Flavonoid-Drug Interactions: Effects of Flavonoids on ABC Transporters

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Flavonoids

- **Basic Structure:**

- **Subclasses:**
  - The most abundant polyphenols present in human diet (vegetables, fruits, red wine and tea)

- Flavones
- Flavonols
- Flavanones
- Isoflavones
- Chalcones
Flavonoids

- Epidemiological studies:
  reduced risk of cancer, coronary heart disease, and osteoporosis
- Biochemical and Pharmacological activities:
  anti-oxidant;
  anti-viral;
  anti-carcinogenic;
  anti-inflammatory;
  anti-angiogenic;
  anti-estrogenic (estrogenic).
Flavonoids Have Little Toxicity

**Toxicity:**

- Long history of consumption with exceptional safety record;
- Extremely large doses used in animal studies;
  
  Acute LD$_{50}$ for rats: 2 g / kg BW by direct injection into blood.

“The margin of safety for the therapeutic use of flavonoids in humans, therefore, is very large and probably not surpassed by any other drug in current use”

Flavonoid Products
Herbal Use in Select Populations

• **HIV infected patients**  
  – 68% of patients used herbs, vitamin, dietary supplements  
  – Consumed herbal remedies to boost immunity, prevent nausea, diarrhea, or weight loss, relieve stress or depression.

• **Post-menopausal women**  
  *(Mahady et al. Menopause 10:65-72, 2003)*  
  – Botanical dietary supplements used by 79% (395/500) of post-menopausal women within the last year.  
    • Commonly used supplements include Soy (42%), green tea (35%), Chamomile (21%), Ginkgo (20%), Ginseng (18%), Echinacea (15%), & SJW (7%).
Flavonoid Products

- Do not need FDA approval
- Drug interactions with conventional drugs have not been evaluated
ABC Proteins

- ATP binding cassette (ABC) superfamily
  - P-glycoprotein (MDR1, ABCB1)
  - Multidrug Resistance Associated Proteins (MRP, ABCC)
  - Breast Cancer Resistance Protein (BCRP, ABCG2)
- Efflux molecules out of cells
- Tumor → multi-drug resistance
- Present in the liver, kidney, BBB, gastrointestinal tract where important for drug disposition
Transporters
- absorption
- efflux

Intestine

Transporters

Metabolism

Transporters

Sandy K Pang
ABC Transporter Expression in the Liver and Gastrointestinal Tract

➢ High expression of MDR1, MRP2 and BCRP in the liver

➢ Expression throughout the gastrointestinal tract

Dietrich et al, Gut 2003, 52:1788
P-glycoprotein

- Two homologous halves
- Each consists of:
  - 6 TM
  - 1 ATP binding site
- Substrate binding sites are located in TMs

Broad substrate specificity:
  - anthracyclines, Vinca alkaloids, epipodophyllotoxins and taxol
  - cyclosporine, digoxin, verapamil etc.

One of the major mechanisms for cancer MDR and drug-drug, drug-food interactions.
General Study Design

- *In vitro studies-* sensitive (no expression) and overexpressing cell lines (characterized) examining accumulation or flux
- *In vitro studies-* effects on the cytotoxicity of chemotherapeutic drugs
- *In vitro studies-* mechanism of interaction; additive effects; SAR/QSAR
- *In vivo studies in animals*
**3H-DNM Accumulation in MCF-7 cells**

**MCF-7/sensitive**

- **Biochanin A**
- **Morin**
- **Phloretin**
- **Silymarin**
- **Verapamil**

**MCF-7/ADR**

- **Control**
- **Biochanin A**
- **Morin**
- **Phloretin**
- **Silymarin**
- **Verapamil**

Flavonoid concentration: 50 µM, Verapamil concentration: 100 µM

Data expressed as mean ±SD, N= 9-12; ***: p < 0.001
Increase of $^3$H-DNM accumulation in P-gp Positive Cells Is Flavonoid Concentration Dependent

DNM accumulation was determined in MDA435/LCC6MDR1 cells.

Minimal Conc. for significant change:
- Biochanin A and morin: 20-30 µM
- Phloretin and silymarin: 30-50 µM
Increase of Doxorubicin Cytotoxicity by Biochanin A

Doxorubicin cytotoxicity was determined in MDA435/LCC6MDR1 cells
Flavonoid-P-gp Interactions

Mechanisms:
- Biochanin A is not a substrate
- Flavonoids affect P-gp ATPase activity or the P-gp ATPase activity induced by verapamil
- Some flavonoids can inhibit P-gp ATP and/or substrate binding
  - Bifunctional binding interactions with nucleotide binding domains at ATP and vicinal substrate binding site (DiPietro et al., 2002)
- No effect on P-gp expression in MCF-7/ADR cells or human hepatocytes following longer incubations for the flavonoids and concentrations examined
Flavonoid-Drug Interactions

- flavone + paclitaxel in rats

- quercetin + paclitaxel in rats
Flavonoid-Drug Interactions

- quercetin + cyclosporine in pigs
- phellamurin + cyclosporine in rats

Hsiu et al. (2002) Life Sciences 72:227-235
Flavonoid-Drug Interactions

- Flavonoid-drug interactions could occur upon coadministration but appear to be substrate dependent (and will be flavonoid dependent)

- However, other factors (for example, other transporters) may be important - there may be poor prediction if only based on their interaction with P-glycoprotein and CYP3A4
MRP1 is a 190-kDa protein encoded by the MRP1 gene. Expressed in most normal tissues in the human body and in several types of tumors such as lung carcinoma, myeloid leukemia, neuroblastoma, and breast cancer.

Substrates of MRP1:

- Endogenous substrates: leukotriene C₄, glutathione disulfide, steroid glucuronides (17β-estradiol 17-β-D-glucuronide)
- Exogenous substrates: daunomycin, vinca alkaloid (vinblastine), methotrexate, fluorouracil, chlorambucil, calcein, drug conjugates
Involvement of GSH in MRP1-mediated transport

Qingcheng Mao
Flavonoids increase the accumulation of $^3$H-VBL in Panc-1 Cells

Nguyen et al, J Pharm Sci 2003
Flavonoids that have no effect on the accumulation of $^3$H-VBL in Panc-1 cells

- Control
- Verapamil
- Apigenin
- Chrysin
- Diosmin
- Luteolin
- Galangin
- Myricetin
- Rutin
- Hesperetin
- Naringenin
- Naringin
- Epigallocatechin
- Daizdein
Inhibitory constants for $^3$H-LTC4 transport in MRP1 membrane vesicles

<table>
<thead>
<tr>
<th>FLAVONOID</th>
<th>Ki (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>myricetin</td>
<td>13.3 ± 2.7 (SD)</td>
</tr>
<tr>
<td>quercetin</td>
<td>8.1 ± 1.7</td>
</tr>
<tr>
<td>naringenin (+ GSH)</td>
<td>20.8 ± 6.4</td>
</tr>
<tr>
<td>kaempferol</td>
<td>2.4 ± 1.6</td>
</tr>
<tr>
<td>apigenin (+GSH)</td>
<td>4.9 ± 0.7</td>
</tr>
</tbody>
</table>

Leslie et al., Mol Pharmacol, 2001
Mechanisms involved in MRP1 Inhibition

- Decreased intracellular GSH appears to be important for some, but not all, flavonoids
- No effects on glutathione S-transferase were observed
- No effects on the expression of MRP1 were seen with longer-term incubations
- Significant effects on MRP1 ATPase activity
- Likely not substrates
- Binding at a substrate or ATP domain is likely also involved
Breast Cancer Resistance Protein (BCRP)

- A new member of ABC transporter superfamily;
- Also known as ABCP (ABC transporter in placenta), MXR (mitoxantrone-resistance protein), ABCG2 (the 2nd family of ABC subgroup G)

Broad substrate specificity;
- mitoxantrone, topoisomerase I inhibitors, methotrexate, topotecan, zidovudine, lamivudine, flavopiridol, sulfate conjugates, omeprazole, genistein
Breast Cancer Resistance Protein (BCRP)

- **Expression in tumors:**
  - leukemia: AML
  - solid tumors: colon cancer, lung cancer, myeloma, endometrial tumor, etc.

  *Role in clinical MDR*

- **Expression in normal tissues:**
  - placenta
  - intestine (expression level is higher than P-glycoprotein)
  - liver canalicular membrane
  - brain microvessels

  *An important determinant for drug disposition*

Steinbach et al. (2002) Leukemia 16: 1443-1447  
BCRP Clinical Implications

• Apparent oral bioavailability: 40.0% ⇒ 97.1%

Breast Cancer Resistance Protein (BCRP)

GF120918 + topotecan in P-gp knockout mice, oral administration

Plasma concentration of topotecan

Biliary excretion of topotecan

A total of 20 naturally occurring flavonoids were studied.

<table>
<thead>
<tr>
<th>Flavones:</th>
<th>Isoflavones:</th>
<th>Flavanones:</th>
</tr>
</thead>
<tbody>
<tr>
<td>apigenin</td>
<td>biochanin A</td>
<td>hesperetin</td>
</tr>
<tr>
<td>chrysin</td>
<td>daidzein</td>
<td>naringenin</td>
</tr>
<tr>
<td>luteolin</td>
<td>genistein</td>
<td>silybin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>silymarin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>epigallocatechin (EGC)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>epigallocatechin gallate (EGCG)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>naringin</td>
</tr>
<tr>
<td>Flavonols:</td>
<td>Chalcones:</td>
<td></td>
</tr>
<tr>
<td>fisetin</td>
<td>phloretin</td>
<td></td>
</tr>
<tr>
<td>kaempferol</td>
<td>phloridzin</td>
<td></td>
</tr>
<tr>
<td>morin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>myricetin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>quercetin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Mitoxantrone Accumulation In MCF-7 Cells

Mitoxantrone: 3 µM
Flavonoid: 50 µM
N = 6 or 7
Correlation Between MX Accumulation in H460 MX20 and MCF-7 MX100 Cells

$R^2 = 0.74$, $p < 0.05$
## Mitoxantrone Cytotoxicity in MCF-7 Cells

<table>
<thead>
<tr>
<th>compounds</th>
<th>MCF-7/sensitive</th>
<th>MCF-7 MX100</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 µM</td>
<td>50 µM</td>
<td>2.5 µM</td>
</tr>
<tr>
<td>control</td>
<td>5.30 ± 2.22</td>
<td></td>
<td>199 ± 19.3</td>
</tr>
<tr>
<td>apigenin</td>
<td>3.66 ± 0.52</td>
<td>3.44 ± 0.35</td>
<td>219 ± 10.0</td>
</tr>
<tr>
<td>BA</td>
<td>2.40 ±0.27***</td>
<td>1.86 ±0.35***</td>
<td>107 ± 17.6***</td>
</tr>
<tr>
<td>chrysin</td>
<td>0.95 ± 0.46***</td>
<td>18.8 ± 0.06***</td>
<td>6.25 ± 2.13***</td>
</tr>
<tr>
<td>genistein</td>
<td>6.98 ± 0.81</td>
<td></td>
<td>148 ± 23.2</td>
</tr>
<tr>
<td>kaempferol</td>
<td>6.10 ± 0.96</td>
<td></td>
<td>196 ± 20.9</td>
</tr>
<tr>
<td>hesperetin</td>
<td></td>
<td></td>
<td>88.6 ± 20.8***</td>
</tr>
<tr>
<td>naringenin</td>
<td></td>
<td></td>
<td>189 ± 11.6</td>
</tr>
<tr>
<td>silymarin</td>
<td></td>
<td></td>
<td>99.1 ± 50.2***</td>
</tr>
<tr>
<td>FTC</td>
<td>2.30 ± 0.29***</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N = 1 experiment performed in quadruplicate in MCF-7/sensitive cells
N = 3 independent experiments performed in triplicate in MCF-7 MX100 cells
Combined Effects of Flavonoids on BCRP-mediated Transport

- Characterization of dose-response profiles of individual flavonoids and flavonoid combinations;
- Calculation of $EC_{30}$, $EC_{50}$ and $EC_{70}$;
- Analysis of potential interactions using isobologram and Berenbaum’s interaction index methods;
- Equal molar concentration of each constituent is present in the combinations.
Dose-Response Profiles for Single Flavonoids

- Apigenin
- Biochanin A
- Chrysin
- Hesperetin
- Kaempferol
- Genistein
- Naringenin
- Silymarin
Dose-Response Profiles for Flavonoid Combinations

AB: apigenin + biochanin A
BC: biochanin A + chrysins
ABC: apigenin + biochanin A + chrysins
ABCGK: apigenin + biochanin A + chrysins + genistein + kaempferol
ABCGKHNS: all the eight flavonoids investigated

The concentration values indicate the concentrations for each individual flavonoid in the combination
### EC<sub>50</sub>, EC<sub>30</sub>, EC<sub>70</sub> for Increasing MX Accumulation

<table>
<thead>
<tr>
<th>Flavonoids</th>
<th>EC&lt;sub&gt;30&lt;/sub&gt; (µM)</th>
<th>EC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>EC&lt;sub&gt;70&lt;/sub&gt; (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apigenin (A)</td>
<td>0.97 ± 0.38</td>
<td>1.66 ± 0.55</td>
<td>2.86 ± 0.80</td>
</tr>
<tr>
<td>Biochanin A (B)</td>
<td>0.70 ± 0.47</td>
<td>1.62 ± 1.02</td>
<td>3.72 ± 2.24</td>
</tr>
<tr>
<td>Chrysin (C)</td>
<td>0.24 ± 0.07</td>
<td>0.39 ± 0.13</td>
<td>0.61 ± 0.23</td>
</tr>
<tr>
<td>Genistein (G)</td>
<td>8.91 ± 2.35</td>
<td>14.9 ± 2.69</td>
<td>25.0 ± 2.58</td>
</tr>
<tr>
<td>Hesperetin (H)</td>
<td>7.12 ± 1.39</td>
<td>12.4 ± 2.21</td>
<td>21.8 ± 3.59</td>
</tr>
<tr>
<td>Kaempferol (K)</td>
<td>3.79 ± 0.33</td>
<td>6.04 ± 0.09</td>
<td>9.67 ± 0.65</td>
</tr>
<tr>
<td>Naringenin (N)</td>
<td>17.5 ± 2.36</td>
<td>32.0 ± 3.22</td>
<td>59.1 ± 10.5</td>
</tr>
<tr>
<td>Silymarin (S)</td>
<td>10.6 ± 1.01</td>
<td>33.7 ± 2.78</td>
<td>109 ± 28.0</td>
</tr>
<tr>
<td>AB</td>
<td>0.39 ± 0.04</td>
<td>0.81 ± 0.17</td>
<td>1.69 ± 0.55</td>
</tr>
<tr>
<td>BC</td>
<td>0.15 ± 0.08</td>
<td>0.32 ± 0.16</td>
<td>0.69 ± 0.32</td>
</tr>
<tr>
<td>ABC</td>
<td>0.16 ± 0.08</td>
<td>0.27 ± 0.01</td>
<td>0.48 ± 0.09</td>
</tr>
<tr>
<td>ABCGK</td>
<td>0.14 ± 0.07</td>
<td>0.23 ± 0.08</td>
<td>0.40 ± 0.10</td>
</tr>
<tr>
<td>ABCGKHNS</td>
<td>0.13 ± 0.06</td>
<td>0.20 ± 0.10</td>
<td>0.34 ± 0.19</td>
</tr>
</tbody>
</table>

- N = 3 independent experiments performed in triplicate
- Concentrations for combinations indicate the concentration for individual flavonoid in the combination

Zhang et al. Pharm Res, 2004
Berenbaum’s Interaction Index Method

Berenbaum’s Interaction Index

\[ I = \sum \frac{D_{x,i}}{EC_{x,i}} \]

- \( EC_{x,i} \): the concentration of the constituent “\( i \)” to produce \( x \) effect;
- \( D_{x,i} \): the concentration of the constituent “\( i \)” in the combination that will produce \( x \) effect.

\( I = 1 \), additive;
\( I < 1 \), synergistic
\( I > 1 \), antagonistic.

Interactions were additive

SAR and QSAR Study

Objective:

- To identify structural elements required for potent BCRP inhibition;
- To derive a QSAR equation for the prediction of flavonoid-BCRP interaction activity.
Conclusions

- Flavonoids can inhibit human BCRP with flavonoids such as chrysin, biochanin A, apigenin and benzoflavone having IC$_{50}$ values in the sub- or low µM range.

- Multiple flavonoids result in additive inhibition of BCRP.

- The diversity of flavonoids allow the determination of SAR and QSAR for these compounds. SAR studies indicated the importance of lipophilicity, the placement of hydroxyl groups and the 2,3 double bond.
Effects of flavonoids on topotecan pharmacokinetics in vivo
Effect of GF120918 on Topotecan PK in SD Rats

- Animal: SD female rats (180~220 g)
- Dosing regimen:
  - Control: vehicle: glycofurol, oral
  - Treatment: 50 mg/ kg GF120918, oral
    3 min later, topotecan 2mg/ kg, oral
    (in saline containing 5% glucose)
  - For iv dosing: topotecan (1mg/ kg)
- Topotecan analysis: validated HPLC method

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n=7)</th>
<th>GF120918 (50mg/ kg) (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( AUC_{0-360} ) (ng/ ml·min)</td>
<td>1.74 ± 0.86 ( (\times 10^4) )</td>
<td>7.65 ± 3.78 ( (\times 10^4) )**</td>
</tr>
<tr>
<td>( AUC_{0-\infty} ) (ng/ ml·min)</td>
<td>1.80 ± 0.89 ( (\times 10^4) )</td>
<td>7.91 ± 356 ( (\times 10^4) )**</td>
</tr>
<tr>
<td>Tmax (min)</td>
<td>52 ± 85.3</td>
<td>75 ± 30</td>
</tr>
<tr>
<td>Cmax (ng/ ml)</td>
<td>86.4 ± 42.9</td>
<td>257 ± 154*</td>
</tr>
<tr>
<td>terminal ( T_{1/2} ) (min)</td>
<td>127 ± 20.0</td>
<td>167 ± 65.1</td>
</tr>
<tr>
<td>F (%)</td>
<td>29.7± 14.8</td>
<td>130 ± 58.8**</td>
</tr>
</tbody>
</table>
Effect of Chrysin on Topotecan PK in SD Rats

**EC$_{50}$ in MCF-7 MX100:** 0.39 ± 0.13 µM

**substrate:** mitoxantrone

- **Animal:** SD female rats (180~220 g)
- **Dosing regimen:**
  - **Control:** vehicle: glycofurol, oral
  - **Treatment:** 5 or 50 mg/kg chrysin, oral
  - 3 min later, topotecan 2mg/kg, oral (in saline containing 5% glucose)

### Parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n=7)</th>
<th>Chrysin (5mg/kg) (n=3)</th>
<th>Chrysin (50mg/kg) (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC$_{0-360}$ (ng/ml•min)</td>
<td>1.74 ± 0.86 ($\times 10^4$)</td>
<td>1.29 ± 0.24 ($\times 10^4$)</td>
<td>0.88 ± 0.83 ($\times 10^4$)</td>
</tr>
<tr>
<td>AUC$_{0-\infty}$ (ng/ml•min)</td>
<td>1.80 ± 0.89 ($\times 10^4$)</td>
<td>1.34 ± 0.27 ($\times 10^4$)</td>
<td>0.93 ± 0.85 ($\times 10^4$)</td>
</tr>
<tr>
<td>Tmax (min)</td>
<td>52 ± 85.3</td>
<td>35.0 ± 22.9</td>
<td>75.0 ± 82.2</td>
</tr>
<tr>
<td>Cmax (ng/ml)</td>
<td>86.4 ± 42.9</td>
<td>68.3 ± 32.2</td>
<td>36.0 ± 37.9</td>
</tr>
<tr>
<td>terminal T1/2 (min)</td>
<td>127 ± 20.0</td>
<td>139 ± 40.4</td>
<td>173 ± 47.7</td>
</tr>
<tr>
<td>F (%)</td>
<td>29.7 ± 14.8</td>
<td>22.1 ± 4.47</td>
<td>15.3 ± 14.1</td>
</tr>
</tbody>
</table>
Effect of Chrysin on Topotecan PK in mdr1a/1b (-/-) mice

- Animal: mdr1a/1b (-/-) mice (23~26.5 g)
- Dosing regimen:
  
  **Control:** vehicle: olive oil, oral
  **Treatment:** 50 mg/kg chrysin, oral

  3 min later, topotecan 2mg/kg, oral (in saline containing 5% glucose)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n=4)</th>
<th>Chrysin (50mg/kg) (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC$_{0-360}$ (ng/ml·min)</td>
<td>4.56 ± 3.95 (×10³)</td>
<td>4.17 ± 2.93 (×10³)</td>
</tr>
<tr>
<td>AUC$_{0-\infty}$ (ng/ml·min)</td>
<td>5.01 ± 3.96 (×10³)</td>
<td>4.65 ± 2.98 (×10³)</td>
</tr>
<tr>
<td>Tmax (min)</td>
<td>45.0 ± 46.4</td>
<td>33.7 ± 18.9</td>
</tr>
<tr>
<td>Cmax (ng/ml)</td>
<td>45.2 ± 47.3</td>
<td>50.8 ± 45.8</td>
</tr>
<tr>
<td>terminal T1/2 (min)</td>
<td>63.6 ± 42.7</td>
<td>90.6 ± 20.5</td>
</tr>
</tbody>
</table>
Possible Reasons for the *In vitro* and *In vivo* Discrepancy

- Metabolism
- Substrate dependence
- Species difference
- Inhibition of topotecan uptake transporter
**Effect of 7,8-benzoflavone (BF) on Topotecan PK in SD Rats**

- **EC₅₀ in MCF-7 MX100:** 0.07 ± 0.02 µM
  - substrate: mitoxantrone

- **Animal:** SD female rats (180~220 g)

- **Dosing regimen:**
  - **Control:** vehicle: glycofurol, oral
  - **Treatment:** 10 or 50 mg/kg BNF, oral
  - 3 min later, topotecan 2mg/kg, oral
    - (in saline containing 5% glucose)

### Parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n=7)</th>
<th>Benzoflavone (10 mg/kg) (n=9)</th>
<th>Benzoflavone (50 mg/kg) (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC₀-₃₆₀ (ng/ml·min)</td>
<td>1.74 ± 0.86 (×10⁴)</td>
<td>2.04 ± 0.48 (×10⁴)</td>
<td>2.50 ± 1.14 (×10⁴)</td>
</tr>
<tr>
<td>AUC₀-∞ (ng/ml·min)</td>
<td>1.80 ± 0.89 (×10⁴)</td>
<td>2.15 ± 0.43 (×10⁴)</td>
<td>2.57 ± 1.15 (×10⁴)</td>
</tr>
<tr>
<td>Tmax (min)</td>
<td>52.0 ± 85.3</td>
<td>82.4 ± 91.4</td>
<td>107 ± 91.2</td>
</tr>
<tr>
<td>Cmax (ng/ml)</td>
<td>86.4 ± 42.9</td>
<td>98.0 ± 45.7</td>
<td>101 ± 50.9</td>
</tr>
<tr>
<td>terminal T1/2 (min)</td>
<td>127 ± 20.0</td>
<td>150 ± 63.6</td>
<td>127 ± 37.3</td>
</tr>
<tr>
<td>F (%)</td>
<td>29.7 ± 14.8</td>
<td>35.5 ± 7.33</td>
<td>42.5 ± 7.09</td>
</tr>
</tbody>
</table>
Possible Reasons for the *In vitro* and *In vivo* Discrepancy

- Metabolism
- **Substrate dependence**
- Species difference
- Inhibition of topotecan uptake transporter
Effect of Flavonoids on Topotecan Accumulation in MCF-7 cells

- Accumulation time: 10 min;
- Topotecan concentration: 5 µM;
- Cells were harvested and sonicated;
- Topotecan in cell lysate was assayed by HPLC;
- Accumulation were normalized by protein content;
- N = 4

Chrysin and BF can inhibit BCRP-mediated efflux of topotecan
Possible Reasons for the *In vitro* and *In vivo* Discrepancy

- Metabolism
- Substrate dependence
- *Species difference*
- Inhibition of topotecan uptake transporter
Effect of Flavonoids on Topotecan Accumulation in MDCK-bcrp1 Cells

- Accumulation time: 10 min;
- Topotecan concentration: 5 µM;
- Cells were harvested and sonicated;
- Topotecan in cell lysate was assayed by HPLC;
- Accumulation were normalized by protein content;
- N = 4

Chrysin and BF may only have weak inhibition activity on mouse bcrp1
Summary

- Chrysin and BF did not change topotecan PK in rats or mice
- Tentative explanation for the discrepancy: species difference with respect to inhibition of topotecan (species difference not seen with mitoxantrone)
- Other possibilities could not be excluded, such as involvement of other transporters
Flavonoids are widely-present in food and herbal products.

Inhibitory interactions occur with P-glycoprotein, MRP1 and BCRP. These interactions may be beneficial for the reversal of multidrug resistance in cancer. Flavonoids may also increase the bioavailability and decrease the clearance of drugs.

Concentrations achievable in vivo in the gastrointestinal tract are likely high enough to result in significant interactions with ABC transporters. This is particularly true with respect to flavonoid concentrations after herbal medicines.