Strategies in drug delivery:
Intestines to intracellular organelles

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Number of the new drugs accepted in Japan

「国立医薬品食品衛生研究所・医薬品医療機器審査センター（PMDEC）」
**Only in Japan?**

Number of the new drugs accepted in USA

![Graph showing the number of new drugs accepted in USA from 1997 to 2004.](image)

*Source: [http://www.fda.gov/cder/](http://www.fda.gov/cder/)
Year 2004: Figure available to March, pro-rata to year end*

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**Reasons of the failure in drug development at the pre-clinical or clinical stage**


- Lack of Efficacy - 30%
- Animal Toxicity - 11%
- Adverse Effects in Man - 10%
- Commercial Reasons - 5%
- Poor ADME Properties - 39%

PhRMA- FDA Benchmarking Survey, (2001)

- Lack of Efficacy - 24%
- Safety Issues - 25%
- Marketing Issues - 24%
- Poor ADME Properties - 27%
Increase in molecular weight and lipophilicity of new drugs

Molecular weight of new drugs

Year

0 100 200 300 400 500
Molecular Weight


Lipophilicity of new drugs

Year

0 1 2 3
\text{cLogP}


Trend of new drug candidates

Physicochemical properties
molecular weight
Log P
solubility
chemical stability
nonspecific binding

Basic PK properties
membrane permeability
metabolic stability
enzyme inhibition or induction
protein binding
transporter affinity ….

Nowadays, most of new compounds synthesized in pharmaceutical companies have some problems in their physicochemical or PK properties. However, if all such compounds are withdrawn, no new drugs will be developed.

Chemical Optimization
DDS technology
Strategy of Drug Delivery

Absorption

Distribution

Metabolism

Excretion

Improve of drug absorption
absorption enhancement
controlled release
new administration route

Drug targeting
to the tissue
to the cell
to the organelle

Strategies in drug delivery: Intestines to intracellular organelles

Faculty lecture

Dr. Shinji Yamashita (Setsunan University)
Issue: Oral Drug Absorption

Dr. Valentino J. Stella (University of Kansas)
Issue: Prodrug approach

Dr. Yukio Kato (Kanazawa University)
Issue: Transporter

Dr. Jeffrey Krise (University of Kansas)
Issue: Intracellular delivery

Student presentation

Arik Dahan (The Hebrew University of Jerusalem)
ADVANTAGES AND LIMITATIONS OF AN IN-VITRO LIPOLYSIS MODEL AS A PREDICTIVE TOOL IN THE DEVELOPMENT OF LIPOID BASED ORAL FORMULATIONS FOR LIPOPHILIC DRUGS

Christopher J. Endres (University of Washington)
THE ROLE OF THE EQUILIBRATIVE NUCLEOSIDE TRANSPORTER 1 (ENT1) IN RIBAVIRIN DISPOSITION IN MICE
**Process of oral drug absorption**

**Formulation**
- Brush Border Membrane
- Solution
- Microsome
- BABA Solution
- dissolution
- permeation
- first-pass metabolism

**Liver**

**BA**

**Macroscopic analysis of drug absorption**

- $D$
- $k_{dis}$
- Solution
- $C_{intestine}$
- $CL_{perm}$
- Absorption $F(\%)$

$AUC_t = \int_0^t C_{intestine} dt$

$FD = AUC_0^t \times CL_{perm}$
Permeability-Solubility relation in drug absorption

Dose: 100 mg, Suspension: particle radius = 10 μm

39.8 Percent of 33093 medicinal chemistry compounds have low solubility (<= 20 μg/mL)
How to improve the oral absorption of poorly water-soluble drugs

Formulations
- micronization, solid dispersion
- additives (surfactants, cyclodextrin …)
- lipid based formulation (self-emulsifying system …)

Salt-form

Prodrug

Cost performance
User friendly
Stability

Effect of micronization on intestinal absorption of poorly soluble drugs

Solubility Cs = 1.0 (µg/ml)

Fraction absorbed in human (%) vs. size $r_0$ (µm)

$P_{eff}$ : permeability to human intestine (cm/sec x 10^{-4})
Effect of Solubility on intestinal absorption of poorly soluble drugs

![Graph showing the effect of solubility on intestinal absorption.](image)

\[ r_0 = 1.0 \text{ (µm)} \]

- \( P_{eff} = 5.0 \)
- \( P_{eff} = 3.0 \)
- \( P_{eff} = 1.0 \)
- \( P_{eff} = 0.5 \)
- \( P_{eff} = 0.1 \)

Fraction absorbed in human (%)

Cs (µg/ml)

\[ \text{Peff : permeability to human intestine} \quad (\text{cm/sec x } 10^{-4}) \]

Oral drug delivery of poorly soluble drugs

In order to develop the efficient system for oral delivery of poorly-soluble drugs, not only the solubility but also the membrane permeability must be taken into consideration.
**Caco-2 monolayer used for drug permeation assay**

Origin: Colon carcinoma (Human)

Monolayer: 16-21 days culture

Permeability of highly soluble drugs across Caco-2 monolayer well correlates to human absorption.

![Caco-2 monolayer diagram](image)


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**Difficulties to assess the oral absorption of poorly water-soluble drugs in vitro**

Technical difficulties to measure the membrane permeability

- How to dissolve the drugs to apply the membrane
- High adsorption (non-specific binding) of drugs to the experimental apparatus

Intra- and inter individual variations in *in vivo* absorption

- Effect of food intake
- Effect of various formulations
**Dissolution/permeation system (D/P system)**

- **Apical side**
  - pH 6.5
  - 8.0 mL
  - 200 rpm
  - Dissolution phase
  - Diffusion phase
  - Stirring
  - Caco-2 monolayer

- **Basal side**
  - pH 7.4
  - 5.5 mL
  - 200 rpm
  - Detection phase

**Pictures of Dissolution/Permeation system**

- Apical chamber
- Basal chamber
- D/P system
- D/P system with stirring system
- Basal side
- Apical side
- Looking from above view point
- Looking from side view point
### Experimental conditions of the D/P system

#### Fluid volume
- **Human**: 500 - 1000 mL
- **D/P system**: 8.0 mL


#### Apical side | Basal side
| Volume   | 8.0 mL | 5.5 mL |
| pH       | 6.5    | 7.4    |
| Medium   | Simulated intestinal fluid | 4.5 w/v% BSA<sup>b</sup> |
| Stirring rate | 200 rpm | 200 rpm |

**Applied amount**: 1/100 of clinical dose

<sup>a</sup> HBSS supplemented with 19.45 mM glucose and 10 mM HEPES.

### Simulated intestinal fluid used in D/P system

#### Fasted State Simulated Intestinal Fluid (FaSSIF)
#### Fed State Simulated Intestinal Fluid (FeSSIF)

<table>
<thead>
<tr>
<th>pH</th>
<th>NaTC&lt;sup&gt;*&lt;/sup&gt;</th>
<th>Lecithin</th>
<th>Osmolality (mOsm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FaSSIF</td>
<td>6.5</td>
<td>3 mM</td>
<td>0.75 mM</td>
</tr>
<tr>
<td>FeSSIF</td>
<td>5.0</td>
<td>15 mM</td>
<td>3.75 mM</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>pH</th>
<th>NaTC&lt;sup&gt;*&lt;/sup&gt;</th>
<th>Lecithin</th>
<th>Osmolality (mOsm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FaSSIF&lt;sub&gt;mod&lt;/sub&gt;</td>
<td>6.5</td>
<td>3 mM</td>
<td>0.75 mM</td>
</tr>
<tr>
<td>FeSSIF&lt;sub&gt;mod&lt;/sub&gt;</td>
<td>6.5</td>
<td>15 mM</td>
<td>3.75 mM</td>
</tr>
</tbody>
</table>

<sup>*</sup> NaTC: Sodium taurocholate
### Permeated amount of various drugs in the D/P system

<table>
<thead>
<tr>
<th>Drug</th>
<th>Clinical dose (mg)</th>
<th>Applied amount (mg)</th>
<th>Permeated amount Fasted (% of dose/2 h)</th>
<th>Permeated amount Fed (% of dose/2 h)</th>
<th>Human Abs. Fasted (% of dose)</th>
<th>Human Abs. Fed (% of dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acyclovir</td>
<td>200</td>
<td>2.0</td>
<td>0.049</td>
<td>0.029</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Atenolol</td>
<td>50</td>
<td>0.5</td>
<td>0.097</td>
<td>0.103</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>100</td>
<td>1.0</td>
<td>8.393</td>
<td>—</td>
<td>83</td>
<td>—</td>
</tr>
<tr>
<td>Chlorpheniramine</td>
<td>6</td>
<td>0.06</td>
<td>5.364</td>
<td>—</td>
<td>80</td>
<td>—</td>
</tr>
<tr>
<td>Danazol</td>
<td>100</td>
<td>1.0</td>
<td>0.125</td>
<td>0.250</td>
<td>24</td>
<td>76</td>
</tr>
<tr>
<td>Griseofulvin</td>
<td>250</td>
<td>2.5</td>
<td>0.640</td>
<td>—</td>
<td>80</td>
<td>—</td>
</tr>
<tr>
<td>Griseofulvin</td>
<td>500</td>
<td>5.0</td>
<td>0.244</td>
<td>0.295</td>
<td>40</td>
<td>78</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>50</td>
<td>0.5</td>
<td>6.295</td>
<td>1.742</td>
<td>85</td>
<td>85</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>40</td>
<td>0.4</td>
<td>4.268</td>
<td>3.119</td>
<td>95</td>
<td>95</td>
</tr>
<tr>
<td>Nadolol</td>
<td>80</td>
<td>0.8</td>
<td>0.124</td>
<td>—</td>
<td>34</td>
<td>—</td>
</tr>
<tr>
<td>Piroxicam</td>
<td>10</td>
<td>0.1</td>
<td>15.659</td>
<td>14.695</td>
<td>95</td>
<td>95</td>
</tr>
<tr>
<td>Pranlukast</td>
<td>225</td>
<td>2.25</td>
<td>0.072</td>
<td>0.040</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Propranolol</td>
<td>10</td>
<td>0.1</td>
<td>4.430</td>
<td>3.401</td>
<td>95</td>
<td>95</td>
</tr>
<tr>
<td>Warfarin</td>
<td>5</td>
<td>0.05</td>
<td>10.793</td>
<td>9.751</td>
<td>94</td>
<td>94</td>
</tr>
</tbody>
</table>

### Correlation between in vivo human absorption and in vitro permeated amount in the D/P system

**Predicted absorption (%)**

\[
\text{Abs}_{\text{max}} \times \text{PA}_{\text{r}} = \frac{\text{Abs}_{\text{max}} \times \text{PA}_{\text{r}} \times \text{PA}_{50'} + \text{PA}_{\text{r}}}{\text{PA}_{50'} + \text{PA}_{\text{r}}} \quad \text{Eq. 2}
\]

- Abs: Maximum absorption (100%)
- PA: Permeated amount of 50% absorption
- PA: Permeated amount in the D/P system (% of dose/2 h)
- r: Hill’s coefficient
**Properties of three drugs**

<table>
<thead>
<tr>
<th></th>
<th>Albendazole</th>
<th>Quazepam</th>
<th>Nateglinide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Charge</td>
<td>Base</td>
<td>Base</td>
<td>Acid</td>
</tr>
<tr>
<td>cLog D*</td>
<td>3.0</td>
<td>4.9</td>
<td>1.2</td>
</tr>
</tbody>
</table>

**Solubility (µg/mL)**

<table>
<thead>
<tr>
<th></th>
<th>Water</th>
<th>FaSSIFmod</th>
<th>FeSSIFmod6.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albendazole</td>
<td>0.32</td>
<td>1.2</td>
<td>8.1</td>
</tr>
<tr>
<td>Quazepam</td>
<td></td>
<td>3.8</td>
<td>5.5</td>
</tr>
<tr>
<td>Nateglinide</td>
<td></td>
<td>&gt; 75.0</td>
<td>&gt; 75.0</td>
</tr>
</tbody>
</table>

**Papp (x10^-6 cm/sec)**

<table>
<thead>
<tr>
<th></th>
<th>Caco-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albendazole</td>
<td>38.7 ± 2.8</td>
</tr>
<tr>
<td>Quazepam</td>
<td>106.3 ± 14.0</td>
</tr>
<tr>
<td>Nateglinide</td>
<td>15.4 ± 2.6</td>
</tr>
</tbody>
</table>

* cLog D was calculated by Pallas 3.0.
† Conditions of permeation study were following:
apical side: TM with 1% DMSO (pH 6.5) and basal side: TM with 4.5% BSA (pH 7.4).

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**Effect of food on oral absorption of albendazole, quazepam and nateglinide in the clinical study**

<table>
<thead>
<tr>
<th>Drug &amp; Food state</th>
<th>Dose (mg)</th>
<th>Cmax (µg/mL)</th>
<th>Tmax (h)</th>
<th>AUC (ng·h/mL)</th>
<th>AUC ratio (Fasted/Fed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albendazole</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasted state</td>
<td>400</td>
<td>0.25</td>
<td>2.3</td>
<td>1340</td>
<td>4.0</td>
</tr>
<tr>
<td>Fed state</td>
<td></td>
<td>1.11</td>
<td>3.8</td>
<td>5298</td>
<td></td>
</tr>
<tr>
<td>Quazepam</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasted state</td>
<td>20</td>
<td>28.0</td>
<td>3.0</td>
<td>291</td>
<td>1.6</td>
</tr>
<tr>
<td>Fed state</td>
<td></td>
<td>81.2</td>
<td>2.0</td>
<td>468</td>
<td></td>
</tr>
<tr>
<td>Nateglinide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasted state</td>
<td>60</td>
<td>3.09</td>
<td>1.9</td>
<td>6.93</td>
<td>0.9</td>
</tr>
<tr>
<td>Fed state</td>
<td></td>
<td>2.05</td>
<td>2.3</td>
<td>6.54</td>
<td></td>
</tr>
</tbody>
</table>

Data taken from literatures.
Estimation of food-effect on oral absorption of albendazole, quazepam and nateglinide from in vitro study in D/P system

<table>
<thead>
<tr>
<th>Drug &amp; Food state</th>
<th>D/P system</th>
<th></th>
<th>In vivo</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Applied amount (mg)</td>
<td>Permeated amount (%)</td>
<td>Estimated absorption (%)</td>
<td>AUC ratio (Fasted/Fed)</td>
</tr>
<tr>
<td>Albendazole</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasted state</td>
<td>4.0</td>
<td>0.041</td>
<td>14</td>
<td>3.5</td>
</tr>
<tr>
<td>Fed state</td>
<td>0.122</td>
<td>0.778</td>
<td>49</td>
<td>3.5</td>
</tr>
<tr>
<td>Quazepam</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasted state</td>
<td>0.2</td>
<td>1.174</td>
<td>75</td>
<td>1.2</td>
</tr>
<tr>
<td>Fed state</td>
<td>0.778</td>
<td>11.109</td>
<td>97</td>
<td>1.0</td>
</tr>
<tr>
<td>Nateglinide</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasted state</td>
<td>0.6</td>
<td>14.712</td>
<td>97</td>
<td>1.0</td>
</tr>
<tr>
<td>Fed state</td>
<td>11.109</td>
<td>100</td>
<td>1.0</td>
<td>0.9</td>
</tr>
</tbody>
</table>

† Permeated amount (% of dose/2 h)
‡ Predicted absorption was calculated by Eq. 2
**Effect of dosage form on oral absorption of danazol in human in vivo**

<table>
<thead>
<tr>
<th>Formulation &amp; Food state</th>
<th>Dose (mg)</th>
<th>$C_{\text{max}}$ (ng/mL)</th>
<th>AUC (ng·h/mL)</th>
<th>Absorption (% of dose)</th>
<th>Ratio (Fasted/Fed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capsule</td>
<td>Fasted state</td>
<td>100</td>
<td>37 ±16</td>
<td>204 ±125</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Fed state</td>
<td>100</td>
<td>101 ±42</td>
<td>639 ±269</td>
<td>76</td>
</tr>
<tr>
<td>Emulsion</td>
<td>Fasted state</td>
<td>100</td>
<td>155 ±55</td>
<td>779 ±189</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>Fed state</td>
<td>100</td>
<td>126 ±56</td>
<td>844 ±194</td>
<td>100</td>
</tr>
</tbody>
</table>


---

**Estimation of formulation-effect on oral absorption of danazol from in vitro study in D/P system**

[Graph showing permeated amount (% of dose/2 h) vs. human absorption (% of dose) for different formulations: Gelucire (97%), Fed State (74%), Fasted State (40%), Powder (30%), and 0.125, 1.101, 1.787]
**Estimation of formulation-effect on oral absorption of danazol from in vitro study in D/P system**

<table>
<thead>
<tr>
<th>Dosage form</th>
<th>D/P system</th>
<th>In vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td>&amp; Food state</td>
<td>Applied amount</td>
<td>Permeated amount</td>
</tr>
<tr>
<td>Crude powder</td>
<td>(mg)</td>
<td>(% of dose/2 h)</td>
</tr>
<tr>
<td>Fasted state</td>
<td>1.0</td>
<td>0.125 ±0.003</td>
</tr>
<tr>
<td>Fed state</td>
<td></td>
<td>0.250 ±0.006</td>
</tr>
<tr>
<td>Gelucire 44/14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasted state</td>
<td>1.0</td>
<td>1.101 ±0.089</td>
</tr>
<tr>
<td>Fed state</td>
<td></td>
<td>1.787 ±0.078</td>
</tr>
</tbody>
</table>

**Summary**

- Oral formulations to improve BA of poorly-soluble drugs should be designed based on the physicochemical properties of drugs (including permeability).

- In addition to the low solubility, nonspecific binding of drugs might cause the large deviations in apparent permeability.

- *In vitro* D/P system can be the useful tool to evaluate the absorption of poorly soluble drugs and the effects of food and formulation on it.