Models to evaluate oral drug absorption

Professor Shinji YAMASHITA, Ph.D.

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Models to evaluate oral drug absorption

Professor Shinji Yamashita, Ph.D.
Faculty of Pharmaceutical Sciences, Setsunan University
45-1, Nagaotoge-cho, Hirakata, Osaka 573-01, Japan

Factors affecting Oral Drug Absorption

<table>
<thead>
<tr>
<th>Membrane Permeability</th>
<th>Chemical and Biological Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solubility</td>
<td>Luminal Volume and Contents</td>
</tr>
<tr>
<td>Chemical and Biological Stability</td>
<td>Luminal pH</td>
</tr>
<tr>
<td>Dose / Formulation</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1.

However, today, because of the very limited time – I only have 25 minutes for my talk – I’d like to focus on the issue of the membrane permeability of drugs. I think it is one of the most important factors to determine oral drug absorption.

In order to arrive at the most appropriate method to evaluate drug absorption, we first have to set out the purpose of each absorption study.

The over-riding purpose for the industry nowadays is to select a good candidate for oral drugs at the drug discovery stage. For this, we need a very rapid high-throughput screening (HTS) system to establish the oral drug absorption, and alongside such a system, we usually need an in-vitro method by which we can estimate the intrinsic drug factors – the membrane permeability, the solubility and the stability – very quickly. So the in-vitro screening system can be very useful, and looks set to become
even more so, now that the development of dedicated computer software is starting to open up new possibilities at the discovery stage.

The next need for a drug absorption study appears as we reach the preclinical or preformulation stage. Here, we need to consider more closely the in-vivo absorption of drugs, especially in humans, so the system used must predict the total bioavailability of drugs after oral administration in humans. This system must also take into account factors such as the fluid conditions in the GI tract, and also the drug’s formulation.

So for this screening, the in-vitro system must have a good correlation to in-vivo absorption and also to in-situ or in-vivo animal studies. In fact, sometimes it might be appropriate to carry out the research in humans at the preclinical stage.

The third and final purpose of a drug absorption study is to guarantee the quality of oral drug products, an issue related to the scientific regulatory requirements. This is also the point at which we have to be able to guarantee the BA (bioavailability) and BE (bioequivalence) of oral dosage forms.

For this stage of the research we need to establish standard methods which are recognized world wide — which, of course, is why the development of good ICH (International Conference on Harmonisation) guidelines and also the biopharmaceutical classification system, BCS, is so important. The concept of BCS is highly relevant to this stage, as you know.

So the next question is, what type of method, or what type of system is most appropriate for the different purposes of drug absorption studies? To answer it, we need to know next the advantages or disadvantages of the methods to evaluate oral drug absorption.

Now, many methodologies are available, from the in-vivo intubation study in humans to the in-vitro permeation study using the culture cell system, based in particular on the well-known Caco-2 monolayers approach, or the artificial membrane. There are now two popular methods for estimating drug permeability to the intestinal membrane: the in-situ perfusion study and the in-vitro permeation study using Caco-2 monolayers; two very useful methods for a drug absorption study.

Today I’d like to focus on these two methods and to demonstrate their advantages and disadvantages. I’ll also show you some data from my own research into ways of improving the potential of these methods in drug absorption screening.
The plots shown in Figure 2 are very famous, and are taken from work by Professor Amidon and also Professor Artursson concerning the relationship between the permeability of drugs and the fraction absorbed in humans. The left-hand plot presents data from an in-situ perfusion study using rat intestine, and the one on the right shows an in-vitro permeation study using Caco-2 monolayers. Now, both methods can predict the fraction absorbed in humans from the permeability found, so Figure 2 makes it seem very easy to develop a perfusion or Caco-2 study for drug screening.

So let me begin with the in-situ intestinal perfusion study in small animals. Now, three kinds of in-situ methods are widely used for estimating the drug permeability to the intestine: the loop method, re-circulating perfusion and the single-pass perfusion method. Each method has its advantages and disadvantages. For example, with loop method, drug movement – drug absorption – is not a steady state, and volume change cannot be corrected. So this method is not suited to kinetic analysis of the data.

But in the case of drugs with a very low permeability to the intestine, using a long period of incubation with loop method significantly increases absorbed fraction – something that is very difficult to achieve by using single-pass perfusion method. So each method has its advantages and disadvantages.

Various permeability parameters can be obtained by these methods, such as % of absorption during the defined period, Ka, the first-order rate constant of absorption, and the drug concentration ratio before and after the perfusion. These parameters are, of course, related to the membrane permeability, but they do also include factors related to the experimental conditions.

So if we want to compare the results of these methods against each other, or to predict in-vivo drug absorption from the permeability data, we have to arrive at the intrinsic permeability of the drugs.

Professor Amidon has already shown a very good equation to calculate the intrinsic permeability of drugs from the single-pass perfusion method. Figure 3 also shows a simple equation for calculating the intrinsic permeability from the data of a loop or perfusion study. I have no time to go through the equation in detail, but by using this simple method it’s very easy to exclude the effect of experimental conditions on the permeability, and so obtain the intrinsic permeability for the drug.
Figure 4 shows the permeability of cephalexin calculated from a number of studies using different methods and carried out under varying conditions. The calculated permeability was plotted against the cephalexin concentration. Cephalexin is a well-known drug, a kind of β-lactam antibiotic, which is transported by a peptide transporter. Although the data from the loop method which we ran shows some deviation from the results in this figure, the different sets of data reported by several different laboratories enable me to demonstrate the concentration-dependent permeability of cephalexin.

So this result simply indicates the importance of estimating the intrinsic permeability for each drug, in order to predict drug absorption.

However, in the in-situ drug absorption study, we used to calculate the permeability of drugs from the disappearance rate of drugs from the intestinal tract. Thus it’s sometimes very difficult to consider the permeability of drugs those undergo hydrolysis by esterase or a protease in the intestinal tract, or metabolism by CYP or other enzymes in the intestinal epithelium.

In order to get some information on the real absorption – I mean the absorption into the blood – we developed a vascular perfusion method using animal intestine. Figure 5 shows our schema for the vascular perfusion study, using rat jejunum. With this method we can estimate simultaneously both the disappearance of drug from the intestine and the appearance of drug in the blood.

Figure 6 is an example using this vascular perfusion method. I used MKA (metokephamid), a peptide drug with five amino acids. From the result, it is very clear that MKA underwent extensive degradation before absorption; the absorbed fraction in the blood is only 1 percent of the dose. The absorption of this drug can be improved by using a peptidase inhibitor such as puromycin or benstatin, which
significantly reduce the hydrolysis of this drug by enzymes and increase its absorption into the blood.

This perfusion study is a little bit difficult to perform because of the very complex follow-up methodology required, but by using this method we can estimate the real absorption of drugs like peptides or other presystemically-unstable drugs.

I’d like to move on now to Figure 7, an in-vitro permeation study with a model intestinal membrane. Many in-vitro model membranes are now available for in-vitro permeation studies, including human, dog or pig cell lines. The immortalized intestinal cell line has recently become available, and the artificial lipid membrane is also very useful for in-vitro drug permeation studies.

Caco-2 monolayer is now one of the key methods within this group, as many researchers have already shown its high potential for evaluating in-vivo absorption from the permeability of drugs. Also, this monolayer, this cell line, is already well characterized, meaning that we now have access to a huge data pool for studying the relationship between permeability and absorption.

However the Caco-2 cell monolayer is not a perfect system for estimating all kinds of drug absorption because Caco-2 monolayers show very low expression of the transporter or enzymes. In addition, the long culture period that’s necessary to get a good monolayer, usually 21 days, is labor intensive, time consuming and not compatible to the high throughput screening. So we tried to improve this disadvantage of Caco-2 monolayers by using the techniques of gene transfection as well as other confidential techniques. Today I’m going to show you some data for improving the feature of Caco-2 monolayers.

Now, a newly developed culture protocol compatible with the HTS screening system – 3-DAY BIO-COAT HTS Caco-2 ASSAY SYSTEM – has been launched by the Beckton Dickinson company. With this system, Caco-2 monolayers can be obtained within only 3 days by adding a differentiation promoter, sodium butyrate. This system provides a means to increase the efficiency of high-throughput screening of drug absorption by delivering a rapid and automated system that is compatible with HTS.

However, from the research in our laboratory using this system, we found that the Caco-2 monolayers formed in 3 days are much leakier than the monolayers obtained by the 21-day culture protocol and this feature of the monolayer makes it difficult to rank order the permeability of compounds having a low to moderate permeability. So we tried to improve it by modifying the protocol of culture to get good monolayers. Protocol 2, in which we used 5 days, not 3, for the culture with the medium containing the serum enabled us to obtain satisfactory data.

As shown in Figure 8, the method using 3-day culture by Beckton Dickinson shows that mannitol, atenolol and cephalexin all have very high and almost the same levels of permeability, suggesting that it is very difficult to put the permeability of these

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Permeability (x 10^-7 cm/sec)</th>
<th>21 days</th>
<th>3 days</th>
<th>Protocol 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mannitol</td>
<td>4.08 ± 0.80</td>
<td>21.6 ± 3.14</td>
<td>7.82 ± 0.738</td>
<td></td>
</tr>
<tr>
<td>Atenolol</td>
<td>4.25 ± 0.33</td>
<td>21.6 ± 4.51</td>
<td>6.94 ± 0.534</td>
<td></td>
</tr>
<tr>
<td>Propranolol</td>
<td>64.20 ± 15.3</td>
<td>64.5 ± 8.67</td>
<td>56.30 ± 4.060</td>
<td></td>
</tr>
<tr>
<td>Cephalexin</td>
<td>8.42 ± 0.47</td>
<td>19.8 ± 4.95</td>
<td>13.70 ± 1.370</td>
<td></td>
</tr>
</tbody>
</table>

Mannitol: Apical pH 7.4, Basolateral pH 7.4
Other drugs: Apical pH 6.0, Basolateral pH 7.4

Figure 8.
compounds into rank ordering. However, our new 5-day protocol produces data compatible with the results of 21-day culture, where cephalexin permeability is high, as compared to mannitol and atenolol. So this data suggests that modifying the protocol of the Caco-2 cell culture allow us to obtain more appropriate properties of the monolayers very easily.

The next project I did was to develop a cell-based assay system for drug transport, specifically aimed at drugs transported by an oligopeptide transporter (PepT1). Because this transporter transports peptide and peptide mimetic drugs into cells with proton, transport of these drugs causes acidification in the cells. So by monitoring the pH change inside the cells I think it’s possible to detect some information on the transport of those drugs.

We have detected the intracellular pH of Caco-2 cells by monitoring the fluorescent intensity of an appropriate fluorescence-probe by utilizing the Fluorometric Imaging Plate Reader (FLIPR, Molecular Devices Corp.). This system can provide very rapid evaluation of intracellular pH change, the speed is 96 wells /15 min. Also, this system dose not require the effort to measure the drug concentration by using HPLC (high-performance liquid chromatography) or other determination systems.

Figure 9 illustrates typical data showing the fluorescence change, which means a pH change in the Caco-2 cells. The addition of cefazolin (CEZ), which is not a substrate of PepT1, to the apical surface of cell monolayer showed no decrease of the intracellular pH. On the other hand, cephalexin (CEX), a good substrate of PepT1, caused a gradual decrease in the fluorescence intensity, indicating a pH change in the cell. Then I calculated the change in fluorescence intensity and tried to correlate it with the permeability of each drug measured by rat perfusion study.

Figure 10 shows, not straight, but saturated relationship obtained between the drug permeability and the change in the intercellular pH. Maybe this saturation pattern is due to the low expression level of the PepT1 in Caco-2 cells. But by measuring the intercellular pH change, we can determine the transport of these kinds of drugs very quickly, which can be applied to the high-throughput screening system.

The latest project we are attempting is to establish an evaluation system for oral absorption of poorly water-soluble drugs. In this project we are trying to develop an in-vitro system which can evaluate dissolution and permeation of drugs simultaneously. The purpose is twofold: to evaluate the oral absorption of poorly-soluble drugs from in-vitro experiments with Caco-2 monolayers, and to screen the most effective formulation for improving the absorption of those kinds of drugs.

Figure 11 shows a very simple approach for expressing the process of drug in the intestine. Drugs are absorbed in line with the dissolved concentration in the intestine and the final absorbed percent could be determined by those parameters, the permeability (permeation clearance) of drugs to the intestinal membrane and the AUC (area under the
curve) of drugs in the intestine up to time T; time T means the transit time of drugs in the intestine. According to this scheme of drug absorption, we developed a new chamber system for absorption screening of drugs having a very low solubility in water.

The Caco-2 monolayers are mounted between the donor and the receptor chambers and the drugs are applied as solid powder or as various types of formulation. By measuring the permeated amount of drugs across the monolayer during the defined period, time T, we can predict the \textit{in-vivo} absorption of this kind of drugs from the solid dosage form. I have no time today to show you the results of this work but we are now getting very interesting results and they will be presented at the next FIP Millennial World Congress in April.

So, to conclude. My presentation has covered many kinds of techniques available to evaluate oral drug absorption. The key to success in the absorption study is to choose the most appropriate method and/or system for the purpose of each study and, of course, it’s very important to try to improve the system for yourselves, by adjusting the data more to your purpose.

Before ending my talk, I would like to express my appreciation to my co-workers and my students for their great contribution to the research. Thank you for your attention.

**Thomas H. Kissel, Philipps University of Marburg, Germany:**

Thank you, Professor Yamashita, maybe we have time for one question.

**Professor Gordon Amidon, University of Michigan, USA:**

Shinji, I think the fluorescence technique is very exciting but you were using that just for PepT1, carrier-mediated transport, right? I think in your abstract you said it worked for salicylic acid so maybe it works for carboxylic acids, right? But is there a general pH change for ‘passive’ compounds?

**Professor Shinji Yamashita, Setsunan University, Osaka, Japan:**

I tried two types of drugs. One is the one I showed, the PepT1 substrate, the other is a monocarboxylic acid, like salicylic acid or benzoic acid. It involves very sensitive matter, the mechanism of absorption of monocarboxylic acid, carrier-mediated transport by MCT1 or passive mechanisms I have proposed. But anyway, that type of acid drug can change the intercellular pH.

Now I’m trying to use cationic drugs with this system, but it may prove impossible with a neutral drug. It’s very difficult to get a good surrogate marker for the transport of passively absorbed drugs.

Monitoring the intracellular pH is only one idea, and there may be other ways of achieving more efficient high-throughput screening based on the cell-based assay. But it’s very difficult to get a good surrogate marker for the transport of passively absorbed drugs, I think.