answer that we have enhanced the absorption without making any modification.

So, to finish my talk, from the point of view of pharmaceutical development we really believe that a lipid formulation system can take us from a Class 2 molecule to Class 1. It can be applied to poorly soluble actives, as I have shown, or to the related problems of poorly bioavailable actives, or to difficult-to-formulate products – remember to use the approach with peptides – and to actives that also have some inter-subject or intra-subject variability, as I demonstrated with the Neoral study.

Biological *In-Vitro* evaluation

Test Article	Percent Recovery	Average N=3	Potential
ACTIVE DMSO	17	3.79	High
Lipid form S1	68	1.74	High
Lipid form S2	65	2.14	High
Reference	3	≤ 0.1	Low

Figure 14.

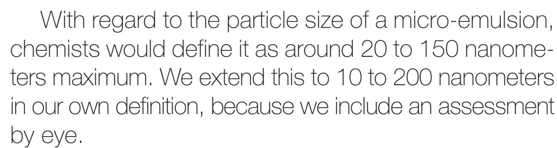
I have reached the end of my talk, and I thank you for your attention.

Chair: Roland Daumesnil, Capsugel Inc., North Carolina, USA: We have some time now for questions. I'm not going to give up… Thank you, sir.

Kakashi, Toyo Capsule Co Ltd., Japan: In our company we deal with many requests to increase the dissolution because we are manufacturers of soft gelatin capsules. I have a basic question. In the definition of a micro-emulsion that you talked about, what type of measurement method is used and what is the particle size?

Dr. Hassan Benameur, Development Center Director, Capsugel R&D Division Colmar France: If I understand correctly, you want the definition of a micro-emulsion and also the size of the droplets. I'll demonstrate this by referring back to Figure 2, the hypothetical phase diagram. If you truly have a micro-emulsion it will only be found in the area indicated in the Figure. So, by definition, a micro-emulsion is a system with a specific composition consisting of a surfactant, co-surfactant and oily phase and water phase. In the case of an oil-in-water micro-emulsion, the continous phase is water with oil inside; with a water-in-oil emulsion you will get a continuous lipophilic phase with water inside, again with surfactant and co-surfactant. So there are always four components.

From your question I can tell where the confusion is coming from. The surfactant/oil axis in the Figure does not represent a micro-emulsion, but a self-emulsifying or a self-micro-emulsifying system. It means that when water is added, you get a more organized system. With a micro-emulsion you always have four components: surfactant, co-surfactant, oily phase and water.



With regard to the particle size of a micro-emulsion, chemists would define it as around 20 to 150 nanometers maximum. We extend this to 10 to 200 nanometers in our own definition, because we include an assessment by eye.

Kakashi, Toyo Capsule Co Ltd., Japan: In that case, what are the conditions of measurement, or the concentration used in measuring the particle size? Could you elaborate on that point? What conditions do you apply?

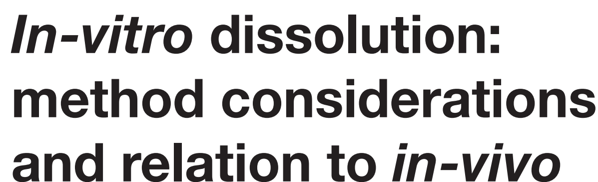
Dr. Hassan Benameur, Development Center Director, Capsugel R&D Division Colmar France: The conditions to measure the particle size are clear. If you are using an oil-in-water micro-emulsion you use the diluability factor that you would use in your dissolution method. It's mainly that you make a 900-milliliter micro-emulsion and you take your fraction and you do the particle size measurement. If you are making a water-in-oil micro-emulsion, this time you have to do the measurement without any dilution and then you have your particle size measurement.

Referring to the Novartis formulation of cyclosporine A , the particles size giving in the figures is obtained after dilution in water for the Sandimune SEDDS and Neoral pre concentrated microemulsion. Indeed, in the pre concentrated microemulsion ethanol is used as hydrophilic phase. So when diluted with water the system stay organized as microemulsion (O/W).

Chair: Roland Daumesnil, Capsugel Inc., North Carolina, USA: I think we have to stop. Thank you, Hassan. I'm sure there will be additional questions during the panel discussion. We are going to have a break now.

In-vitro dissolution: method considerations and relation to *in-vivo*

Professor James POLLI



In-vitro dissolution: method considerations and relation to *in-vivo*

Pr. James Polli

Associate professor, University of Maryland.

Noriko Yamanouchi, Capsugel Japan: Ladies and gentlemen, please be seated. We are resuming the session. We are very pleased to have Professor Hashida of Kyoto University as chair of the session, Invited Lecture II-c. Thank you.

Chair, Professor Mitsuru Hashida, Kyoto University, Japan: This is the last of the Invited Lectures sessions, and we are very pleased to have two speakers. So far, we have talked about formulation and formulation technology and various innovative approaches. The last two speakers will be talking about regulatory issues and the approach to evaluate formulations. They will also be discussing issues about global standards and requirements.

The first speaker in this session is James Polli of the University of Maryland School of Pharmacy, where he is associate professor. Professor Polli acquired a PhD under Dr. Amidon at the University of Michigan and worked with Professor Amidon on oral dosage formulation and evaluation. He also frequently collaborates with FDA and is active in the area of regulatory affairs. He will be sharing with us his expertise in those two areas. In relation to the regulations he will be discussing *in-vivo/in-vitro* correlation. Professor Polli.

Professor James Polli, Associate Professor, University of Maryland School of Pharmacy: Thank you Professor Hashida for that kind introduction. It is a great privilege for me to be here and I appreciate the fact that the organizers have invited me to this great conference. What I would like to speak with you about *in-vitro* dissolution methodology, and also how we can relate *in-vitro* dissolution to *in-vivo* plasma profiles.

I'll talk about dissolution method considerations, and really we'll be talking about three things. First, we'll talk about dose and sink-condition considerations; secondly, we'll talk about a biphasic system dissolution system to provide sink-conditions and, thirdly, we'll discuss product-specific dissolution methods. After speaking about dissolution methods, we'll talk about *in-vitro/in-vivo* correlation methods and two particular IV/IVC methods: one that follows the spirit of the FDA guidance on IV/IVC and, secondly, a deconvolution approach to the IV/IVC form.

Dr. M. Pernarowski was a Canadian pharmaceutical scientist who is credited with discovering the basket dissolution method. Dr. Pernarowski has often commented on the role of dissolution, and I would just like to highlight one particular comment from him. He says: 'A discriminating method for determining the dissolution characteristics is an excellent research tool… Products with poor dissolution characteristics are obviously poor candidates for the market place or for clinical trials…'

Perhaps one of the most interesting things he has to say, in the context of poorly soluble drugs, is: 'Laboratory tricks whose sole purpose is to increase the rate of solution… are not always a guarantee that the formulated product will be biologically available'. I will try to refer back to this comment later in our discussion.

From Dr. Pernarowski's comments on dissolution, it appears there are two purposes of *in-vitro* dissolu-

tion testing. First, dissolution can serve as a quality control function for the manufacturing process and, secondly – perhaps a higher role – dissolution can serve as a surrogate, or a predictor, of *in-vivo* bioequivalence.

To some extent, these can be very differing roles. These two different roles can be assessed by your answer to the following question: should dissolution detect differences in formulation, or should dissolution detect differences in bioavailability? Is it that dissolution should be discriminating with regard to differences in formulation, or do we want dissolution to actually reflect only those changes that manifest in *in-vivo* plasma profiles?

It appears that many misunderstandings in dissolution may have to do with one person's perception of the role of dissolution versus another person's perception. I've been fortunate to speak with many people who have several years of experience in dissolution testing, and here are some observations that I've written down from speaking with them.

The first is that in early product development, there's a tendency for too much confidence in dissolution testing. The belief is that if we measure many physicochemical parameters, perhaps we can use dissolution to tell us whether formulation will be good or poor, even if we've made no effort to relate *in-vitro* dissolution to *in-vivo* product performance. Then during late formulation development, perhaps by being previously burnt or disappointed in early dissolution result, there's a tendency for too little confidence in dissolution. And a third observation is that unfortunately there is no one simple universal dissolution test method for every formulation that may enter your laboratory.

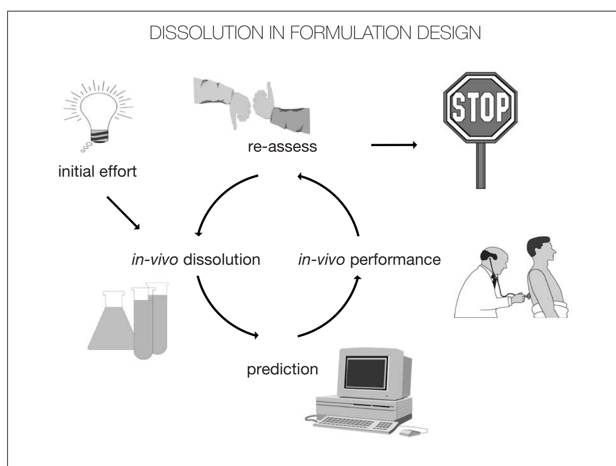


Figure 1.

Product Development Process

stage 1	product concept/ target	**assumed IVIVR**
	prototype	
	pilot PK	**retrospective IVIVR**
stage 2	define formulation	
	IVIVR PK study	**prospective IVIVR**
	IVIVR defined	
	process optimization	
stage 3	scale-up/pivitol PK/reg	
stage 4	SUPAC	

from J. Devane and J. Butler

Figure 2.

This process is something you think about in your laboratory: the role of dissolution and the design of an *in-vitro* dissolution test, based upon drug solubility and the formulation, including its excipients. From these considerations, we start off with an initial idea as to what the dissolution system should be. If we decide that dissolution should serve as a surrogate for *in-vivo*, then *in-vitro* dissolution testing should aim to mimic *in-vivo* dissolution (*Figure 1*). Then, based upon underlying biopharmaceutical properties, we can perhaps scale *in-vivo* dissolution – at least in our minds – to how it will perform *in-vivo*.

Hopefully, we will have the opportunity to test the product *in-vivo* and then we assess whether the dissolution test is performing as we hoped it would. And, of course, we actually need to go through this process many times before we have a good understanding of how *in-vitro* dissolution can actually mimic *in-vivo* dissolution and hence be a surrogate for *in-vivo* absorption.

The same schematic is represented in *Figure 2*, as a time-line of the product development process. There are various stages in product development – an early stage, a middle stage and a late stage. Early in product development, we have to estimate what the role of dissolution will be in product performance.

After testing the early-phase product in early PK studies, we learn something and typically revise our understanding of how the formulation is performing *in-vivo*, and hence how we should tweak or change our dissolution test method in order to serve as a better test. So there's a natural course of events where we have to change our understanding of how we can perform dissolution in the best fashion.

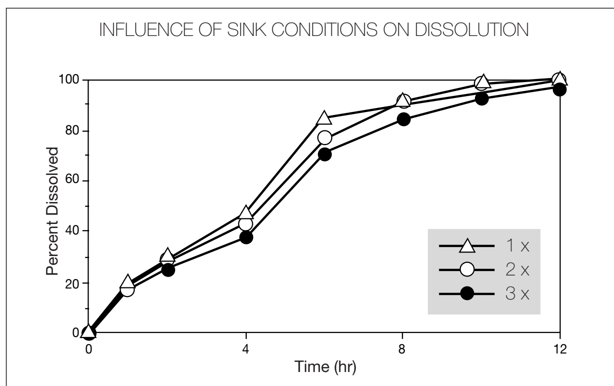


Figure 3.

What are the favorable properties of a dissolution test? Dissolution should be analytically and experimentally simple, since there's a lot of analysis to do. Arguably, one does *in-vitro* dissolution with the purpose of mimicking *in-vivo* dissolution. Obviously, dissolution is not a permeability test. By itself, it's not a test that's able to predict bioavailability; that would require additional information. But *in-vitro* dissolution provides the opportunity to mimic *in-vivo* dissolution and, perhaps most importantly, assess for factors that may influence *in-vivo* dissolution. If there are formulation factors that are important *in-vivo*, we would like our *in-vitro* test to be sensitive to those factors.

There are a couple of issues with regard to dissolution testing. For instance, if we're trying to mimic *in-vivo* dissolution, what if a drug does not dissolve completely? There is the classic example of griseofulvin. Griseofulvin's absorption is incomplete because of incomplete dissolution due to low solubility. An estimate is that the bioavailability of griseofulvin is about 30 or 40 percent, due to incomplete dissolution of griseofulvin *in-vivo*. So the natural question is, what should be the extent of dissolution in the *in-vitro* test for griseofulvin? Should it be 30 percent or 40 percent? We typically assumption that *in-vitro* dissolution should go to completion, even if *in-vivo* dissolution is not complete. This is an outstanding issue.

Similarly, we frequently design the dissolution test to provide sink conditions. This is most problematic for poorly soluble drugs. To attain complete dissolution of a poorly soluble drug, one frequently adds a solubilizing agent – for example, surfactant – and unfortunately we know that surfactant can decrease the sensitivity of the dissolution test.

Another issue with regard to dissolution is that we want everything to be the same across dosage strengths. There could be a 10-fold range in dose strength, including poorly soluble drugs. Yet we have this historical tendency to prefer the same test method and the same specifications, even though we know that dose is an important fundamental barrier to complete dissolution, especially for poorly soluble drugs.

Figure 3 is an example where we have three different products. It's really the same product, a poorly soluble drug, at three different dose levels: level 1X; an intermediate level, 2X, where there's twice as much drug in the dosage form, and also a three-fold dose level, 3X. This is a multi-particulate product where particulates are encapsulated, using a capsule, and that way there are three dose levels available.

The 3X dose is the slowest-dissolving. Next comes the intermediate, that is, the 2X dose The most rapidly dissolving dosage form is the lowest dose. These are intended to be extended-release products so, for an extended-release product, these variations are really significant, particularly considering that the only difference between these products is really just the number of particulates.

Why does dose slow dissolution? Based upon the solubility of the drug and our test conditions, using 900 ml, the two highest doses, the 2X dose and 3X dose, do not provide sink conditions. Meanwhile, the 1X dose provides sink conditions. I think we are all familiar with the basic dissolution equation in *Figure 4*, where dissolution is a function of the solubility of the drug, and also it considers the amount of drug that's already in solution. So, by applying that model we can perhaps determine what's going on.

We took the data for the 2X product and the 3X product and apply the dissolution model to fit the data. *Figure 5* is the observed data for the 3X product and the fitted profile. *Figure 6* is the observed data for the 2X product and the fitted profile. We're happy with the fits.

Influence of Sink Conditions on Dissolution

Dose	Sink Conditions ?
1 X	Yes
2 X	No
3 X	No

$$dM/dt = \frac{AD}{h}(C_s - C)$$

Figure 4.

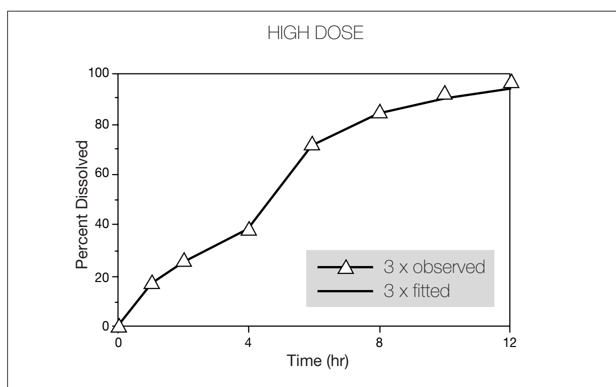


Figure 5.

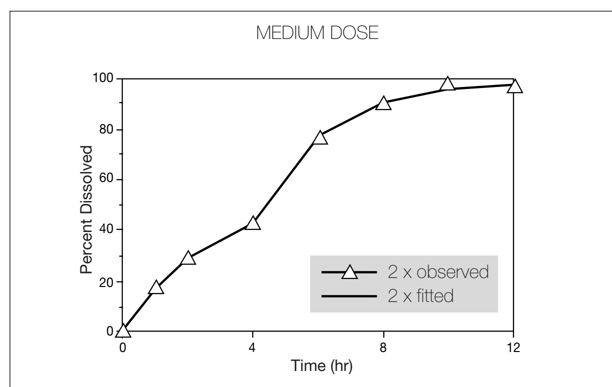


Figure 6.

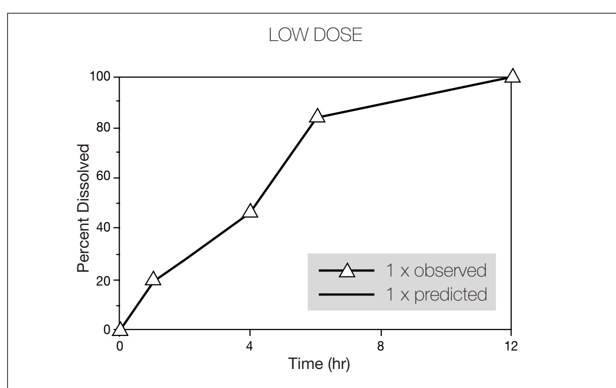


Figure 7.

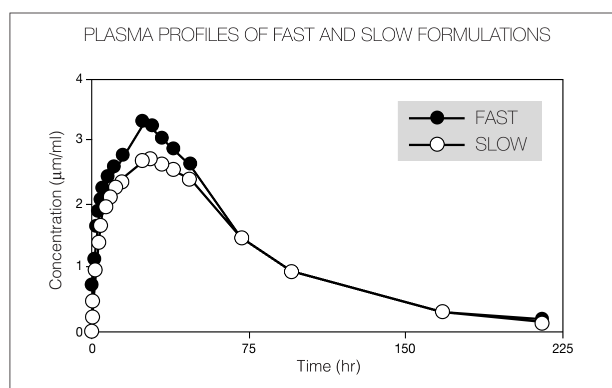


Figure 8.

Then we asked, what would be the predicted dissolution profile for the low-dose product? The 1X product did not have problems with sink conditions. Using the model based upon the 2X and the 3X dose, what we predicted is the dissolution profile in *Figure 7*, which pretty much matches the observed profile. These results suggest that the lower doses dissolve quicker since they are not limited by dissolve drug.

And so the question we come back to is, what should we do? Since the formulations are essentially identical but only differ in the amount of drug, it would be reasonable to expect the lower-dose products to actually dissolve quicker, and allow specifications that follow that observation.

Figure 8 is another example. There is a fast dissolution formulation version of a product, where the particle size is relatively small. There's another formulation with larger drug particle size. We'll call them a fast formulation and a slow formulation. There plasma profiles are reproducible, with the fast formulation yielding a higher C-max. The fast formulation dissolves more rapidly than the slow product, although the difference is modest. So what was needed here was a highly sensitive dissolution method to discriminate between these somewhat similar but reproducibly bio-inequivalent products.

Given Dr. Pernarowski's comments with regard to the overuse of surfactants in dissolution testing, we designed a biphasic dissolution system (*Figure 9*), where we had 800 ml of buffer along with 100 ml of octanol, which functioned as a sink. The octanol layer negated the need for surfactant, yet provided sink conditions for this poorly soluble drug.

We first wanted to understand the basic performance of this biphasic system. At time zero, drug solution was added to the buffer, and we monitored drug distribution between the phases. Over a short time, approximately an hour or two, about 15 percent of the drug remained in the buffer. Meanwhile, the rest of the drug partitioned into the octanol. *Figure 10* shows disposition kinetics of drug within the biphasic system.

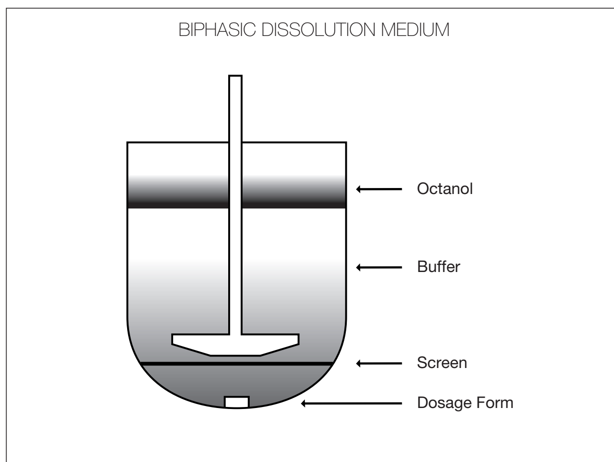


Figure 9.

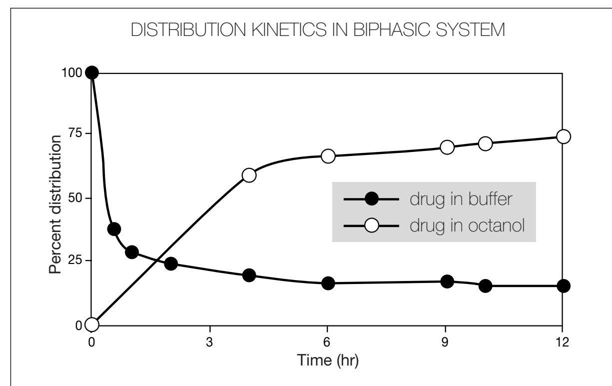


Figure 10.

Since we anticipated that the differences between the fast and the slow formulations were due to particle size, we assessed if the system was sensitive to particle-size. In evaluating fine particles and coarse particles, *Figure 11* shows their percent dissolved versus the time profile in the biphasic system. We were happy with this result.

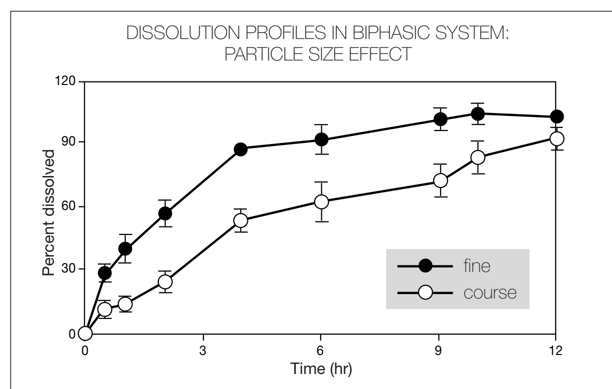


Figure 11.

To assure that these results were not an artifact of the biphasic system, we essentially repeated the study in buffer only, using a small amount of drug, a small amount of fine particles and also a small amount of coarse particles, such that sink conditions were not a problem. Buffer was able to discriminate the fine particles from the coarse particles (*Figure 12*). We felt this biphasic system had some chance, although the use of octanol presents an environmental challenge.

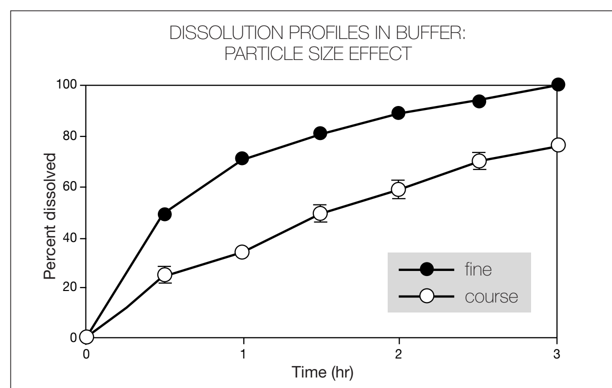


Figure 12.

Figure 13 shows the dissolution profile of the fast extended-release formulation in the biphasic system. We monitored the amount of drug in the buffer. We also monitored the amount of drug in the octanol phase,. We added them together. *Figure 13* illustrates the cumulative dissolution profile of the fast formulation. Very interestingly, we always saw about the same constant level of drug in the buffer phase, regardless of how much drug had been dissolved up to any particular time.

Figure 14 is the dissolution profile from the slow formulation in the biphasic system, showing drug in buffer, drug in the octanol phase and the total drug-release profile.

Then we compared fast and slow (*Figure 15*). The fast formulation, which showed a slight but significantly higher C-max than the slow formulation *in-vivo*, actually did perform as a fast formulation in this *in-vitro* test. A surfactant-based system was not able to discriminate between the two formulations.

Just one last comment with regard to dissolution methods. Perhaps it's fair to say that dissolution is product-specific. This would be one point of view.

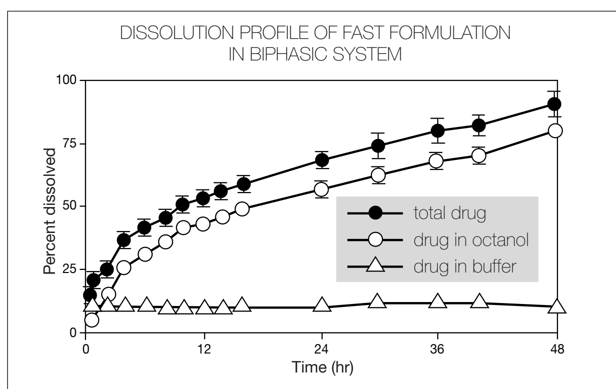


Figure 13.

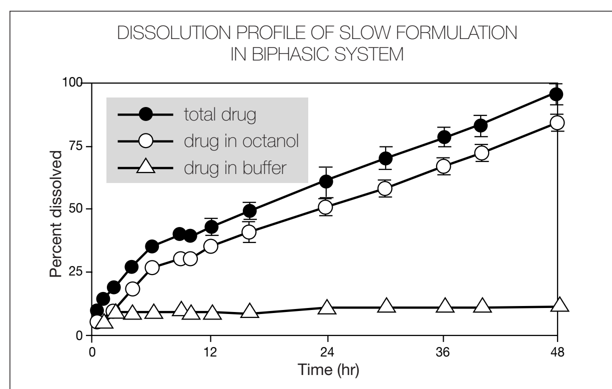


Figure 14.

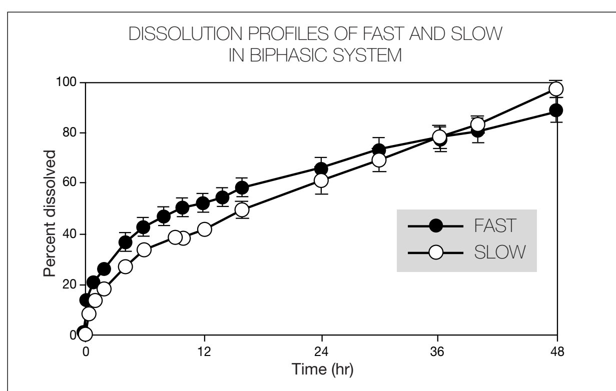


Figure 15.

Since the relevance of the dissolution method depends upon the drug substance as well as the actual drug product itself (for example, the release mechanism), dissolution testing will generally be considered to be drug-product specific. The experimental evidence for such a broad statement is that unfortunately we still have never found a universal dissolution test that is applicable to a wide range of dosage forms.

And the implication of the idea that dissolution is product-specific is that perhaps it would be best to do some dissolution early on – maybe not too much, but focus on getting some *in-vivo* data as early as possible. This approach will allow for an early evaluation of what dissolution is able to tell us about *in-vivo*.

To further give evidence for the notion of product-specific dissolution, there are many marketed versions of theophylline extended-release capsules. The United States Pharmacopeia (USP) accepts 10 different dissolution tests. These 10 tests have eight different dissolution media. They range from simulated gastric fluid (SGF) for one hour, then pH 6 phosphate; through pH 3 phosphate for 3_ hours, then pH 7.5 phosphate; to pH 4.5 phosphate with octoxynol 9; and pH 7.5 simulated intestinal fluid (SIF), without enzyme. These 10 different dissolution tests have 10 different dissolution criteria, 10 different sample times and acceptance intervals.

One dramatic aspect is that two of the formulations have the same test method and media, so everything's experimentally identical. Clinically, they are also identical in that they are both intended to be given every 12 hours. So everything is similar about these products in terms of their dissolution method, dissolution media and clinical utilization.

However, they differ dramatically in their specifications. Specifications for one product allow 85 to 115 percent at five hours, while the other product is much slower-dissolving and is allowed 50 to 80 percent at five hours. Clearly, these two products are different. Even though they perform the same *in-vivo*, they perform very differently in *in-vitro* tests, but meanwhile are still safe and effective products.

So those were some comments with regard to dissolution methodology.

What I would like to talk about next is *in-vitro/in-vivo* correlation. In particular, we will talk about an important document that the FDA issues. In 1997, the FDA issued its *in-vitro/in-vivo* correlation guidance, which is applicable to both new drugs and generic drugs.

The guidance describes what data is necessary for an IV/IVC, and formulation requirements. It discusses predictability evaluation – the IV/IVC has to show predictability – and the guidance says there are two areas of application. First, an IV/IVC will allow for biowaivers; that is, waivers of *in-vivo* bioequivalence studies. Also, an IV/IVC will allow one to justify dissolution specifications.

Predictability evaluation

- **internal predictability vs. external predictability**
  - dependent on amount of data, therapeutic index, range of release rates studied

$$\% \, PE = \frac{\text{observed value} - \text{predicted value}}{\text{observed value}} \bullet 100\%$$

Figure 16.

So, briefly, an overview of IV/IVC in the context of this guidance. It says that one can take *in-vitro* dissolution release, couple it with a model for IV/IVC which essentially is a pharmacokinetic model, and predict *in-vivo* plasma profiles. If one is able to do that with accuracy, one is then allowed to use dissolution as a replacement for *in-vivo* bioavailability studies.

The term *in-vitro/in-vivo* correlation refers to a predictive mathematical model describing the relationship between an *in-vitro* property of an extended-release dosage form, and also a relevant *in-vivo* response.

This IV/IVC guidance talks about various levels of IV/IVC. There's an FDA Level A correlation definition, based on convolution. I would just caution you that the Level A definition in the FDA guidance is extremely different from the USP Level A definition. For the FDA, they say a Level A correlation is a predictive mathematical model for the relationship between the entire *in-vitro* dissolution profile and the entire *in-vivo* response profile, that is the plasma profile. So it has to be a predictive model that maps all dissolution data to all PK plasma data.

The guidance addresses methodology as far as data formulation requirements are concerned. As I read it, essentially three formulations are needed, and these formulations need to be different. The scope of this guidance has to do with trying to identify the important formulation variables, so there needs to be some effort to vary the important formulation variables, and they presumably will have different *in-vitro* release profiles.

There are dissolution data requirements. Formulations have to employ the same dissolution system, which preferably does not have a pH greater than 6.8. There has to be a sample size of at least 12 and there needs to be a fair difference, at least 10 percent, between the fast, medium and slow formulations. Plasma data is needed in humans and, preferably, it would be advantageous to have crossover PK data, and a reference dosage form.

An important component is predictability evaluation (*Figure 16*). The IV/IVC has to demonstrate that it can predict plasma profiles. There are two terms described: internal predictability and external predictability. There are circumstances when one can do internal predictability; for example, if your drug is a broad therapeutic index drug. If your drug is a narrow therapeutic index drug, then you need to do external predictability.

Internal predictability concerns demonstrating that you can adequately fit the data used in your IV/IVC model building. External predictability is more challenging, in that you need to show that you can predict a formulation PK profile, when that formulation was not used to build the IV/IVC model. The measure that is used to assess prediction is the percent prediction error, and that's the observed value minus the predicted value over the observed value, so it's a percent.

Internal predictability is the only requirement if you have a drug with a wide therapeutic index, provided three formulations are studied. If only two formulations are studied, a limited benefit is given without external predictability. As far as the specifications are concerned, if you have three formulations – fast, medium and slow – the average predicted error has to be less than 10 percent across all three formulations for the C-max as well as the AUC. Additionally, the prediction error for each individual formulation must be less than 15 percent.

If the drug possesses a narrow therapeutic index, if internal predictions are inconclusive, or if only two different formulations were studied in IV/IVC development, you need to subject your IV/IVC to external predictability. It's preferred that the formulation subjected to external predictability is one that has a different release rate than the formulas that contributed towards building the model in the first place. It may or may not

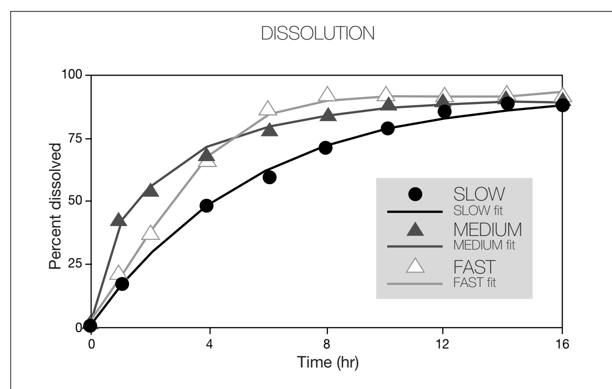


Figure 17.

be acceptable if the formulation for external predictability analysis has the same release but different manufacturing aspects, or is the same product but from a different lot.

The passing criterion is 10 percent. You are allowed a 10 percent prediction error for the C-max and AUC in order to demonstrate external predictability. A prediction evaluation of between 10 and 20 per cent is inconclusive, and more data is needed. A result greater than 20 percent fails.

Figure 17 is an example of IV/IVC. Following the FDA guidance, there are three formulations: slow, medium and fast. *Figure 18* shows the plasma profiles of these slow, medium and fast formulations. The goal is to take the dissolution data and come up with a pharmacokinetic model, an IV/IVC model, that fits the plasma profiles.

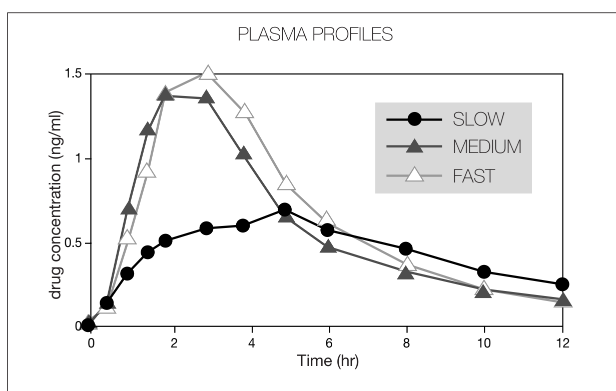


Figure 18.

Figure 19 gives the pharmacokinetic metrics, the C-max and AUC. Since the slow and fast formulations are used to build an IV/IVC model, we'll perform internal predictability analysis on those two formulations. The third formulation in the middle, the medium formulation, will be use to evaluate external predictability. Hopefully the slow and fast will fit, and if so, we'll see if the model can predict the medium formulation.

Predictability evaluation

Formulation	Cmax (ng/mL)	AUC_{48hr} (ng hr/mL)
SLOW	0.680	8.61
MEDIUM	1.38	9.27
FAST	1.50	9.46

Figure 19.

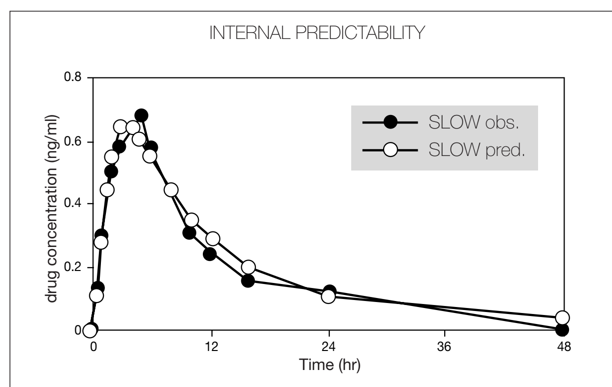


Figure 20.

The slow formulation was one of our two formulations used to build the model. *Figure 20* shows the slow observed plasma profiles, and the model was able to match the profile. The other formulation that was used to build the IV/IVC was the fast formulation (*Figure 21*). Again, we have observed data and the IV/IVC is trying to match the observed data.

We need to calculate prediction errors. We have observed C-max values for our two formulations, and we have predicted C-max values from the IV/IVC. We can determine the C-max prediction error: what is the relative difference between our observed and predicted values? These values are each less than 10 percent, so it passes (*Figure 22*).

We calculated the internal predictability AUC for the two formulations that went into the model-building, slow and fast (*Figure 23*). Again, we are looking at the percent prediction error. They are both under 10 percent, so it passes the acceptance criteria.

Since the model was able to pass internal predictability for the two formulations, we then took the third formulation and put its dissolution profile into the IV/IVC model, yielding the predicted profile in *Figure 24*.

Figure 25 provides the percent prediction errors. Errors are unusually low. In this regard, this not a typical example, since results are better than normal, that's for sure. However, these results exemplify an approach to carry out the FDA guidance on IV/IVC, which is a convolution approach emphasizing plasma profiles.

There is a second way to do an IV/IVC. Early in product development, we're interested in drug absorption per se, rather than plasma profiles, since a lot of our laboratory testing is focused on drug absorption itself. With an interest in drug absorption, de-

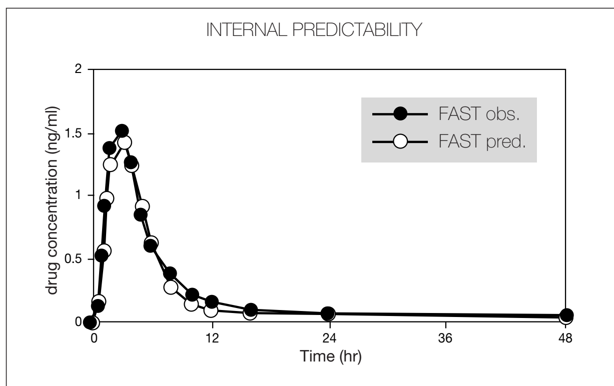


Figure 21.

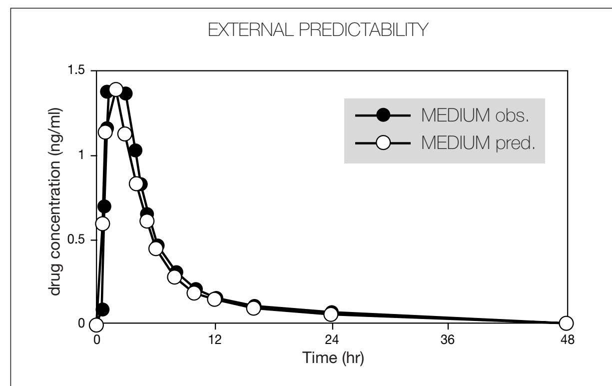


Figure 24.

Internal Predictability: Cmax

Formulation	Observed Cmax (ng/ml)	Predicted Cmax (ng/ml)	%PE
SLOW	0.680	0.643	5.4
FAST	1.50	1.43	4.7

Figure 22.

Internal Predictability: AUC

Formulation	Observed AUC_{48hr} (ng hr/mL)	Predicted AUC_{48hr} (ng hr/mL)	%PE
SLOW	8.61	9.20	– 6.9
FAST	9.46	9.17	3.1

Figure 23.

convolution approach to IV/IVC is an approach to emphasize.

This deconvolution approach is built on a very simple model for oral absorption. There is a dissolution step and a permeation step. From this simple model, we derive an equation where fraction absorbed is a function of the fraction dissolved (*Figure 26*). As more drug dissolves, more drug is absorbed.

However, the nature of the relationship between absorption and dissolution is affected by a couple of parameters. In particular, this relationship is modulated by the term 'alpha'. Alpha is simply a ratio of the two rate constants (permeation and dissolution). In this two-step model, we have a rate constant for permeability as well as a rate constant for dissolution.

External Predictability

Metric	Observed	Predicted	%PE
Cmax (ng/ml)	1.38	1.40	– 1.4
AUC_{48hr} (ng hr/mL)	9.27	9.19	0.8

Figure 25.

Model

- F_a is the fraction of the total amount of drug absorbed at time t,
- f_a is the fraction of the dose absorbed at t = infinity,
- alpha is the ratio of the first-order apparent permeation rate coefficient (k_p^{app}) to the first-order dissolution rate coefficient (k_d), and
- F_d is the fraction of drug dose dissolved at time t.

Polli, J.E., Crison, J.R., and Amidon, G.L. (1996): A novel approach to the analysis of in-vitro-in-vivo relationships. J. Pharm. Sci. 85:753-760

$$F_a = \frac{1}{f_a}\left(1 - \frac{\alpha}{\alpha - 1}(1 - F_d) + \frac{1}{\alpha - 1}(1 - F_d)^{\alpha}\right)$$

Figure 26.

Large alpha values indicate permeability is much faster than dissolution, which is to say that dissolution is rate-limiting (*Figure 27*). At the other extreme, very small alpha values indicate permeability is very slow compared to dissolution, so that would be a permeation rate-limited absorption product. We can take the equation and vary alpha, in a simulation-wise fashion.

Alpha

$$\alpha = \frac{k_p^{app}}{k_d}$$

- **large alpha**: dissolution rate-limited absorption
- **small alpha**: permeation rate-limited absorption
- **alpha = 1**: mixed rate-limited absorption

Figure 27.

Figure 28 plots the family of curves that result from the deconvolution IV/IVC equation. Since this model encompasses the classic USP Level A model, the upper line or curve may look very familiar to you. This line of unity is the classic USP Level A IV/IVC. A straight-line relationship is obtained only when alpha is very large. The only time we see this linear relationship between absorption and dissolution is when dissolution is rate-limiting. As the drug is dissolving, it's being immediately absorbed, because the permeability is several-fold faster than dissolution.

I suggest that scientists have been frequently disappointed with *in-vitro/in-vivo* correlation, particularly for immediate-release products, in part since an incorrect model is applied. The classic model has been the USP model. Unfortunately, the USP model Level A is only useful for products that are dissolution rate-limited. Many products, particularly immediate-release products, may not be dissolution rate-limited.

I would like to thank several graduate students at the University of Maryland, and my collaborators in

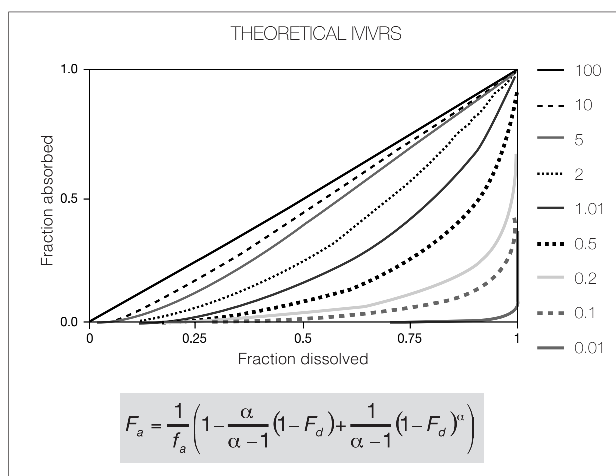


Figure 28.

dissolution, Dr. Shah and Lawrence Yu at the FDA. Most importantly, I appreciate being here with you and being invited. Shinji and Roland, thank you very much for inviting me. I appreciate your hospitality. Thank you.

Chair, Professor Mitsuru Hashida, Kyoto University, Japan: Thank you very much, Professor Polli. In the first half of his presentation he gave useful general considerations on dissolution methodologies, and in the second part he talked about *in-vitro/in-vitro* correlation and examined several perspectives in explaining a unique concept of the correlation. Thank you very much. Since it is a very rare opportunity, I would like to open the floor for discussion. Yes?

Hamaura, Sankyo Pharmaceuticals: Professor Polli, thank you very much for the insightful discussion. I have two questions. The first is about the definition of sink conditions. The definition of sink conditions may vary, so what is your definition of sink conditions when you describe them in your biophasic system?

Professor James Polli, Associate Professor, University of Maryland School of Pharmacy: I am under the impression that there are at least two definitions. I think most pharmaceutical scientists say it's a factor of 10, minimally. If the concentration of solution reaches one-tenth that of the solubility (or more), then you no longer have sink conditions. I believe the British Pharmacopoeia uses a value of three, or one-third, and here I use the one-third value. When the concentration of the dissolving drug exceeded one-third of the drug's solubility, then it was deemed to be non-sink.

Hamaura, Sankyo Pharmaceuticals: Thank you very much. I have a second question. In your talk, you mentioned that IV/IVC is difficult to undertake for an immediate-release formulation. In *Figure 2*, where you showed the IV/IV relationship with PK studies, the PK study was mentioned as part of an IV/IVC. Well, if there's no problem in formulation then the formulation may not be changed from Phase 1 to the end. And yet we are expected to change the formulation, and we have to see the PK in humans to look at the IV/IVC. Do we waive that study or not?

Professor James Polli, Associate Professor, University of Maryland School of Pharmacy: Your question has to do with when should one do an IV/IVC? I think I understand your question, and I hope I don't put regulators on the spot, but broadly speaking it seems as if we have things backwards. It's interesting that the FDA has an IV/IVC guidance on extended-release formulations.

You say you have had the fortunate opportunity to have formulations that are not very sensitive to formulation changes. Arguably, that's a very good position to be in. If one had to give a biowaiver based upon dissolution, wouldn't it make sense to do it for formulations that represent low risk?

Meanwhile, we have an IV/IVC guidance for relatively high-risk products, extended-release products. While IV/IVCs for extended-release products can be very appropriate, it would appear unusual that immediate-release products are not provided a biowaiver option via IV/IVCs since immediate-release products are intrinsically lower risk products, compared to extended-release products. Maybe we have arrived at this circumstance since whenever we've subjected immediate-release products to IV/IVC, we always get bioequivalence.

There was a large collaborative study of immediate release formulations performed at the University of Maryland. This was headed by Larry Augsburger and the FDA, and in all cases there was bioequivalence between a fast, medium and slow formulation, in spite of real formulation differences. At some level, such results are disappointing, since there are differences *in-vitro* but they're all the same *in-vivo*. But should we really be disappointed in that? Many immediate-release products are water soluble, and I suggest that their dissolution kinetics are not overtly important. Kinetically, dissolution is often rapid, such that you can change dissolution and yet it does not have a big impact *in-vivo*. In that sense, it's good not to have a correlation between dissolution and bio.

Hamaura, Sankyo Pharmaceuticals: If I may take a different perspective… From the QC perspective, quality control, of course the specification of the dissolution has to be determined, and there, the formulation may be changed. Do you believe that the PK study is needed, to determine the PK in the specification?

Professor James Polli, Associate Professor, University of Maryland School of Pharmacy: It's my opinion that, for may products, we frequently don't have a good understanding of the role of dissolution in absorption kinetics. I do think that dissolution is very quick for some products, such that slowing dissolution has no ill effect.

The analogy that comes to my mind is that in the US there are two very wealthy people. There's Bill Gates and the financier Warren Buffett from Nebraska. Bill Gates is twice as rich as Warren Buffett. But as far as I'm concerned, they're both very wealthy.

At some level we can't simply concern ourselves with the fact that there's change, but we need to be more sensitive to the importance of that change. If a product is not dissolution rate-limited and dissolution is slowed, that change certainly may not matter. I think that because we often want dissolution to do everything all the time, we're excluding other important considerations, such as the role of gastric emptying and intestinal permeability kinetics.

Chair, Professor Mitsuru Hashida, Kyoto University, Japan: Thank you very much. Can Professor Sugiyama please be quick?

Professor Yuichi Sugiyama, University of Tokyo: I'm not so familiar with this field, so I'm sorry to ask such a very, very basic question. You mentioned that when people are interested in drug absorption they use the deconvolution method, and when they are interested in overall pharmacokinetics they use the convolution method. That's what I do not necessarily understand because I am not a specialist in this field, although I know pharmacokinetics very well. So, when you use the deconvolution method to assess drug absorption, what is the rate function, what is the output function? And when you apply the convolution method, what is the input function, what is the rate function? That's my very, very basic question.

Professor James Polli, Associate Professor, University of Maryland School of Pharmacy: I think I understand the nature of your question and I would agree with you that both of those procedures are more similar than they are different.

Professor Yuichi Sugiyama, University of Tokyo: Yes, yes, convolution is just the mirror…

Professor James Polli, Associate Professor, University of Maryland School of Pharmacy: But I will say this. Sometimes, in the laboratory, we're generating dissolution data. We're also generating permeability data, and we're interested in drug absorption. We are less concerned with distribution and elimination. We want to learn about how dissolution is affecting drug absorption, how permeability is affecting drug absorption. So our emphasis is really on drug absorption.

Meanwhile, perhaps very much later in drug development, maybe at the registration stage, we play a game. We want to match plasma profiles; I want AUC and C-max to be the same. That's a different circumstance than maybe early on in product development where we're collecting permeability data and dissolution solubility data. However, the underlying pharma-

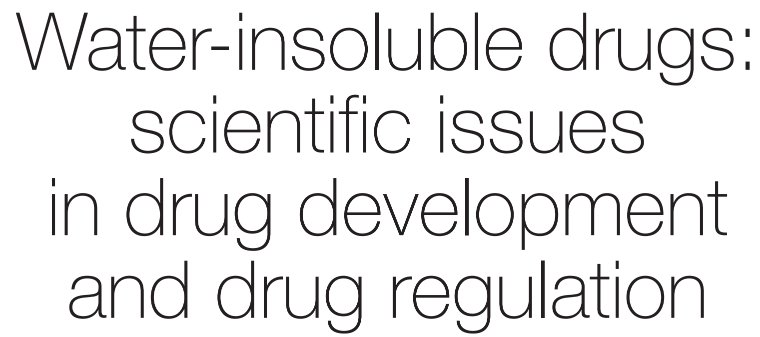
cokinetic methods are essentially the same. But they emphasize two different things.

Professor Yuichi Sugiyama, University of Tokyo: I don't necessarily understand what you said, but I can discuss the data with you. What I want to know is, what is the input function, and what is the rate function for the convolution method? And, for the deconvolution method, what is the output function and what is the rate function? That is my question. But I can talk to you later.

Professor James Polli, Associate Professor, University of Maryland School of Pharmacy: Thank you. We could have a whole symposium on IV/IVC methods…

Chair, Professor Mitsuru Hashida, Kyoto University, Japan: Thank you very much. I'd like to close the lecture now. Thank you, Professor Polli.

The next speaker is Dr. Lawrence Yu. Let me introduce Dr. Yu. His details are included in your handout. Currently, he works as Director for Science in the Office of Generic Drugs at CDER, part of the FDA. There are a lot of very active people working in the Office of Generic Drugs and Dr. Yu is a leader of that Office. Recently, his name has been frequently cited in various society meetings, or we often hear from him directly at these meetings. He will be talking about Water-insoluble Drugs: scientific issues in drug development and drug regulation.



Water-insoluble drugs: scientific issues in drug development and drug regulation

Dr. Lawrence YU

Water-insoluble drugs: scientific issues in drug development and drug regulation

Dr. Lawrence Yu

Director for Science, Office of Generic Drugs, CDER, Food and Drug Administration, USA.

Dr. Lawrence Yu, Director for Science, Office of Generic Drugs, CDER, Food and Drug Administration, USA: Thank you. Good afternoon, everyone. Thank you, Dr. Hashida, for your kind introduction. I'd also like to thank the organizers for inviting me. I want to thank Capsugel for your continued effort in developing formulation science. It is my pleasure and privilege to be here to give a talk entitled, Water-insoluble drugs: scientific issues in drug development and regulatory evaluation. Since I am from the FDA I am required to provide a disclaimer: 'Opinions expressed in this presentation are those of the speakers and do not necessarily reflect the view or policies of the US FDA'. What this specifically means is that neither the FDA nor myself are responsible for what I am going to say for the next 45 minutes!

What Is the BCS?

- The BCS is a scientific framework for classifying drugs based on their aqueous solubility and intestinal permeability.

Biopharmaceutics Class	Solubility	Permeability
I	High	High
II	Low	High
III	High	Low
IV	Low	Low

Figure 1.

My talk will cover four aspects, namely: the Biopharmaceutical Classification System (BCS): the scientific basis for biowaiver or biowaiver extensions; limits to oral drug absorption, to understand what causes poor oral drug absorption; examples of the delivery of poorly water-soluble drugs and, finally, a very brief discussion of the challenges to regulatory evaluation.

Let's start with the BCS scientific basis for biowaiver or biowaiver extensions (*Figure 1*). What is the BCS? The BCS is a scientific framework for classifying drugs, based on their aqueous solubility and intestinal permeability. Basically, there are two parameters, solubility and permeability. Each parameter has two levels, high and low. The combination of two parameters and two levels creates four Classes, namely Classes I, II, III and IV. Class I is high solubility/high permeability, Class II is low solubility/high permeability, Class III is high solubility/low permeability and, finally, Class IV is low solubility and low permeability.

In August 2000 the FDA issued a guidance entitled, Waiver of *in-vivo* Bioavailability and Bioequivalence Studies for Immediate-release Solid Oral Dosage Forms, Based on a Biopharmaceutical Classification System, or BCS. Please note that the waiver refers to the studies, not the requirements of bioequivalence or bioavailability; it simply is a waiver of *in-vivo* bioavailability/bioequivalence studies. What has this guidance specifically discussed? If the solid oral dosage form meets certain criteria, *in-vivo* bioavailablity or bioequivalence studies can be waived (*Figure 2*). First, rapid dissolution; namely, if more than

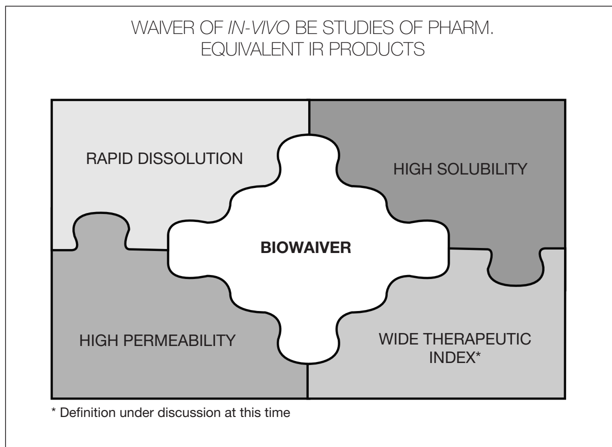


Figure 2.

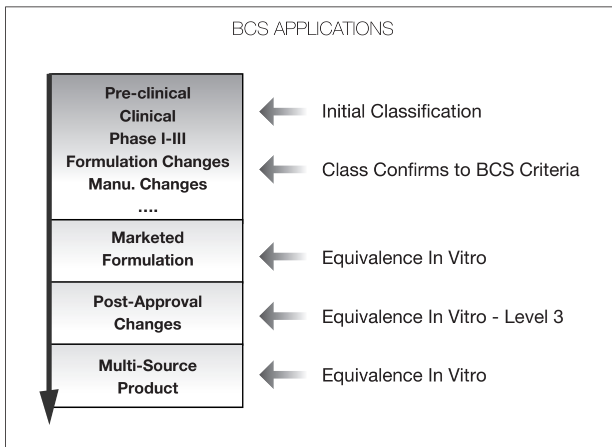


Figure 3.

85 percent dissolves in 30 minutes at the three dissolution media, pH 1.2, pH 4.5 and pH 6.8. Second, high solubility; namely, that the highest strength of the drug dissolves in less than 250 ml aqueous media in a pH range of 1 to 7.5. Third, high permeability; namely that the drug's extent for intestinal absorption is equal to or more than 90 percent. Finally, the FDA always uses a risk management approach. As a result, only drugs with a wide therapeutic index are included. Although at this point we have not reached a consensus on the definition of a wide therapeutic window drug, we are still investigating. So this is the guidance.

Where or when can it be used? It can be used anywhere, from pre-clinical to Phase IV and in multi-source drugs or generic drug applications (*Figure 3*). The BCS has received tremendous support from the scientific community, especially from the FDA Advisory Committee for Pharmaceutical Science, other experts, FDA staff and public workshops. We've run at least four or five workshops on this topic.

Concerns expressed at these public workshops, along with comments on the BCS guidance, suggest that the approach is overly conservative. As you can see, this is an initial step towards biowaivers, so it's natural to be conservative. Therefore we are making a continued effort to relax the solubility class boundary conditions, or maybe extend biowaivers to BCS Class III drugs, namely, high solubility/poor permeability drugs (*Figure 4*). I can hear you asking why is that? Why not a Class II? Why not a Class IV, instead of Class III?

At this point we want to examine Class III drugs (*Figure 5*). Let's review the scientific basis for biowaiver extension for Class III drugs. The oral absorption of Class III drugs is likely limited by their permeability, and less dependent upon the formulation. If dosed in rapidly dissolving solid oral dosage forms,

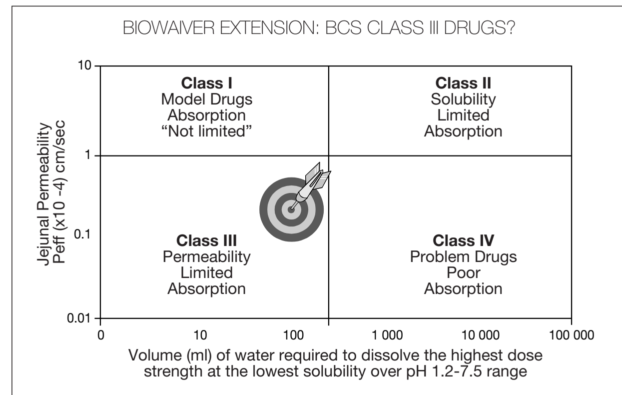


Figure 4.

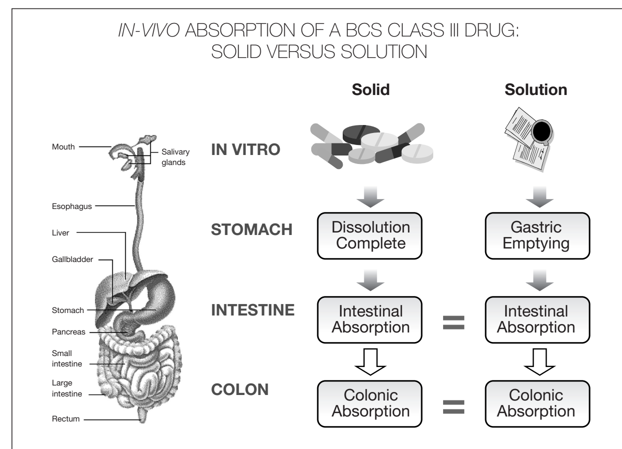


Figure 5.

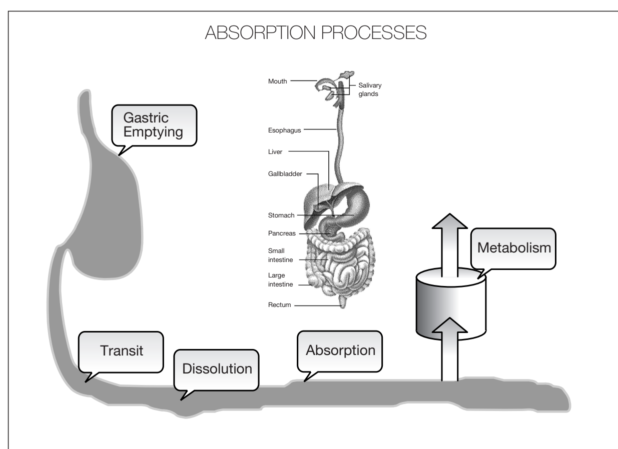


Figure 6.

the dissolution can be complete in the stomach. Thus, whether it's a tablet or a capsule, once it enters the stomach, dissolution essentially takes place there. But a solution will simply go through mixing in the stomach. What left the stomach is a drug solution whether it is dosed in solid dosage forms or in solutions. Since absorption mainly occurs in the small intestine, if excipients have no effect on absorption or GI motility, intestinal absorption is expected to be the same, whether it is dosed from a solid oral dosage form or a solution. Therefore there's no scientific reason to believe that rapidly dissolving immediately release products with highly soluble/poorly permeable drugs could show bio-inequivalence, unless excipients utilized in the solid dosage forms strongly affect the permeability or GI motility.

So we have the following hypothesis for biowaiver extension for Class III drugs. If two solid immediate-release dosage forms of BCS Class III drugs dissolve rapidly at all physiologically relevant conditions and contain no excipients that may affect oral drug absorption, then the bioequivalence of these two solid oral dosage forms is assured. Therefore we believe that, scientifically, a biowaiver can be granted. At this time we are still collecting data to provide more evidence, so that hopefully some day we can convince the scientific committee and the public that for those BCS Class III drugs, a biowaiver can be granted.

Now let's move on to the next topic – the limits to oral drug absorption. Today we have discussed poor oral drug absorption, from perspective of permeability, and from perspective of solubility. I want to go one step further. With respect to solubility, what are actually the causes of poor oral drug absorption? In order to do that, please allow me to go back to review mechanistic models for predicting oral drug absorption.

This is basically a brief description of the absorption process. When administered to the patient, a solid drug such as a tablet or capsule will disintegrate or dissolve in the stomach. Dissolved and undissolved drug empties from the stomach into the small intestine, where dissolution continues. The dissolved drug will cross the intestinal membrane, passes through the liver and reaches the systemic circulation. We use the rate and extent of absorption to characterize the efficacy of these processes. So the fundamental processes of oral drug absorption are transit, dissolution, absorption and metabolism (*Figure 6*).

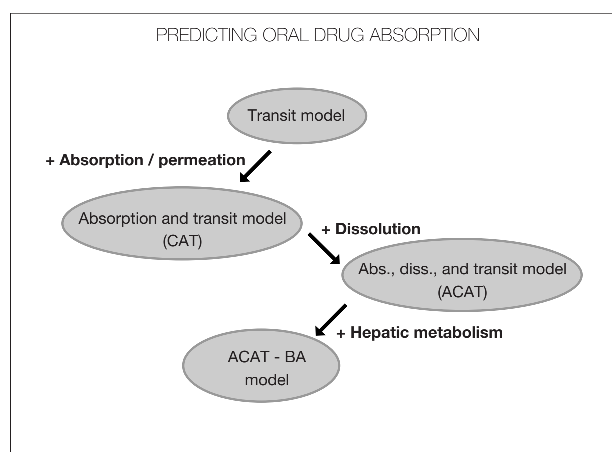


Figure 7.

In order to predict oral drug absorption, we use the so-called a reduction approach, which models and simulates one process at a time (*Figure 7*). How do we go about developing a mathematical process model? First, we develop a transit model. We assume the drug molecules are like a plastic ball, so there's no absorption/intestinal permeability, there's no metabolism, there is no dissolution. It's simply the drug flowing through the intestine. Once the transit model is designed and validated, you then add absorption, and at this point you assume dissolution is very fast and therefore it's not a limiting step. We call it the absorption and transit model, or CAT, for compartmental absorption and transit model. Next you add the dissolution model for poorly soluble drugs and we then have an advanced compartment absorption and transit model (ACAT). Finally, we include the last step, hepatic metabolism, the ACAT-BA, for an advanced dissolution and transit model, with BA standing for bioavailability.

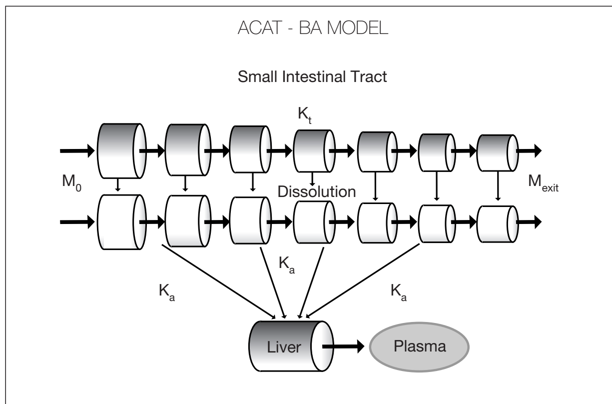


Figure 8.

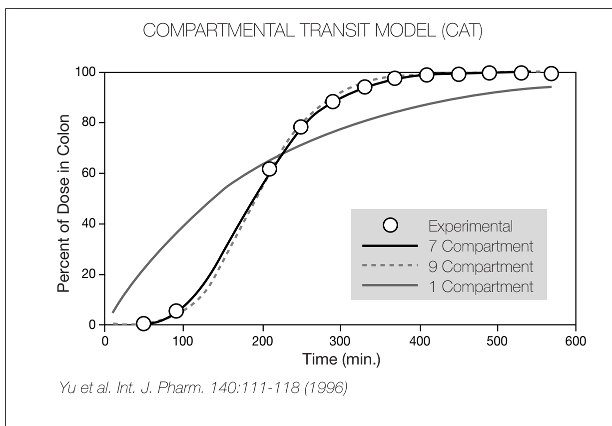


Figure 9.

The final ACAT-BA model consists of seven compartments (*Figure 8*). The top row in the *Figure* stands for the solid drug and the lower row stands for the liquid drug, and we have the liver and the systemic circulation. Now, I'm now giving you equations to describe the ACAT-BA model. If you write down the mathematical equation it can consist of over 16 ordinary differential equations. It appears complicated, but with a computer it's a piece of cake.

Figure 9 is the compartmental transit model (CAT), showing one compartment, nine compartments and seven compartments. Based on human experimental data for small intestine transit time we found that seven compartments best represented the data, and so we regard the seven-compartment model as the transit model that models the drug flowing through the small intestine.

The model can certainly predict transit time. By taking the next step, and adding absorption, you can

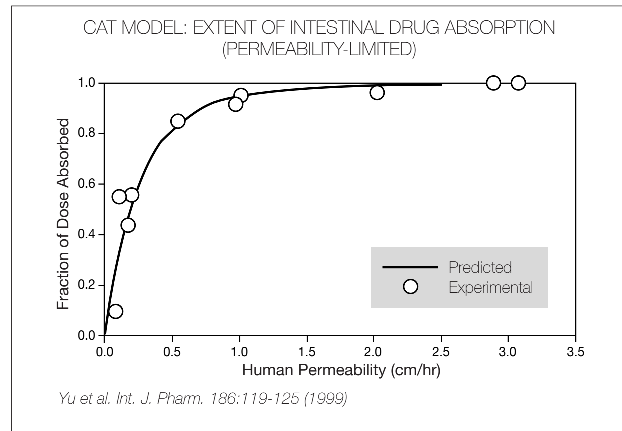


Figure 10.

now also predict the extent of oral drug absorption, or what I call the extent of intestinal absorption (*Figure 10*). At this point, dissolution is very fast, and we can show you a couple of compounds – cefatrizine and ranitidine – where prediction works reasonably well. However, even though the figures appear very good, if you look at low permeability drugs, we have large differences, so it's not perfect.

Not only can we predict the extent of intestinal absorption, we can also predict the rate of absorption for saturable drug absorption, as in the case of cefatrizine in *Figure 11*. As you probably know, cefatrizine is a carrier-mediated transporter. For saturable absorption the extent of bioavailability at 250 mg and 500 mg is about 75 percent, and at 1,000 mg it's about 50 percent. So not only can you predict the rate of passive absorption, you can predict the saturable absorption.

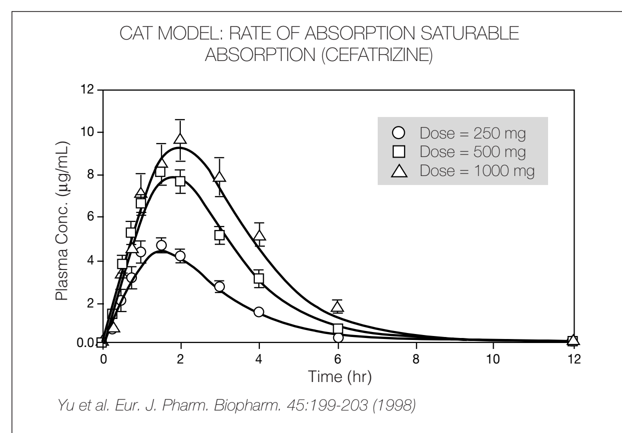


Figure 11.

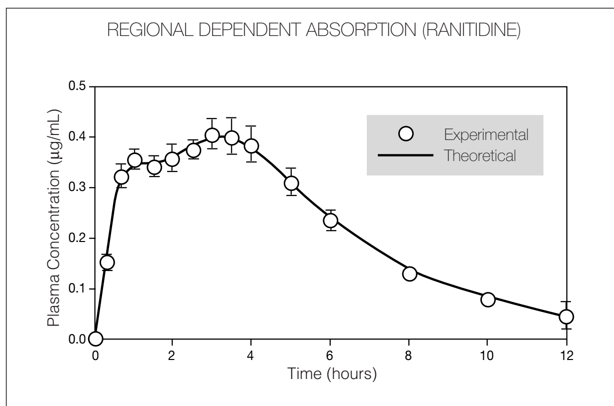


Figure 12.

Because it is made up of seven compartments, we can assign different absorption rate constants to each compartment; for example, the jejunal, duodenal or ileal. Eventually this leads to being able to predict the regional drug absorption, as you can see in the case of ranitidine (*Figure 12*).

As I said, these prediction models consist of over 16 ordinary differential equations. Certainly if I presented this to chemists, as Dr. Lipinski pointed out this morning, they would be likely puzzled and would shake their heads.

Therefore I am trying to develop a very simple scheme, to help our friends in the formulation department, in the pre-formulation department and in the medicinal chemistry department to understand what this specifically means. In order to do that I want to define so-called dissolution-limited absorption and solubility-limited absorption.

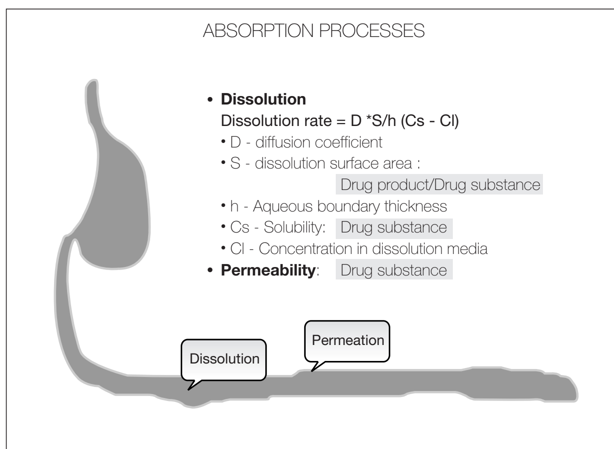


Figure 13.

If we look at the dissolution rate equation in *Figure 13* you have a diffusion coefficient, D, dissolution surface area, S, aqueous boundary thickness, h, solubility, Cs, and the concentration in the dissolution media, Cl. If we look at this equation carefully, we will quickly find there are two key parameters that can bring about significant change. The aqueous boundary thickness or the diffusion coefficient does not usually change that much from drug to drug. So the two fundamental parameters are the dissolution surface area – as you know, reduced particle size increases the surface area – and the solubility. So if slow dissolution is caused by the surface area, I call this dissolution-limited absorption. If slow dissolution is caused by slow solubility, I call this solubility-limited absorption.

Quantitative Estimation of Absorption

- **Dose Volume** $V_{dose} = \frac{M_0}{C_s}$
- **Dissolution Time** $T_{diss} = \frac{phr_0}{3DC_s}$
- **Absorbable Dose** $D_{abs} = P_{eff}\, C_s\, A < T_{si} >$

Figure 14.

Now let's look at the potential utility, taking the definition one step further. In order to do that, we first have to define some parameters which we can use to see whether it is a dissolution-limited absorption, a permeability-limited absorption, or a solubility-limited absorption. In order to do that, we have to define three parameters: dose volume, dissolution time and the maximum absorbable dose *Figure 14*. Dose volume is essentially the same definition as in the Biopharmaceutical Classification System. Dissolution time represents the minimum time required to dissolve a particle. Finally, the maximum absorbable dose is based on the permeability, solubility, absorption, effective absorption surface area, and transit time.

Figure 15 is basically a very simple schema that I have put together. The main transit time of 199 minutes represents the mean human small intestinal transit averaged from 440 data points collected from the literature. The 2 x 10^{-4} centimeters per second is basically the human permeability and represents over 90 percent fraction dose absorbed, or a 90 percent extent of intestinal absorption. Finally, we have the ab-

Limits to Oral Drug Absorption

Rate-limiting steps	Conditions	Comments
Dissolution limiting	T_{diss} > 199 minutes P_{eff} > 2 x 10^{-4} cm/sec D_{abs} >> Dose	The absolute amount of drug absorbed increases with the increasing of the dose.
Permeability limiting	T_{diss} < 50 minutes P_{eff} < 2 x 10^{-4} cm/sec D_{abs} >> Dose	The absolute amount of drug absorbed increases with the increasing of the dose.
Solubility limiting	T_{diss} < 50 minutes P_{eff} > 2 x 10^{-4} cm/sec D_{abs} < Dose	The absolute amount of drug absorbed does not increase with the increasing of the dose.

Figure 15.

sorbable dose over the actual dose. So, under this scenario we can define the dissolution-limited absorption, permeability-limited absorption, and solubility-limited absorption.

Let's review the comments included in the *Figure*, especially when applying the schema to preclinical toxicity evaluation. For dissolution-limited absorption or permeability-limited absorption, the absolute amount of drug absorbed increases with increased doses. However, for solubility-limited absorption – I'm talking about conventional dosage forms here – the absolute amount of drug absorbed does not increase with increased dosing.

I want to give you some examples to show how you can try out the schema. Case Study 1 is a real live case. The dose is relatively low, 5 milligrams. Solubility is 4 micrograms per milliliter, while permeability is high, at 8 x 10^{-4} centimeters per second. Obviously, this is a very lipophilic compound, poorly soluble. In order to improve the bioavailability, over 20 formulations were designed and manufactured, with bioavailabilities varying from 20 to 30 percent.

Sixteen formulations were evaluated in animals over a year; you can imagine how long it takes to evaluate 16 formulations in three to six dogs. And over the year it was found that that all these formulations had a similar animal bioavailability, ranging from 20 to 35 percent. In other words, at the end of over a year's effort, during which numerous studies were conducted, the outcome was basically the same. So, as a formulation scientist, you ask yourself the questions, why, what, and how?

Now in this case, even though the solubility is extremely low, only 4 micrograms per milliliter, in fact absorption is complete, believe it or not, because of the fairly low dose. Because the solubility here is aqueous solubility, solubility *in-vivo* might be tremendously increased. What happened here to cause the low bioavailability is simply hepatic metabolism. The formulation has a limited effect on bioavailability, and a limited number of animals may not show not significant bioavailability improvement (43 percent CV (coefficient of variation)). In other words, whatever kind of formulation you use, unless you inhibit hepatic metabolism, you cannot improve the bioavailability.

Now let's look at Case Study 2. In this case, solubility is even lower, 2 micrograms per milliliter, the dose is 400 milligrams, and permeability is 10 x 10^{-4} centimeters per second. So we have a high dose and, as we discussed this afternoon, the first approach you would try is to reduce particle size, because it's low solubility. It's very natural. It's a simple approach, probably the first approach option all of us would normally take, formulation scientists not excepted.

The particle size was reduced from 20 micrometers to 0.5. As you know, to reach 0.5 micrometers is not a trivial task, and when you conduct *in-vitro* dissolution and find a 25-fold increase you are extremely happy because 25-fold is a very significant improvement. However, after they are given to dogs the results came back, saying, sorry, there's no improvement at all. So again you have to ask yourself the questions why, what and how?

In this case, solubility-limited absorption is the cause; particle size has no significant effect on absorption. What this specifically means is that because it is a high dose, you are simply saturating the small intestine. Therefore, with the reduced particle size you can pretty much reach maximum solubility in the intestine, and that's the most you can do. So you see a significant improvement *in-vitro* because of the sink conditions there. However, you cannot see actual improvement *in-vivo*, because there are no sink conditions.

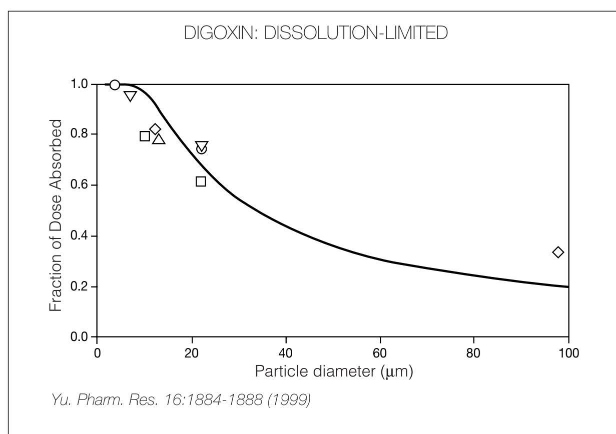


Figure 16.

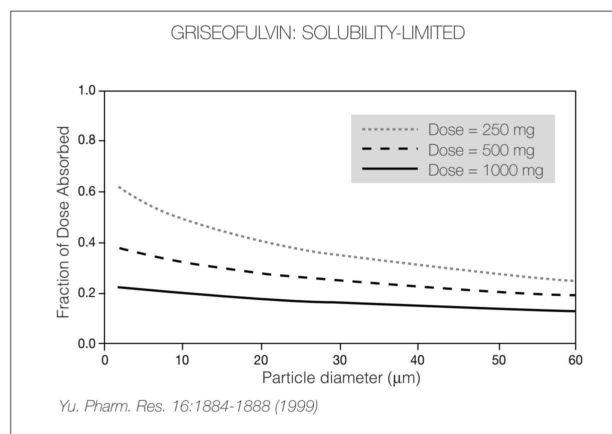


Figure 17.

Figure 16 is the classic example of digoxin, showing dissolution-limited absorption. In this case, therefore, a reduced particle size improves bioavailability, or the fraction dose absorbed.

The next example is griseofulvin (*Figure 17*). At high dose we basically do not see much improvement, because absorption is limited by the solubility. However, at low dose, absorption is limited by both solubility and dissolution, therefore we can see limited improvement. But unlike with digoxin, where it can pretty much reach 100 percent absorption, it is not so in this case.

So far, we have discussed the Biopharmaceutical Classification System, and we have discussed the limits of oral drug absorption. Now let me discuss the delivery of poorly water-soluble drugs.

This afternoon we've heard about a variety of approaches: lipid-based delivery systems, solid dispersion, co-solvents, nanocrystals, prodrugs, salts, and so on. Now I'd like to ask you to pay attention to what we can learn from the absorption of cholesterol (*Figure 18*). Even though its solubility is extremely low, I am sure all of us would like to have a low absorption for cholesterol. If you look at the solubility, it is 12 nanograms per milliliter. How low should it be? It's low enough, isn't it? It has a C-log of at least 9; that's extremely high. However, for whatever reasons, nature designed in such a way that all of us can absorb an average of 3 grams of cholesterol a day. So even if you eat a lot of large pizzas, they can pretty much get absorbed.

Now this is 3 grams, where normally you would say that a dose of 1 or 2 grams for poorly soluble drugs is extremely high. So we can learn from nature. She gives us an indication for the possible delivery of poorly soluble drugs. We are still at the trial and error stage. Hopefully, in the future, formulation science will develop to where, based on drug properties, we can see what kind of approach we need to take to deliver these problem drugs.

The example I want to give now is one that I was involved with before I joined the FDA – amprenavir (APV). This is an HIV protease-inhibitor, and it has a low solubility and is poorly wetted. Conventional oral dosage forms, including the powder-in-capsule form, basically have no detectable plasma levels. So you can see that bioavailability is very close to zero and the dose is extremely high, 1,200 milligrams, twice daily.

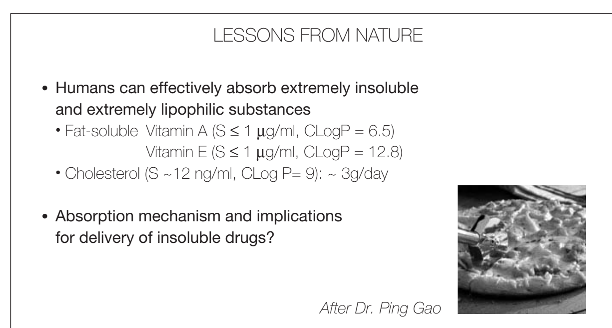


Figure 18.

Now, with this situation, we often use a salt approach. With a PK of around 4 or 5, for example, the salt approach works very well. But in this case the PK of 1.9 (*Figure 19*), it is difficult to make it.

So the approach to take is the self-emulsifying drug delivery system. In fact, a micro-emulsion including vitamin E-TPGS, or tocopheryl polyethylene glycol 1,000 succinate, PEG 400, and polyethylene glycol.

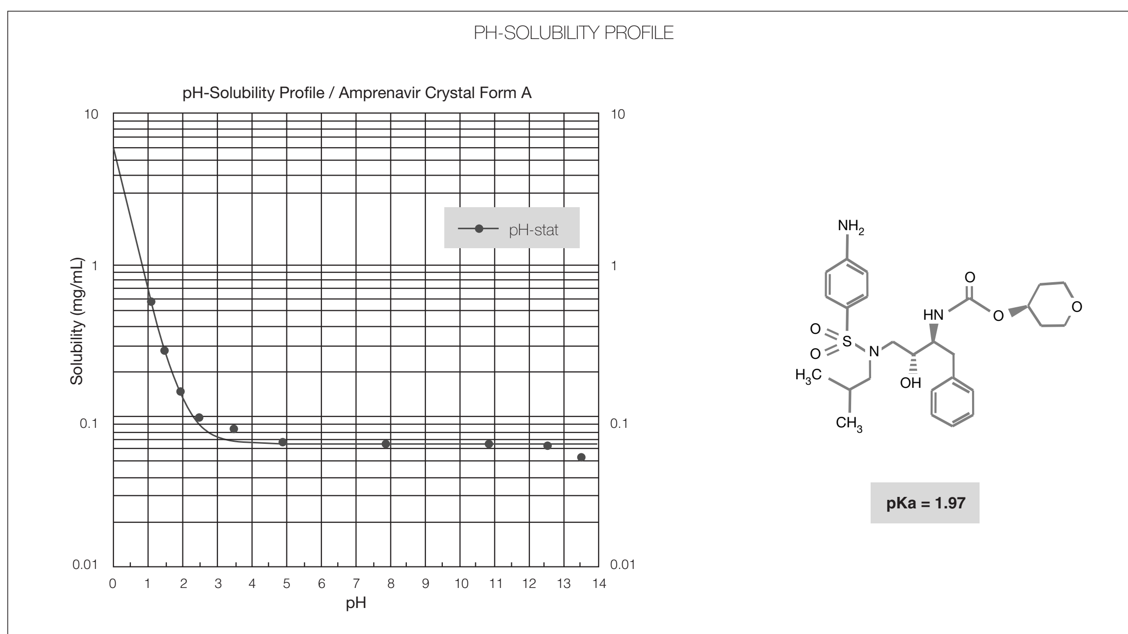


Figure 19.

The initial clinical studies, which utilized amprenavir dissolved in E-TPGS in hard gelatin capsules, had good bioavailability. Because we did not do any IV pharmacokinetics, we would never know the actual bioavailability.

Figure 20 is just to give you an idea of pharmacokinetics in dogs with a variety of formulations. The dry-filled drug capsule gives zero bioavailability. But at the 50 percent point of Vitamin E-TPGS, we pretty much reach the high limit. Now, you might ask, why not use 50 percent of Vitamin E-TPGS? There are all kinds of reasons, one of which is that it is way too expensive. On the hand, 20, 30 or 40 percent of Vitamin E-TPGS give reasonable levels of plasma levels; they have a reasonable bioavailability. So this compound, which is actually water insoluble, the self-emulsifying delivery system provides a reasonable bioavailability and can be developed in a product.

Scientifically, I want to discuss the role of TPGS in the improvement of bioavailability of amprenavir. In this case, the solubility of amprenavir was significantly improved in the presence of TPGS through micellar solubilization, and TPGS also enhanced the absorption or permeability of amprenavir *in-vitro*. So, overall, TPGS enhanced the absorption flux by increasing solubility, and probably also enhanced the permeability.

Pharmacokinetics in Dogs

Formulation	Cmax (μg x hr/mL)	Tmax (μg/mL)	$t_{1/2}$ (hr)	AUC (0 to 24h) (h x μg/mL)	Bioequivalence[a] (%)
Dry Fill Capsule	0	0	0	0	0
Peg 400 Solution	3.85 ± 1.25	1.1 ± 0.9	4.2 ± 1.7	12.2 ± 1.46	47 ± 11
20% Vit E-TPGS	5.41 ± 0.69	1.7 ± 0.6	3.6 ± 0.8	22.1 ± 4.52	86 ± 10
25% Vit E-TPGS	5.03 ± 0.44	1.7 ± 0.6	2.0 ± 0.8	20.6 ± 4.85	84 ± 13
30% Vit E-TPGS	8.24 ± 0.12	1.3 ± 0.6	2.0 ± 0.7	23.5 ± 4.97	93 ± 12
40% Vit E-TPGS	6.92 ± 0.94	1.7 ± 0.6	1.9 ± 0.6	24.4 ± 4.55	98 ± 16
50% Vit E-TPGS	7.63 ± 1.46	1.7 ± 0.6	2.5 ± 1.3	26.8 ± 8.27	
(Hard gelatin capsule)					

Figure 20.

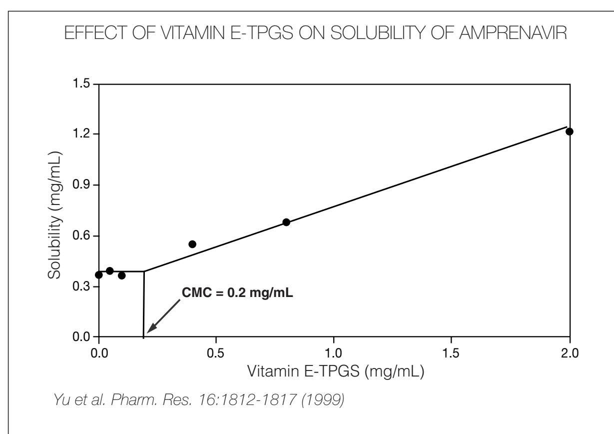


Figure 21.

Figure 21 has been shown before. Basically, the addition of Vitamin E-TPGS improved solubility by around four to 10-fold, and permeability improved two-fold, from five to around 10.

So, in this very brief overview, we have discussed the Biopharmaceutical Classification System, we have discussed limits to oral drug absorption, and we have also used examples to discuss the delivery of poorly water-soluble drugs. Now I would like to talk about the challenges to regulatory evaluation.

In the development of self-emulsifying delivery systems, we face a number of issues. One is the lack of consensus on the appropriate *in-vitro* dissolution methods that are predictive of *in-vivo* absorption. Here, you can look at the dissolution method for Neoral in the USP; in fact, for Neoral, the USP requirement changes to a disruption test instead of dissolution. In other words, what is specifically meant is the test of disruption of the capsule, instead of dissolution of the capsule.

Then there is the lack of scientific rationale for excipient selection. We have been the same levels for a very long period of time. We try and see what happens, but we cannot predict what might be happening. We sometimes have some kind of idea – for example, if we looked at a micro-emulsion versus an emulsion, we probably would know the micro-emulsion is better than the emulsion with the absorption characteristics. However, if we used the same micro-emulsion formulation and changed the excipients, you really wouldn't know what might happen.

Certainly, the number of surfactants and solvents is very limited, and at early-stage formulation development it's pretty much that we let animals lead human beings; we depend upon the *in-vivo* study for screening. We're testing in dogs, we're testing in rats and we see what happens, even though we know animals can not always have good correlations with humans. But this is the best approach available to us right now. Finally, there is a poor understanding of the *in-vivo* drug absorption mechanism via self-emulsifying delivery systems.

So because of those challenges, we really don't know if this is the right or wrong dissolution test. But we know this dissolution test is sufficient for quality control, and that is the usual objective of the dissolution test. We know that, but we do not know whether this dissolution test is sufficient or correlates to the *in-vivo* performance of specific self-emulsifying delivery products.

I want at this point to thank the people who have helped me in this presentation: Dr. Ajaz Hussein of the FDA, Professor Gordon Amidon of the University of Michigan, Professor James Polli of the University of Maryland, and Dr. Arup Roy of Eli Lilly.

My final wish is that some day we will know what kind of formulation, what kind of excipients we should choose, based on drug properties, to give us the right answer to save our time and development costs and to help human beings. But we have not yet reached this stage with respect to self-emulsifying delivery systems. We are at the stage of the art of trial and error, we have not reached the stage that science asks us to do. It depends on you, it depends on all of us to get there.

This concludes my presentation. I welcome any comments and criticism. Thank you for your attention.

Chair, Professor Mitsuru Hashida, Kyoto University, Japan: Thank you very much, Dr. Yu. You mentioned biowaiver extensions to the BCS. You also clarified the limits to oral drug absorption. You talked about modeling, and based upon the modeling you talked about various analyses of the kinetics of absorption and also various delivery systems. You also talked about the challenges to regulatory evaluation. So you covered a broad range of topics in your overview. I would like to invite any comments or questions about your presentation.

Hassan Benameur, Capsugel Inc., North Carolina, USA: Thank you for the excellent presentation you gave us. Let me challenge you. I have two questions for you. One is regarding your Case Study 1, where you said that from the formulation point of view, when an active has a metabolic effect, formulation cannot enhance bioavailability. Is that right?

What we know today is that hepatic metabolism certainly occurs. But it is known that before that, dur-

ing absorption, there is some intestinal metabolism, and we can develop more individual models by using human microsomes to see if we first have intestinal metabolism. And on the art of formulation, if we can show by using human intestinal microsomes that there is a first metabolism at the intestine, can we choose this as a formulation strategy and so enhance the bioavailability? So this is some information about the approach, and I totally agree with you. We are far from the end of these studies, we are still at the beginning.

Dr. Lawrence Yu, Director for Science, Office of Generic Drugs, CDER, Food and Drug Administration, USA: Well, for Case Study 1, investigators found a very limited effect on bioavailability for all the formulations that had been evalauted. I am well aware that some polymers, for example, are able to inhibit gut metabolism, at least *in-vitro*. At this point, in the case of all the 16 formulations that were evaluated, a significantly improvement *in-vivo* was not demonstrated. I do not know whether this is actually a normal occurrence or a very limited occurrence. But based on the data, it suggests that any contribution from gut metabolism is very limited.

Hassan Benameur, Capsugel Inc., North Carolina, USA: Did you check if you got an intestinal metabolism off your active?

Dr. Lawrence Yu, Director for Science, Office of Generic Drugs, CDER, Food and Drug Administration, USA: That certainly is another topic of symposium. Based on my understanding of some of the compounds, in general the intestinal contribution is not that significant for the compounds evaluated.

Chair, Professor Mitsuru Hashida, Kyoto University, Japan: Thank you very much. Any other questions, please?

Walt Walters, Simulations Plus, USA: The compounds that you dealt with apparently do not have the kind of characteristics that perhaps Dr. Benameur's question related to. But there are certainly a large number of compounds on the market that are 3A4 substrates, and certainly potentially there will be more. It's possible that by delaying release of the compound, so that release occurs beyond regions of high 3A4 expression in the upper small intestine, you might actually affect bioavailability by avoiding those regions.

Dr. Lawrence Yu, Director for Science, Office of Generic Drugs, CDER, Food and Drug Administration, USA: That's correct.

Prof. Yuichi Sugiyama: Related to that question, I would like to add a comment. Recently, in my laboratory, based on the literature data, we tried to understand first- pass GI metabolism, particularly for the CIP 3A4 substrate. We used about 12 compounds. Then, from the clinical pharmacokinetic data, after oral and IV administration, we calculated the so-called Fa/Fg as well as the hepatic clearance. Then we plotted the Fa/Fg intrinsic hepatic clearance passes, because in the 3A4 it should be the same in the liver and in the intestine; that is the same iceline.

So what did we find? We are now writing that paper, it's going to appear in a journal very soon. Under conventional microsomal stability studies, 1 milligram of microsomal protein per milliliter is a liver microsome. Our data indicates that if the half-life of microsomal stability is less than five minutes, then you have to worry about first-pass GI metabolism. That is our conclusion, after summarizing all of the information. In that case, according to our findings, whether that compound is a substrate of PGP or not, it does not affect our conclusion. That is just our summary.

Walt Walters, Simulations Plus, USA: It's great information, thank you. So its natural passing is… What is the average half-life of the compound? If we're lasting five minutes, the gut metabolism is significant.

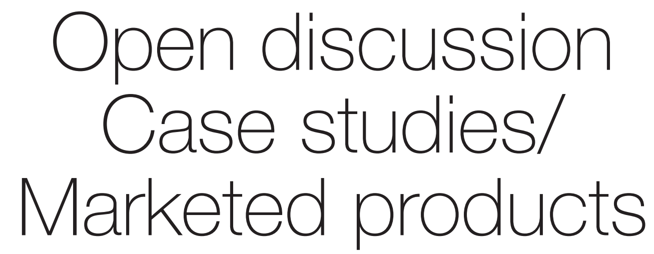
Prof. Yuichi Sugiyama: Less than five minutes means the *in-vitro* microsomal stability studies, using *in-vivo* microsomes…

Dr. Lawrence Yu, Director for Science, Office of Generic Drugs, CDER, Food and Drug Administration, USA: For the majority of compounds which you have investigated, what kind of percentage show that from *in-vitro* they have a half-life lasting five minutes?

Prof. Yuichi Sugiyama: Not so many; I would say less than 30 percent of the cultured 3A4 substrate. That's our result.

Dr. Lawrence Yu, Director for Science, Office of Generic Drugs, CDER, Food and Drug Administration, USA: Pgp has no impact on your conclusion. That is interesting!

Chair, Professor Mitsuru Hashida, Kyoto University, Japan: Thank you very much once again, Dr. Yu. I would now like to close the session.



Open discussion Case studies/ Marketed products

Roland DAUMESNIL

Open discussion
Case studies/Marketed products

Roland Daumesnil

Capsugel Inc.,
North Carolina, USA

Chair, Professor Mitsuru Hashida, Kyoto University, Japan: Ladies and gentlemen, we are very pleased to open the final session. I would like to ask Professor Yamashita and our colleague, Roland Daumesnil, to chair the open discussion session.

Thank you very much for staying with us over many hours. Many topics have been discussed and perhaps your brain is saturated with information. But I'm asking you to stay on because there are some issues that haven't yet been fully discussed or addressed, and we would like to cover those in this session. I would first like to ask my coach here, Roland Daumesnil, to summarize the issues and approaches regarding new and marketed products, and later he will also give us a summary of today's discussions. He says that his presentation will take 20 minutes and I am sure that you will enjoy it. After that, the floor will be open for discussion, with the chance for questions or comments to any of the speakers in today's symposium.

Roland Daumesnil, Capsugel Inc., North Carolina, USA: Let's examine some numbers first. If you look at the value in billions of dollars of the global pharmaceutical market in 2002, total OTC sales plus Rx prescriptions were US $420 billion, split as $53 billion for OTC, and $367 billion for Rx. Not bad. We talk a lot about drug delivery: the drug delivery business represents $50 billion, or 12 percent of the total. What we talked about today, the poorly soluble actives that are on the market, already represent $110 billion; in other words 26 percent of the total value is created by poorly soluble actives.

We have been dealing with poorly soluble actives for years, but there is certainly room for improvement. Remember what Chris Lipinski said? When high-throughput screening started, his team was generating a lot of poorly soluble actives. Of the new molecules launched last year, in 2002, 58 percent were poorly soluble actives. There must be a good reason for this, and I'm sure you got it today. The current pipeline is no different. People continue to approach their work using the techniques we have talked about, and 40 to 50 percent of new molecules under development – even when the Rule of 5 is applied – are poorly soluble.

Going back to the marketed products that account for 26 percent of the total market value, there are about 100 candidates for reformulation among all these poorly soluble actives. Maybe your company has one. Certainly, they can be reviewed with an eye to increasing the bioavailability, improving the PK profile, improving the stability, creating line extensions with functionality and, last but not least, to extending patent protection.

Some of these products are blockbusters.

Let's look at the 12 most important poorly soluble active products already on the market *(Tables 1 and 2)*. Let's start with Pfizer. The first one is atorvastatin, the famous Lipitor. Look at the solubility, 0.11 mg/ml. Also it's not because you have a poorly soluble active that you don't have a blockbuster. The same for simvastatin. These two products, one poorly soluble, the other insoluble with high first-pass metabolites, already accounted for US $13 billion

Marketed products. Candidates				
Generic name	**Trade name**	**Solubility in mg/ml**	**Bioavailability in %**	**Worlwide sales 2001 in Billion $**
Atorvastatin	Lipitor	0,11	***HPFM 20-40***	6,5
Simvastatin	Zocor	Insoluble	**< 5**	6,7
Loratidine	Claritin	Insoluble	0-20	3,2
Celecoxib	Celebrex	Insoluble	?	3,2
Olanzapine	Zyprexa	Insoluble	40	3,1
Sertraline	Zoloft	< 10	20-40	2,4

Table 1.

Marketed products. Candidates				
Generic name	**Trade name**	**Solubility in mg/ml**	**Bioavailability in %**	**Worlwide sales 2001 in Billion $**
Paroxetine	Paxil	5,4	***HPFM ?***	2,5
Cyclosporine	Neoral	0,04	20-40	1,4
Clopidrogel	Plavix	Insoluble	50	1,4
Paclitoxel	Taxol	Insoluble	?	1,2
Clarithromycin	Biaxin	Insoluble	50	1,2
Docetaxel	Taxotere	Insoluble	?	1,0

Table 2.

in 2001. And so the list goes on. All these products could be improved and are going to be improved. Celecoxib is one of them, loratidine is another. Something can be done to give a second life to a product.

Paroxetine is the same (*Table 2*). Cyclosporine – we'll be talking a lot about this product, because for me it represents a unique example. Despite all the issues that surrounded its launch, this product has saved the lives of thousands of people. Plavix (clopidrogel) and the anti-cancer products, Taxol and Taxotere, are also insoluble. They have great value and deserve to be improved, if possible, to give them a patent extension. So with 12 products already on the market which belong to the blockbuster category – meaning they are worth more than $1 billion – this is already saying something important about the potential of insoluble drugs.

We already talked about how to formulate Class II drugs. In reality, we're talking about a lot of systems: creating salts or a prodrug, reducing particle sizes, including the active in a polymer complex, and creating a micro-emulsion or SEDDS. But how many such products are on the market, and using which technology? I checked the Physicians' Desk Reference, the PDR, plus other compendia, and I came up with some interesting results: there are not very many.

What you will indeed see more and more are products based on micro-emulsions, emulsions or self-emulsifying systems, such as cyclosporine, ritonavir and isotretinoin capsules. So let's look at them, plus a few others. I will certainly be talking about amprenavir – I'm not sure I totally agree with Larry Yu's viewpoint. But we are here to be challenged.

Sandimmune. I love this product (*Figure 1*). A lot of people around the world love this product, not because it was a big challenge, a big issue in terms of formulation, but because, as I said before, this product has saved thousands of lives. Without this pro-

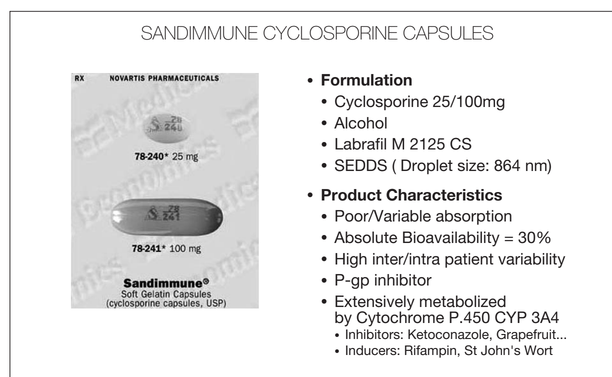


Figure 1.

duct, it is impossible to achieve successful transplants. Novartis developed an emulsion which was, by the way, a self-emulsifying system, not a microemulsion, and Dr. Okamoto told us that the droplet size is around 864 nanometers. It has poor and variable absorption, everybody knows that, as well as low bioavailability and high inter/intra patient variability. It is a P-gp inhibitor – extensively metabolized by cytochrome CYP 3A4.

What does all this mean? It means that, yet again, these concepts are sometimes the terrible reality we have to deal with.

What do inhibitors mean? If you inhibit the P-gp or the cytochrome CYP 3A4, you will increase the concentration in the blood. You can do that with ketoconazole and grapefruit. But what if it is the reverse, if you use an inducer of P-gp like rifampin or St John's Wort? You simply reduce the quantity, the concentration of cyclosporine in the blood, up to the point where you could have a graft loss, a rejection of the transplant. You simply kill the patient.

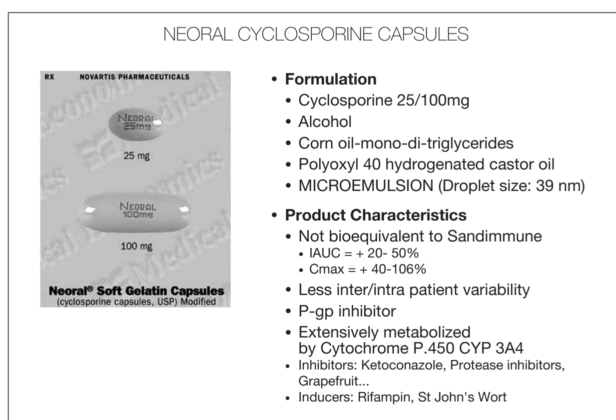


Figure 2.

So, talking about P-gp inhibitors, talking about cytochrome CYP 3A4, and so on, has great value for these kinds of product. They are life-saving products, and looking at the contra-indications and drug interactions is extremely important in better understanding the role of transporters, inhibitors or inducers.

That is why Neoral was developed (*Figure 2*). It is a micro-emulsion with a small droplet size of 39 nanometers. It is not equivalent to Sandimmune because it has between 20 and 50 percent more AUC, and 40 to 106 percent more in terms of the C-max. There is no bioequivalence, and less inter/intra patient variability. Nothing else changes.

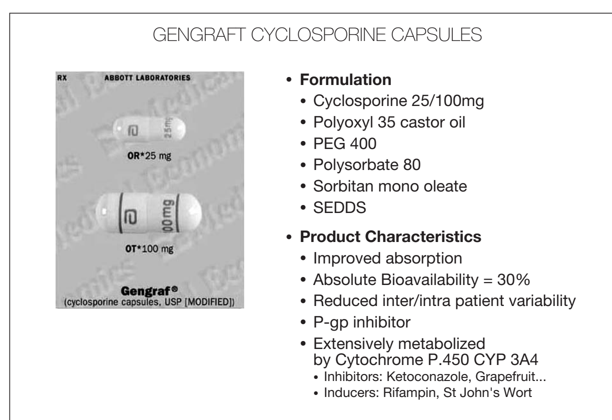


Figure 3.

What is interesting about *Figure 3* is that Gengraft is a generic version of cyclosporine targeted at Sandimmune and Neoral. It is a self-emulsifying system and you can see they use quite different excipients to be able to circumvent Novartis' patent and come up with a formulation bioequivalent to Neoral. Bear in mind that for this kind of product you also have to undertake clinical studies. You will never get approval from the FDA or any ministry of health without doing clinical trials.

Chris Lipinski was talking about the fact that solubility is very dependent on product synthesis, on chemistry synthesis. For some products, this is critical. Norvir in *Figure 4* is an interesting example. Let's talk about it.

Abbott was obliged to change the formulation because it changed the synthesis. The product started to precipitate and the crystals were simply insoluble. So they had to withdraw it from the market. Also, this product is extremely sensitive to the fed condition.

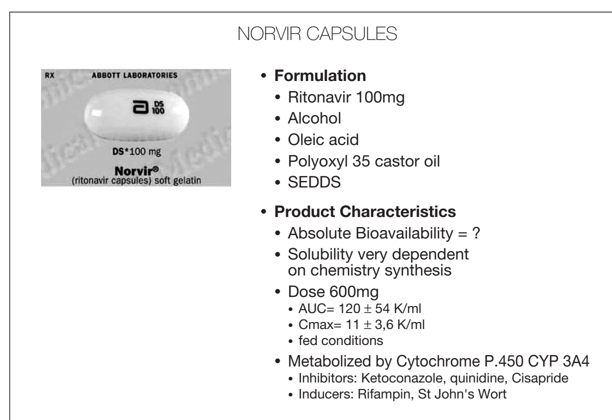


Figure 4.

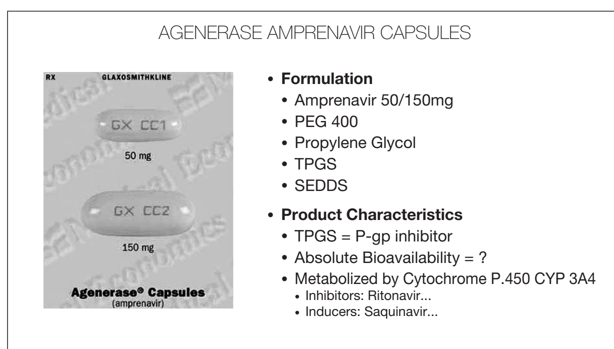


Figure 5.

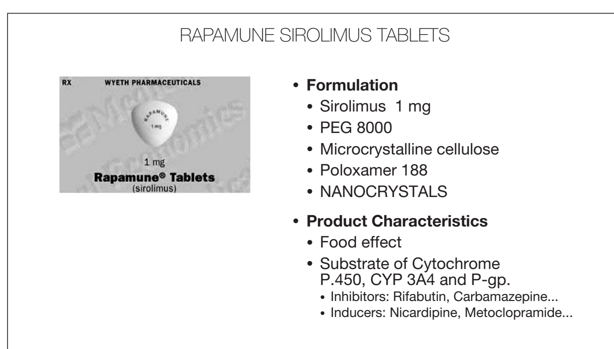


Figure 6.

You can increase the blood concentration by up to 30 percent if you take it with a heavy meal. Once again, cytochrome CYP 3A4 is involved, and the inhibitors and inducers are exactly the same as the ones we discussed before.

Another good example is Agenerase, or amprenavir (*Figure 5*). Dr. Yu, I believe that TGPS is a P-gp inhibitor and that the inclusion of TPGS simply changes the permeability. You are able to increase bioavailability by including TPGS. The active is also metabolized by CYP 3A4. But in this case the inhibitor, ritonavir, and the inducer, saquinavir, are different. They are protease inhibitors.

Rapamune (sirolimus), 1 milligram, is another immunosuppressant used for transplantation (*Figure 6*). The product was launched as a solution. A solution is never the perfect dosage form, because you never know if the patient is going to take a little more or a little less. So they used nanocrytals to include 1mg active into a tablet. This is an interesting example where the nanocrystals really bring something to the product. But remember, the dose is extremely low.

Food effect is important with this drug. Sirolimus is also a substrate of cytochrome CYP 3A4 and P-gp. Professor Sugiyama was talking this morning about their synergistic effects, and here you have a product which is sensitive to both. Once again there are inhibitors that increase the concentration in the blood, while inducers decrease it. In this case, remember that if you decrease the blood concentration, as with cyclosporine, you risk graft loss.

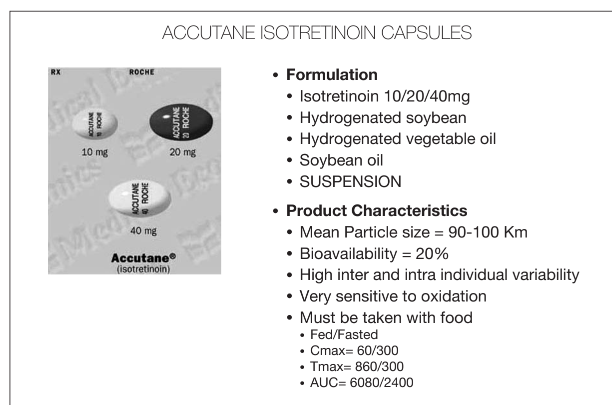


Figure 7.

Isoretinoin is a suspension (*Figure 7*). What is important here is not the dissolution – this is a point for all of you to take in, especially those involved in formulation. Sometimes, you have to forget the dissolution. What discriminates batches is the particle size.

I will go even further. If you are interested there is a dissolution method in the British Pharmacopoeia. It doesn't work. It simply doesn't work. Try it, and call me if it works. This is a typical case where the dissolution method doesn't work. It must be taken with food. It's also very sensitive to oxidation, so the formulation is quite difficult. But if you look at the difference between fed and fasted, bioavailability is roughly five times greater.

Figure 8 (slide 15 on the presentation) is a generic version of isotretinoin. I haven't given the name of the company, because the product has not been launched. As it's not yet on the market, the company did not want me to include its name. It's a generics company in Europe. To develop it they used exactly the same formulation, which is a suspension with surfactant. They got bioequivalence with Accutane, by the way, but at a lower dose, which could be important in terms of cost because isotretinoin is a very expensive active.

What about OTC? You can also do something with an OTC product.

Solufen G is a formulation of 200 mg of ibuprofen, an OTC product (*Figure 9*). It uses Gelucire 44/14,

and you've got a self-emulsifying system with one excipient. I think they were lucky there, because you cannot put more than 200 milligrams into this capsule size. It has excellent stability, a rapid onset of action and it is differentiated from the competition, which is quite tough in this segment.

Among the new approaches still under development, nitric acid-releasing NSAIDs (non-steroidal ant-inflammatories), are simply naproxen linked with nitric oxide. Using a SEDDs system increases the solubility and bioavailability.

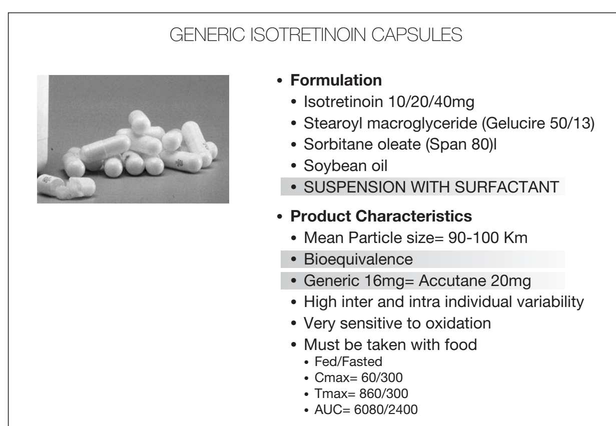


Figure 8.

For celecoxib, Pharmacia used a SEDDS with transcutol and what they got – and this is important for this kind of product – was a rapid onset of action. Don't ask me if Pharmacia/Pfizer is going to launch this product. Even if I knew, I wouldn't tell you, but they say it works very well. If you look at the patent, you will find something very interesting. The patent describes the formulation strategy and how they came to a SEDDS using a ternary diagram. The selection of excipients is also explained in the patent. It is very well done, it's an excellent rationale.

Figure 9.

In the case of Paclitaxel, they decided to put PVP into a SEDDS formulation, and the result was excellent. They increased the solubility and the bioavailability five times.

Last, but not least, elitriptan is an excellent product from Pfizer. This product is very unstable and to have a rapid onset of action you need to create a formulation which I would say is different from a normal tablet. So they developed a SEDDS, and what happened was a rapid onset of action with excellent chemical stability, which was not the case with a lot of the formulations they had investigated.

Including the active in a polymer complex is also an alternative to formulate poorly soluble active. Here we need to mention cyclodextrin. Cyclodextrin is used a lot for injectables but, orally, there is only one marketed product, an oral solution of itraconazole in which you use cyclodextrin to complex the active.

The poorly soluble active represents a superb market. There are 100 marketed active compounds with poor solubility: candidates for reformulation. Look at your portfolio of products, maybe you have one in there. The level of 40 to 50 percent poorly soluble new actives is here to stay. And I think Chris Lipinski's message was very clear; even if you use the Rule of 5 and other filters, it will stay.

There are new technologies available to achieve acceptable absorption: nanoparticles, polymer complexes, micro-emulsions and SEDDS.

But don't just take my word for it. Do the same exercise as I did. Look at the patents, look at all the literature. You will see that self-emulsifying micro-emulsion systems have become an industry reality. We're not talking about the past, but today reality. Why? Because companies dealing with poorly soluble actives understand more and more that they need to investigate alternative dosage forms.

Chair, Professor Mitsuru Hashida, Kyoto University, Japan: That was Roland's presentation. Now we would like to have an overall questions and answers discussion, including his presentation and covering the whole of today. So please raise any questions that you have. We won't restrict any topics, so feel free to direct your questions to anybody, please.

Perhaps it will help if we review the scope of discussion a little? A number of topics were covered today. We discussed various things, but formulation studies were the focus of our presentations and discussions. In the morning Dr. Lipinski made a presentation, questioning the type of activity chemists are

engaged in during pharmaceutical development, and asking how chemists, formulation people and also development scientists can collaborate with each other. And to take this idea further, as Dr. Yu did, to what extent will the formulation study of the future be involved in the drug development process? What contribution can the formulation study make to the discovery stage, for example?

So could anybody with any opinions, any experience to share, please break the ice? Of course, it can be any other topic.

Dr. Soon-ih Kim, Ono Pharmaceutical Co. Ltd, Osaka, Japan: Earlier, I put a question to Dr. Kusai. Now I would like to ask a question to Dr. Lipinski. He said that early formulation should be discouraged as it makes the chemist's work too relaxed. I think that in Japanese companies the specialists in formulation studies become rather confused with that type of statement. So we would like to have Dr. Lipinski confirm that statement again, please.

Dr. Christopher A. Lipinski, Pfizer Inc., USA: Yes. I'll state what I said again. At least in the Pfizer Groton Laboratories, we actually discourage very early discovery formulation work, because there's a danger it will prevent the chemists from doing what is their job, which is to change the chemistry structure so as to make the compounds better absorbed, more soluble and more permeable. I recognize that this is controversial; I know that there are some companies that in fact have early discovery pre-formulation work.

I must say, I influenced what the Groton Laboratories did. But it's my opinion that if you have compounds that are so insoluble and so impermeable that you have to do formulation work in early discovery to get biological activity on those compounds, then perhaps you should consider that you should not even work on those kinds of compounds.

Roland Daumesnil, Capsugel Inc., North Carolina, USA: I have a question for a group of speakers, this afternoon's formulators. If you take only the log-P as a criterion, do you think the strategy in terms of formulation with a log-P of 3 or a log-P of 6 is the same, or would you approach it differently?

Dr. Lawrence Yu, Director for Science, Office of Generic Drugs, CDER, Food and Drug Administration, USA: I'll talk about formulation here, as a regulatory scientist. I think that when you look at the solubility, C log-P, you need to look at how much drug is needed to be effective. So what I'm trying to say is that the question is not that simple. Based on one parameter, you're saying, here you are, here is one simple criterion you may use. But you have to look at the overall picture, you have to look at other parameters such as total dose to make reasonable strategic decisions.

Now I'd like to comment about something that really worries me very much. Between 1995 or 1997 and last year, the cost of drug research and development increased drastically, at least in the USA. I don't know the figures. Meanwhile, the number of approvals by the FDA has drastically decreased.

Basically, things are going in opposite directions. On the one hand, expenditure has gone up, on the other hand the number of new molecules approved by the FDA has declined drastically. I think last year costs rose by as much as 40 percent, while new molecule approvals dropped by over 10 percent. I don't know the precise figures. I think companies should be prepared to do something about it, because we all know these trends cannot continue.

Roland Daumesnil, Capsugel Inc., North Carolina, USA: On average, Dr. Yu, over the last five or six years the overall number of new molecules per year has been around 40, so it's quite low. I'm talking worldwide, not just the USA.

Dr. Lawrence Yu, Director for Science, Office of Generic Drugs, CDER, Food and Drug Administration, USA: I think there are the same trends in Japan and Europe. The number of new molecules approved by regulatory agencies has decreased drastically. The cause is most anybody's guess, and I'm sure that people are looking into it. But I'm also sure that if all of us take a responsible attitude, those trends cannot, and will not, continue.

Sototo Iso, Japan: So what is the cause of such a situation in clinical studies and new molecules? Is it because of ADME, or something else?

Dr. Lawrence Yu, Director for Science, Office of Generic Drugs, CDER, Food and Drug Administration, USA: I really don't know. I think ADME's contribution has drastically decreased lately. I think there have been some break right-off or yellow duster/molecules, but not that many. You know, I've spent eight years in the industry, and I've only seen one molecule which cannot be delivered.

Question from the audience: To follow up on that… I was very pleased to hear your remarks this morning, Yamashita San, about the need for breadth and depth of knowledge, and the need for a multidisciplinary approach to the problems that we have. Listening to Roland's presentation, we saw things where

you have to think about solubility, permeability, P-gp, CYP 3A4 formulation. We have to put all of this together.

Perhaps one of the reasons that the number of approvals is down is that we know more things that can go wrong now than we did some years ago. So we know what to look for that limits the compound from becoming approved.

I think that as an industry the multidisciplinary approach, where we start thinking about all of these things together, rather than one person working in a cubicle over here on toxicity, and another person over there on solubility, is perhaps one of the answers. We need to, I believe, train and educate more generalists who can consolidate the information from the specialists and apply it. Of course, I'm a bit biased, but I believe simulation and modeling is about the only way to pull all of that together and make sense out of it.

Chair, Professor Mitsuru Hashida, Kyoto University, Japan: I completely agree with your comments. Maybe somebody from the Japanese companies can comment on this issue?

Yano, Ono Pharmaceutical Co., Ltd, Osaka, Japan: I'm one of the chemists working under Dr. Kim. We have to look at both the overall aim and the costs at the same time. When we are doing synthesis, we have to try hard to bring the molecules to the very end of development. We also have to look at the formulation, and we have to minimize cost and risk both in the synthesis stage and the formulation stage. I think that is the best method. However, we have not yet found the right approach. It's difficult to find the right approach, and we don't yet know the limitations of what can be successfully developed in the synthesis stage. So those are the constraints. We have to know what the limit is.

Chair, Professor Mitsuru Hashida, Kyoto University, Japan: Thank you very much for your valuable contribution. Anyone else – preferably not from Ono Pharmaceuticals? Ono Pharmaceuticals' strategy may be somewhat unique. Dr. Kikuchi, you discussed the dose number and chemists, for example. I think you have to explain to chemists about the dose number and how such knowledge is used objectively in synthesis, and generate consensus.

Dr. Hiroshi Kikuchi, Principal Investigator, Daiichi Pharmaceutical Co. Ltd, Japan: The compound that I used in the presentation was from five years ago, and people handling the synthesis of course became aware of the dose number and the Rule of 5. But on the other hand, we had some difficulties – I am doing some soul-searching here – because we sweated so much over solubility that we forgot the importance of membrane permeability. If membrane permeability is high, then formulation may be successful even with a relatively low solubility. For quite some time, there was too much stress on solubility. But dose number and the Rule of 5 are very well understood by chemists and in early screening we only have alerts.

In the ninth reformulation approach, I looked at changing the salt of the crystal. Today, we can change the crystal salt and if the salt had been HCL it would have been much easier. But under the Japanese regulations prevailing at the time, it was not easy to switch from one salt to another. In discovery we were working to the standards of the Research Institute of Material Properties which meant that not only the chemical stability but the absorption of a salt to be used in crystalline form had to be checked in monkeys or dogs, and an overall evaluation made before moving ahead. So we made a change to the system, but because it was five years ago we had an older system where we could not change the salt in the crystal.

If we can cope with the problem by changing the salt or doing something with the crystalline form, that should be tried first before reformulation. The salt issue still remains today so far as regulations are concerned. Once a certain type of salt is adopted you can't change it. Is that the case at the FDA, Dr. Yu?

Dr. Lawrence Yu, Director for Science, Office of Generic Drugs, CDER, Food and Drug Administration, USA: Based on current regulations, a salt is a new molecular entity. In other words, I consider a chlorine salt and a free-based one two different molecular entities. Actually, you can gain marketing advantage from this sometimes.

Roland Daumesnil, Capsugel Inc., North Carolina, USA: Professor Sugiyama, you gave a superb presentation as usual. But I'm scared, because you keep on discovering more and more influx/efflux systems or transporter. Do you know what you are doing? You're increasing the number of possible drug interactions. Take your time, take your compendium, take the PDR, and look at all these critical molecules. You have pages of, 'don't take this drug if you belong to a particular patient group', or 'don't take that drug with this product'.

If it continues like this I think that for all products, all molecules with an efflux system, or where a cytochrome metabolism is involved, maybe you will have to say, I don't want to launch it. I'm sure that

there are some pharma companies which are thinking about that, because you limit the product's potential on the market tremendously. I don't know if I'm exaggerating, but that was the way I understood your superb presentation.

Professor Yuichi Sugiyama, University of Tokyo: I don't know whether I should answer this question in English or Japanese. Let me answer in Japanese. Suppose the number of transporters is going to increase in the intestinal tract or the liver… We need to go back to the history of cytochrome discovery 20 years ago, when more and more cytochrome subtypes were being discovered. Today, this is a totally matured research area and our understanding of it is quite extensive. For instance, if there is a drug-drug interaction, CYP3A4 is the important enzyme and if there is genetic polymorphism then 2D6 and 2C19 are the risky CYP isozymes.

Therefore, we are continuing the effort to find new transporters. This is important because, if we can do it, then in five years' time, even if there are many, many transporters, we will be able to identify and narrow down the difficult ones. We will be able to state which ones incur a drug interaction risk and pinpoint the ones that carry a risk of genetic polymorphism. What's important is to clarify the truth. The most important thing is the scientific value. That is my answer.

This question needs to be posed in Japanese and I hope that the translator will do a good job. I'll speak very slowly. Well, we have talked with other pharma companies and they have confirmed what I am about to say. Using excipients, the efflux transporter such as P-glycoprotein is inhibited, and so absorption is enhanced. This is a good strategy.

However, there is the patent, inclusive of the concept of the strategy. What I don't know is the circumstances in which we would be infringing this patent protection. If inhibiting efflux enhances absorption, of course to support or to demonstrate that would be not so easy. Are there any occasions when pharma companies would be hesitant in doing further research on that compound, because of that patent protection? Since I am a layman in the patent area I am putting this question to either the Japanese or the overseas-based pharma companies.

Chair, Professor Mitsuru Hashida, Kyoto University, Japan: Thank you very much. Has anyone had the experience Professor Sugiyama describes?

Dr. Soon-ih Kim, Ono Pharmaceutical Co. Ltd, Osaka, Japan: In this company, three years ago, 3A4 inhibitors were used to increase the plasma concentration. We achieved our aim, but found that there was an established US patent for that application. We asked the CRO about the licensing fee and the royalty was so huge that we dropped the product. That's our experience.

Question from the audience: Well, Dr. Kim, if there's good evidence for an activity then of course you may be infringing a patent. But it's not that clear. You know, there are many, many excipients and there are many, many enzymes that inhibit the influx system and if we say that this function constitutes a patent infringement in itself, then maybe they cannot be used as a substrate. I really do not know how a patent can be applied to the very wide range of reactions and phenomena *in-vivo*. We use TPGS.

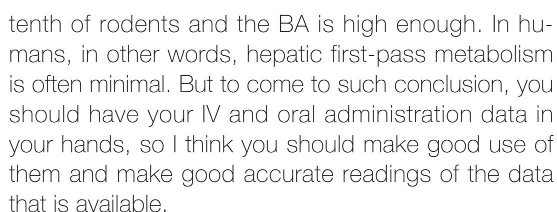
Professor Yuichi Sugiyama, University of Tokyo: May I just make one final comment? This is a question to people working in Japanese and foreign pharmaceutical companies. I have a lot of opportunity to discuss with people in companies about the drug discovery process, and they have a good understanding of PK. Still, I also encounter some other cases that I would like to talk about now, as I would like you to be aware of them.

With the development of LC/MS spectrometry bioavailability screening is carried out extensively in rats, using cassette dosing. Once the IV and oral administration data is available, we get the BA data through that. If you conduct that kind of experiment with rodents to obtain the BA(bioavailability) values, then if the BA is below 10 percent, how can we interpret the results?

Actually such low BA in rodents is not due to the poor GI absorption in many cases, but rather due to first-pass hepatic metabolism or possibly first-pass hepatic eliminations, though we have many exceptions.

There are thus a lot of cases where the BA is very low due to the extensive hepatic metabolism in rodents. Nevertheless, based only on the low BA values, they have tendency to consider that it is merely due to poor intestinal absorption. However, there exist many data available in which the low BA comes from the extensive first-pass hepatic clearance, so you should also conduct other investigations to know the exact mechanism for the low BA values.

In my experience, even for the drugs with low BA in rodents which come from the extensive first pass hepatic metabolism, if you apply that to humans , then the metabolic activity is very often less than one-



tenth of rodents and the BA is high enough. In humans, in other words, hepatic first-pass metabolism is often minimal. But to come to such conclusion, you should have your IV and oral administration data in your hands, so I think you should make good use of them and make good accurate readings of the data that is available.

By doing so, you can know the low BA may come from the poor GI absorption or from the extensive first-pass hepatic metabolism. If you are already doing that, then please overlook my remarks, but listening to some of the comments, I have noticed that there are people who are rather ignorant about this situation. That's the reason why I wanted to bring it up. For example, once permeability and solubility have been correctly evaluated then you will be able to identify the problem from that kind of data , as Dr Yu indicated in Case Study 1.

Question from the audience: Yes, I think that's correct. There are some predetermined PK analysis methodologies available. So whether the reason is insolubility or whether it is the permeability in the GI tract, when the GI absorption is poor, if you apply it to humans you cannot expect the extent of absorption to be higher. But where the BA is low due to an extensive first-pass hepatic metabolism, then in a lot of cases it is only a very small problem when applied to humans.

Lawrence Yu, Director for Science, Office of Generic Drugs, CDER, Food and Drug Administration, USA: In answer to Dr. Sugiyama's comments, I never published this data. The reason I didn't do it is because I was trying to see whether a computer model is better than animal testing or not, and because of other responsibilities I never got round to publishing. I collected about 22 compounds, from 2001 and 2002; because I am at the FDA I have these advantages.

When I speak about correlation from the rat, I'm only talking about bioavailability, I'm not talking about drug disposition. Certainly, animal studies are utilized for drug disposition, to understand where the drug is located. But for absolute bioavailability, the predictive correlation from rats to humans is R2, or 0.19, while it's also about 0.2 from dogs. Actually it's in my computer. So what these figures mean is that absolute bioavailability in animals has no predictive value at all for humans.

Closing remarks

THANKS TO ALL OF YOU. I think it was a superb symposium with a great deal of learnings. We know better about the importance of the influx / efflux system and the mutation of the P-gp which can result in substantial changes in PK profiles. We also learnt that even if you apply Lipinski's rule of five properly, the poorly soluble actives are here to stay.

Another interesting input came from the facts that reduction in particle size doesn't necessary mean increased absorption. Good *in-vitro* profile doesn't mean good *in-vivo* profile. Good *in-vivo* results in rats or dogs doesn't mean good *in-vivo* results on humans.

In other words until you have performed the human clinical trials, do not limit your *in-vivo* tests on one formulation. Test also the alternatives which didn't give you a good profile on animals.

We also learnt that new industrial technologies are now available to achieve an acceptable absorption.

The last but not the least, considering the dissolution test as a predictor of *in-vivo* absorption is wrong. This QC dissolution test doesn't correlate with the *in-vivo* performance. New tests must be developed which will become good *in-vivo* predictor noticeably for class II.

So, thanks to all speakers who made excellent presentations. Thanks to all of you who made this symposium a very interactive one. Thanks to the translators who did a good job and thanks to the Capsugel team who perfectly organized such a superb day.

I'm going to finish with the chairman, my friend Professor Shinji Yamashita who spent a lot of time to prepare this symposium and who as usual efficiently chaired it. He also made sure, with elegance that everybody spoke within the allocated time. He deserves a special thanks on behalf of all of us.

Roland Daumesnil

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