Flavonoid-Drug Interactions: Effects of Flavonoids on ABC Transporters

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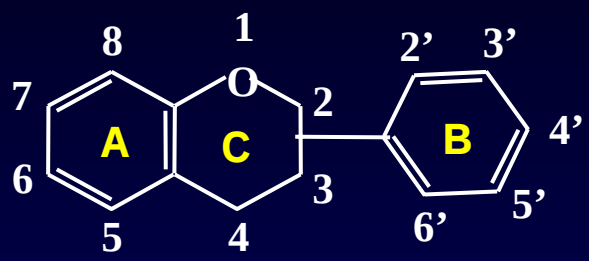
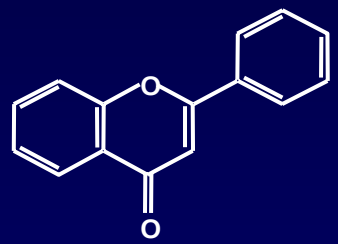
Flavonoids

❖ **Basic Structure:**

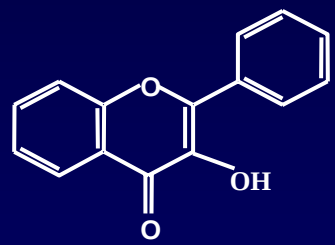


➢**The most abundant polyphenols present in human diet (vegetables, fruits, red wine and tea)**

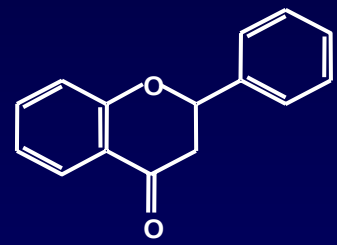
❖ **Subclasses:**



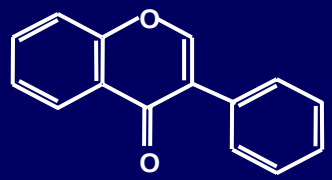
flavones



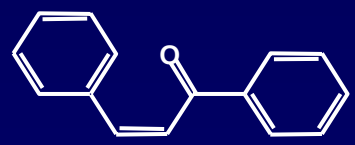
flavonols



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

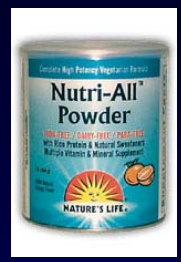
- **Toxicity:**
  - **Long history of consumption with exceptional safety record;**
  - **Extremely large doses used in animal studies;**

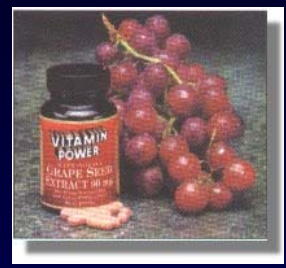
    Acute LD_{50} for rats: 2 g / kg BW by direct injection into blood.

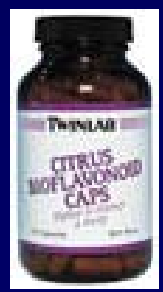
"The margin of safety for the therapeutic use of flavonoids in humans, therefore, is very large and probably not surpassed by any other drug in current use"

Havsteen, (2002), Pharmacology and Therapeutics 96:67-202.

Flavonoid Products







Herbal Use in Select Populations

- **HIV infected patients**
  (Fairfield etal Arch Intern Med 158:2257-2264, 1998)
  - **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

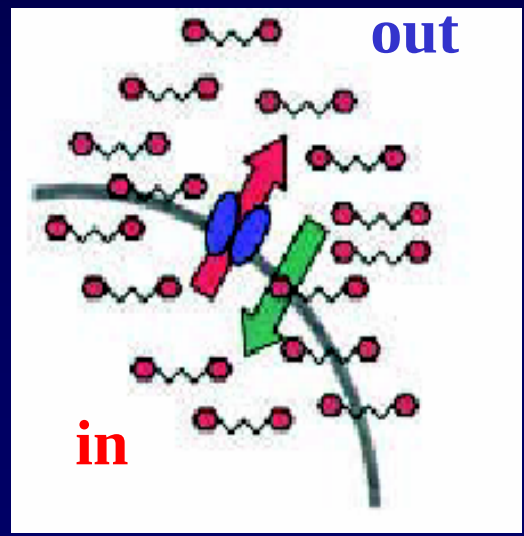
- ATP binding cassette (ABC) superfamily

  P-glycoprotein (MDR1, ABCB1)

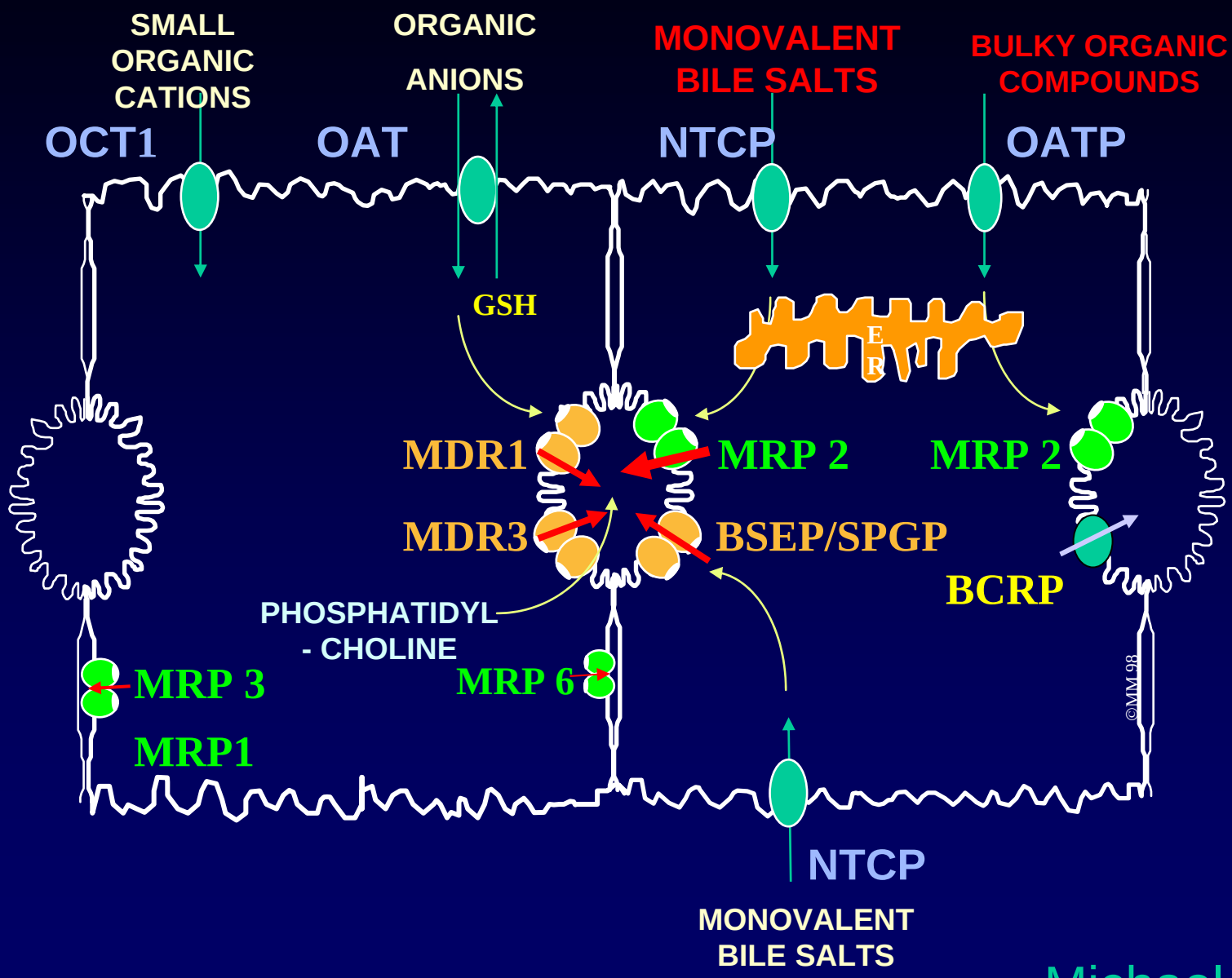
  Multidrug Resistance Associated Proteins (MRP, ABCC)

  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



Hepatocyte



Michael Müller

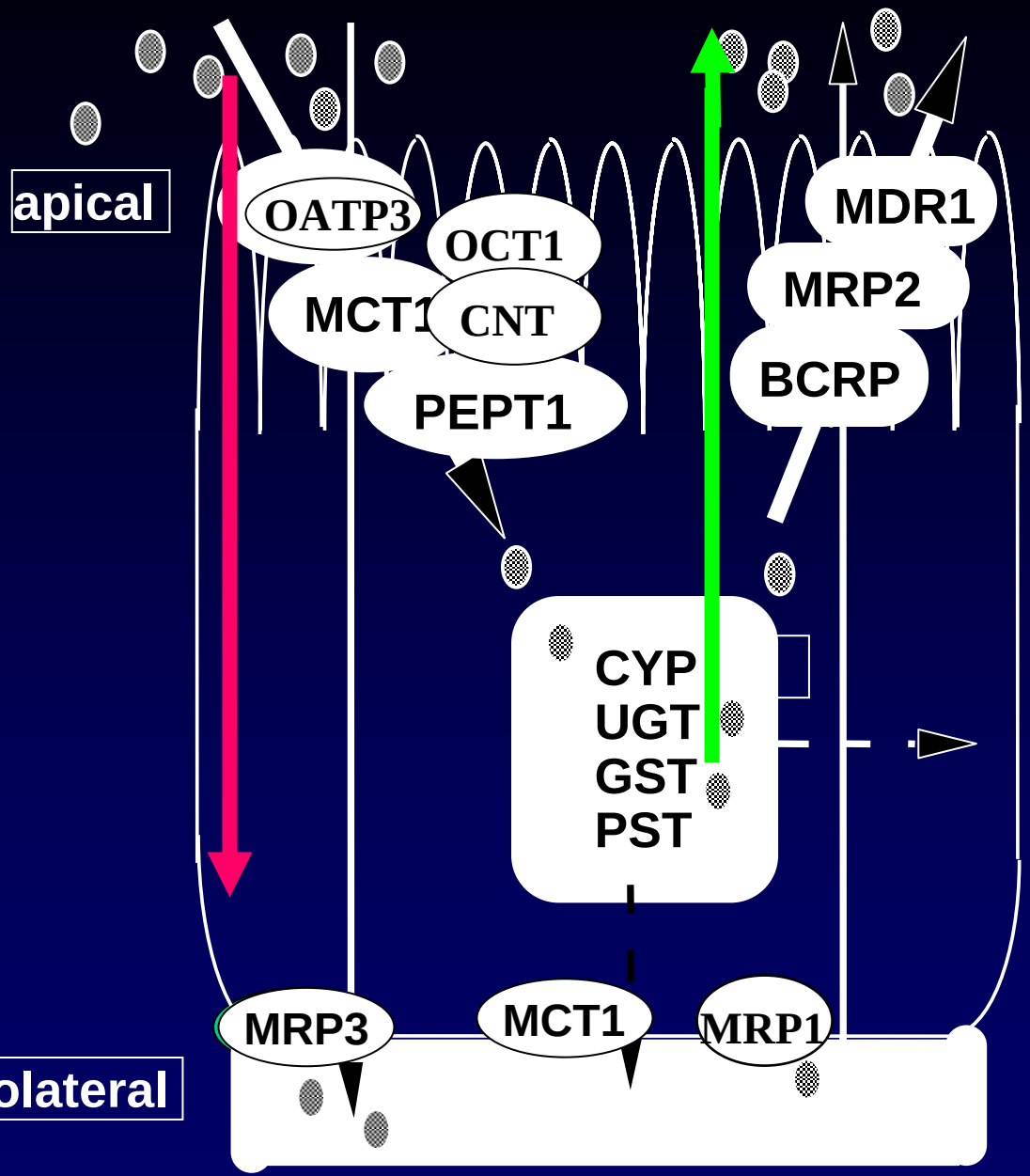
Intestine

Transporters

- absorption
- efflux

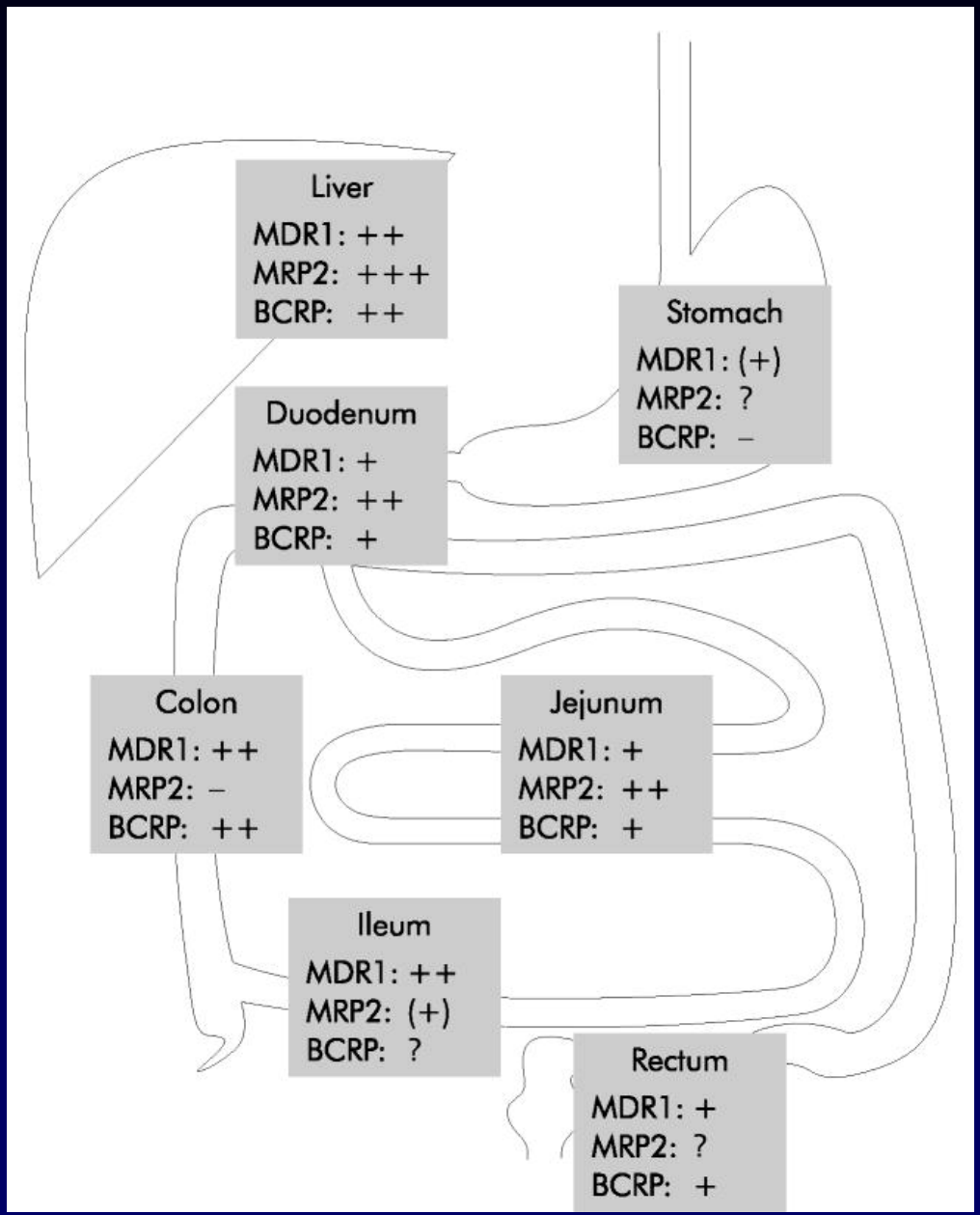
Metabolism

Transporters



Sandy K Pang

ABC Transporter Expression in the Liver and Gastrointestinal Tract

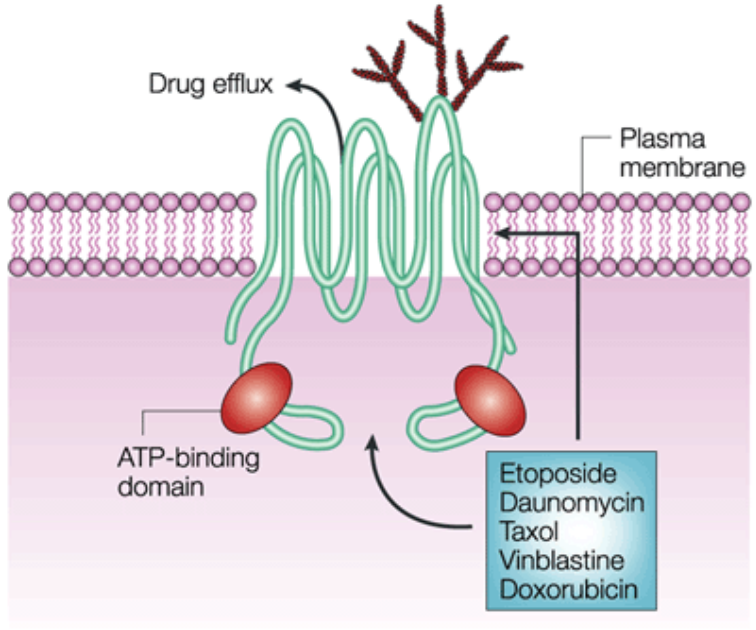


- High expression of MDR1, MRP2 and BCRP in the liver
- Expression throughout the gastrointestinal tract

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:

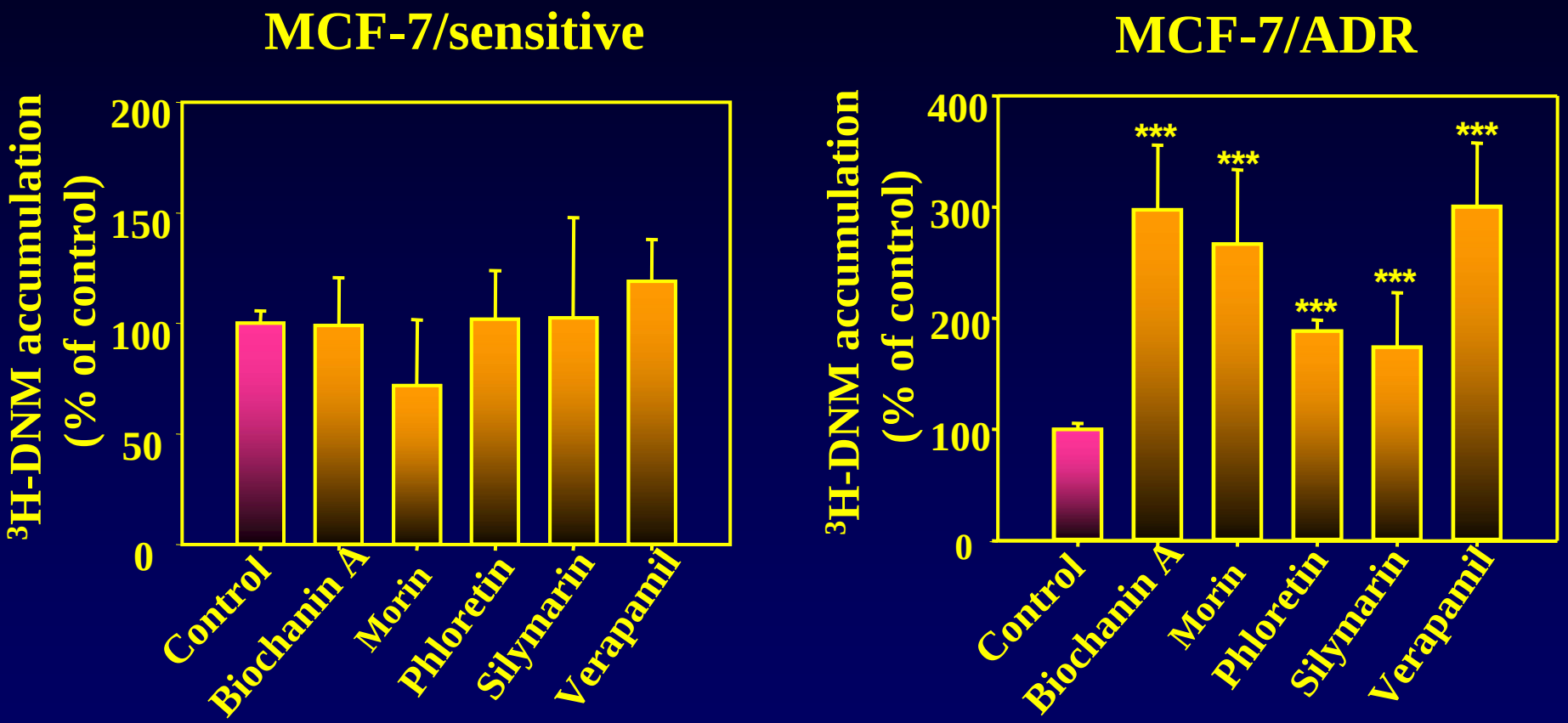
anthracyclines, Vinca alkaloids, epipodophyllotoxins and taxol
cyclosporine, digoxin, verapamil etc.

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

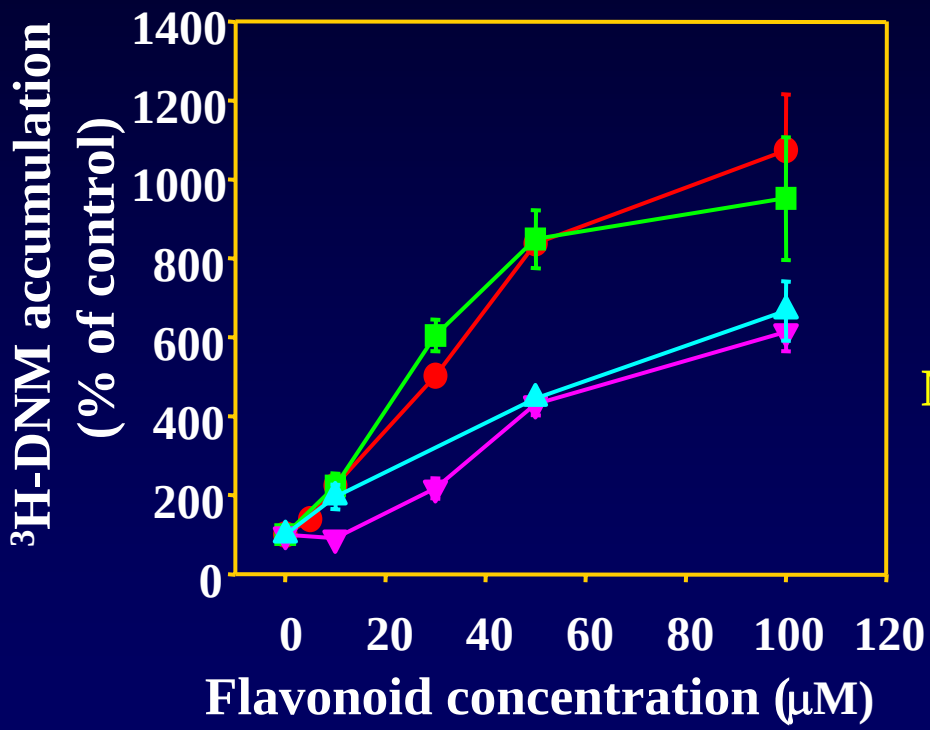
^{3}H-DNM Accumulation in MCF-7 cells



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



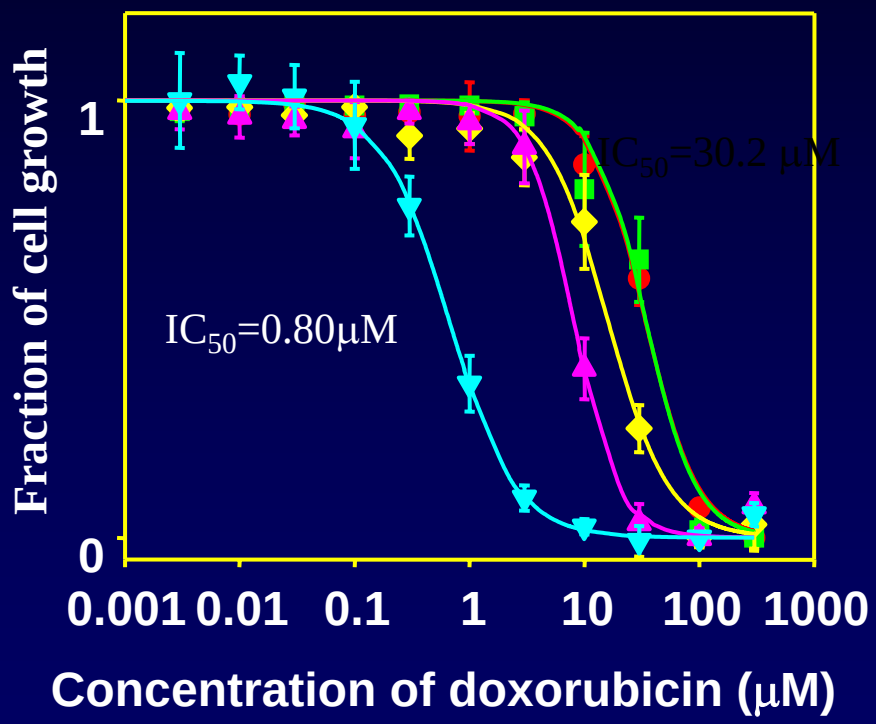
- Biochanin A
- Morin
- Phloretin
- Silymarin

Minimal Conc. for significant change:

Biochanin A and morin: 20-30 μM
Phloretin and silymarin: 30-50 μM

DNM accumulation was determined in MDA435/LCC6MDR1 cells

Increase of Doxorubicin Cytotoxicity by Biochanin A



- Control
- 10 μM biochanin A
- 30 μM biochanin A
- 50 μM biochanin A
- 100 μM biochanin A

Doxorubicin cytotoxicity was determined in MDA435/LCC6MDR1 cells

Flavonoid-P-gp Interactions

Mechanisms:

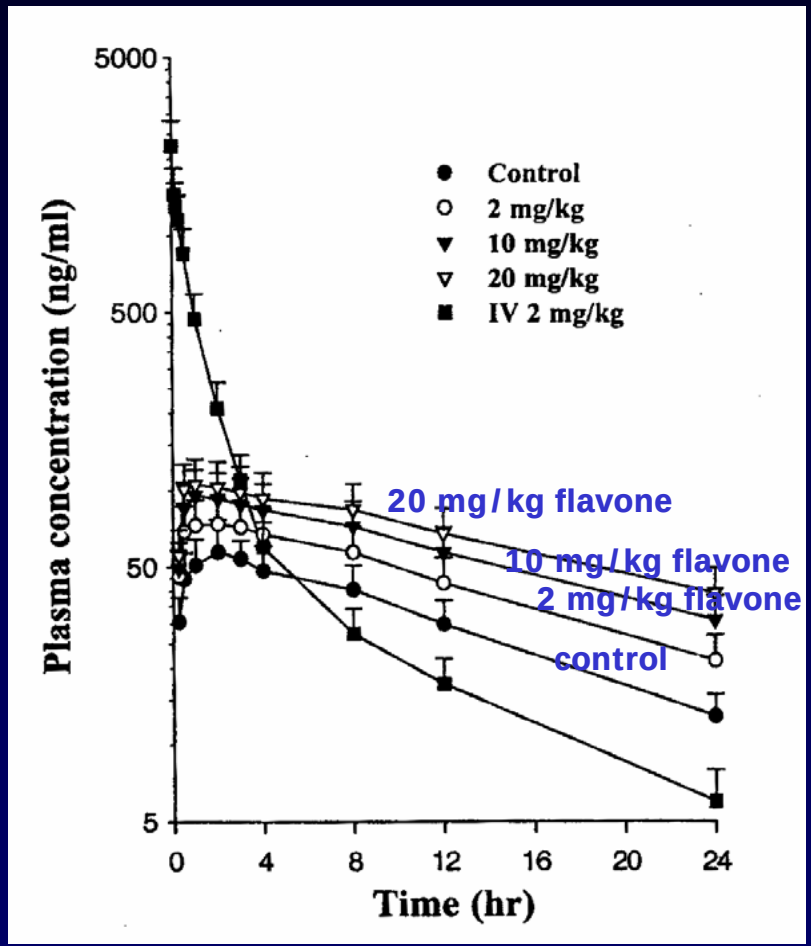
- **Biochanin A is not a substrate**
- **Flavonoids affect P-gp ATPase activity or the P-gp ATPase activity induced by verapamil**
- **Some flavonoids can inhibit P-gp ATP and/or substrate binding**

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**

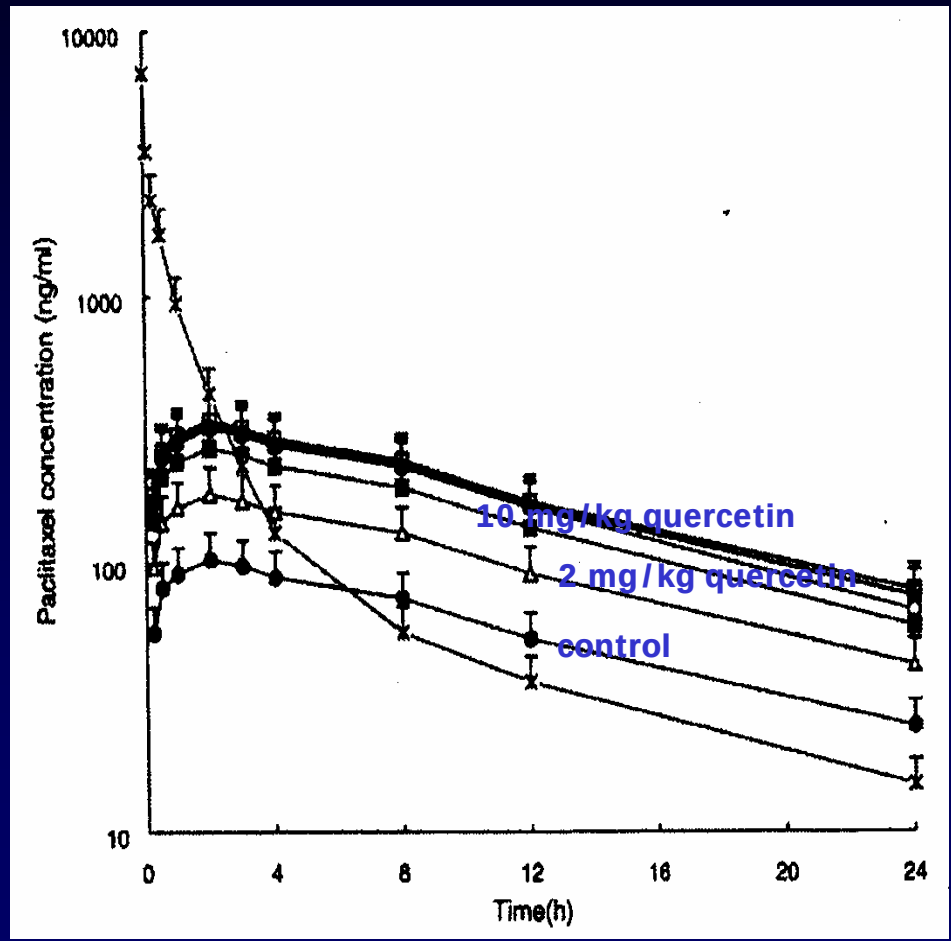
Flavonoid-Drug Interactions

❖ flavone + paclitaxel in rats



Choi et al. (2004) Int J Pharm 275: 165-170

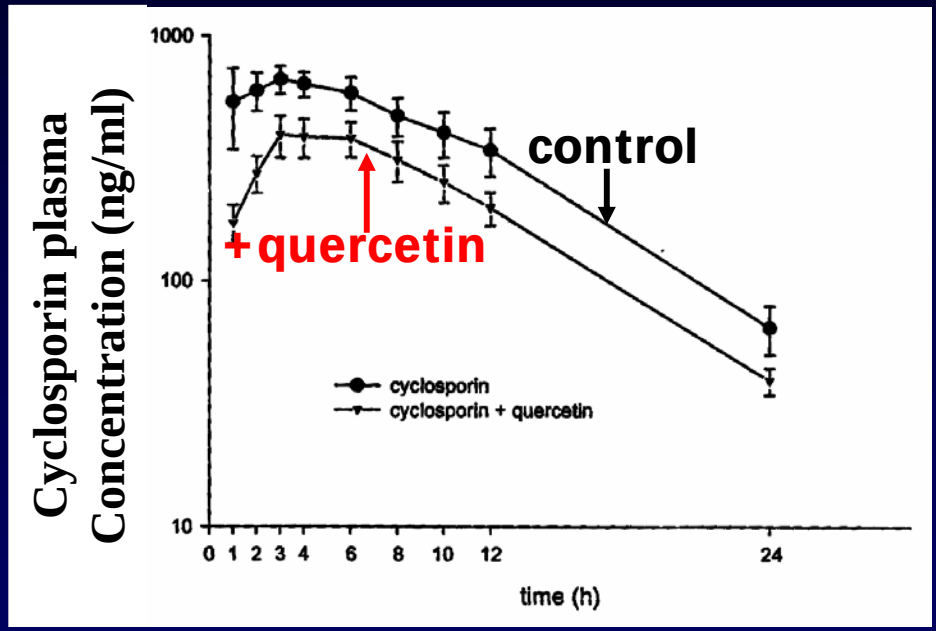
❖ quercetin + paclitaxel in rats



Choi et al. (2004) Eur J Pharm Biopharm 57: 313-8

Flavonoid-Drug Interactions

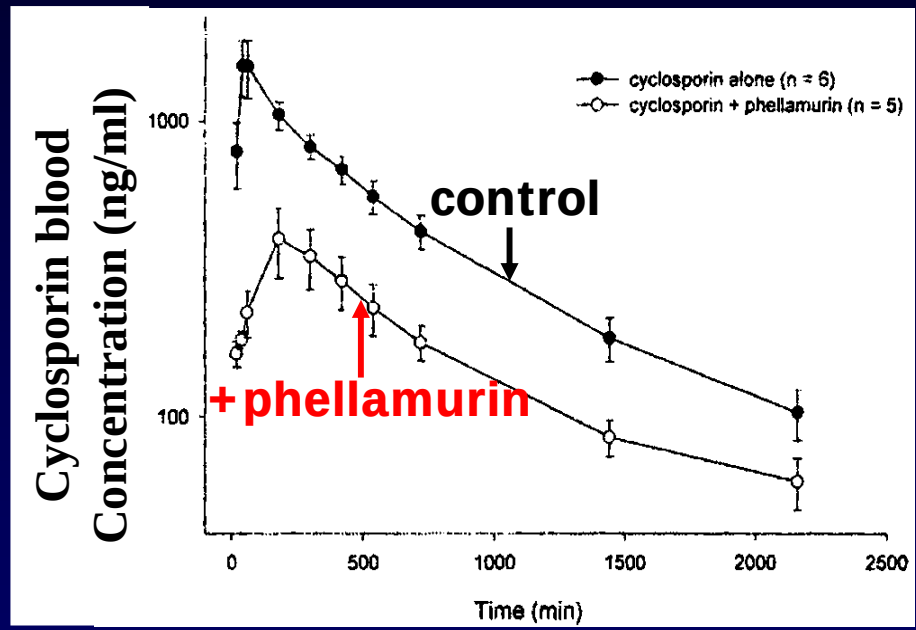
❖ quercetin + cyclosporine in pigs



AUC_{0-3}: 56 % ↓
Cmax: 47 % ↓

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

❖ phellamurin + cyclosporine in rats



AUC: 56 % ↓
Cmax: 77 % ↓

Chen et al. (2002) Planta Med 68:138-141

Flavonoid-Drug Interactions

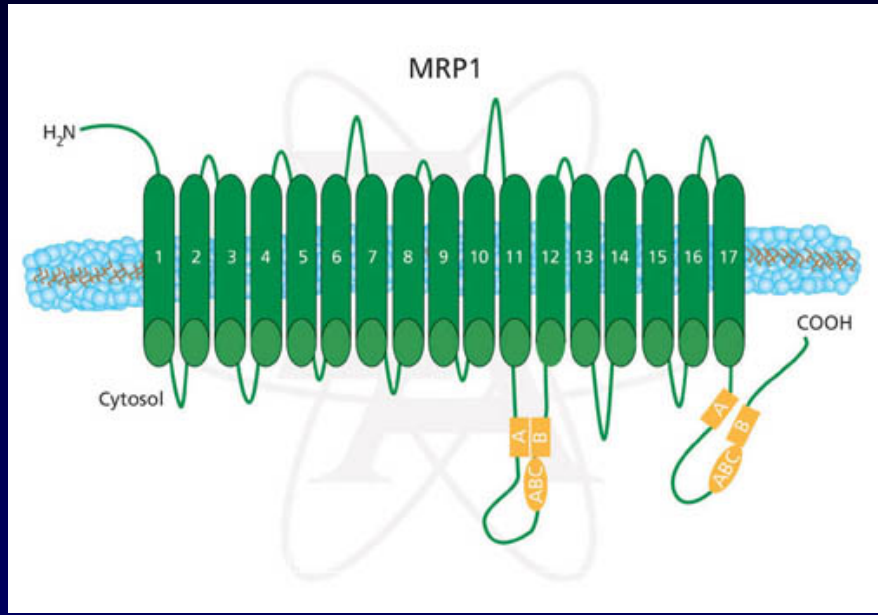
- Flavonoid-drug interactions could occur upon coadministration but appear to be substrate dependent (and will be flavonoid dependent)

- 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

Multidrug Resistance-Associated Protein 1 (MRP1, ABCC1)

MRP1 is a 190-kDa protein encoded by the MRP1 gene.

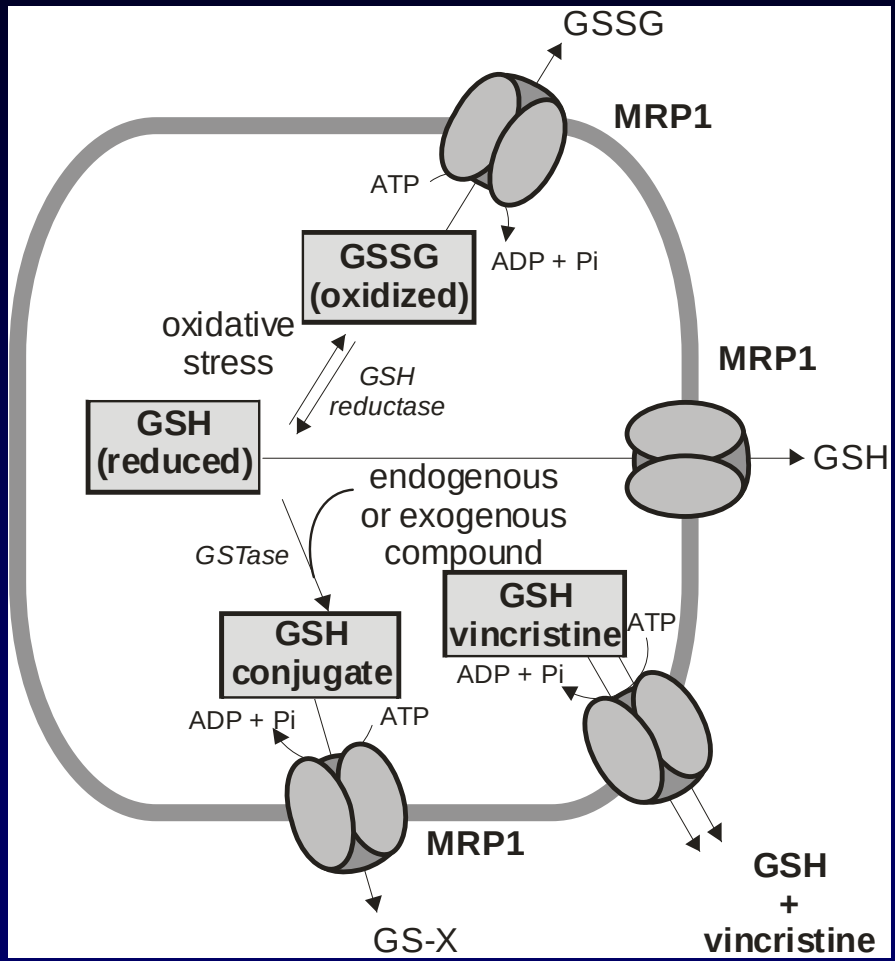
Expressed in most normal tissues in the human body and in several types of tumors such as lung carcinoma, myeloid leukemia, neuroblastoma, and breast cancer



Substrates of MRP1:

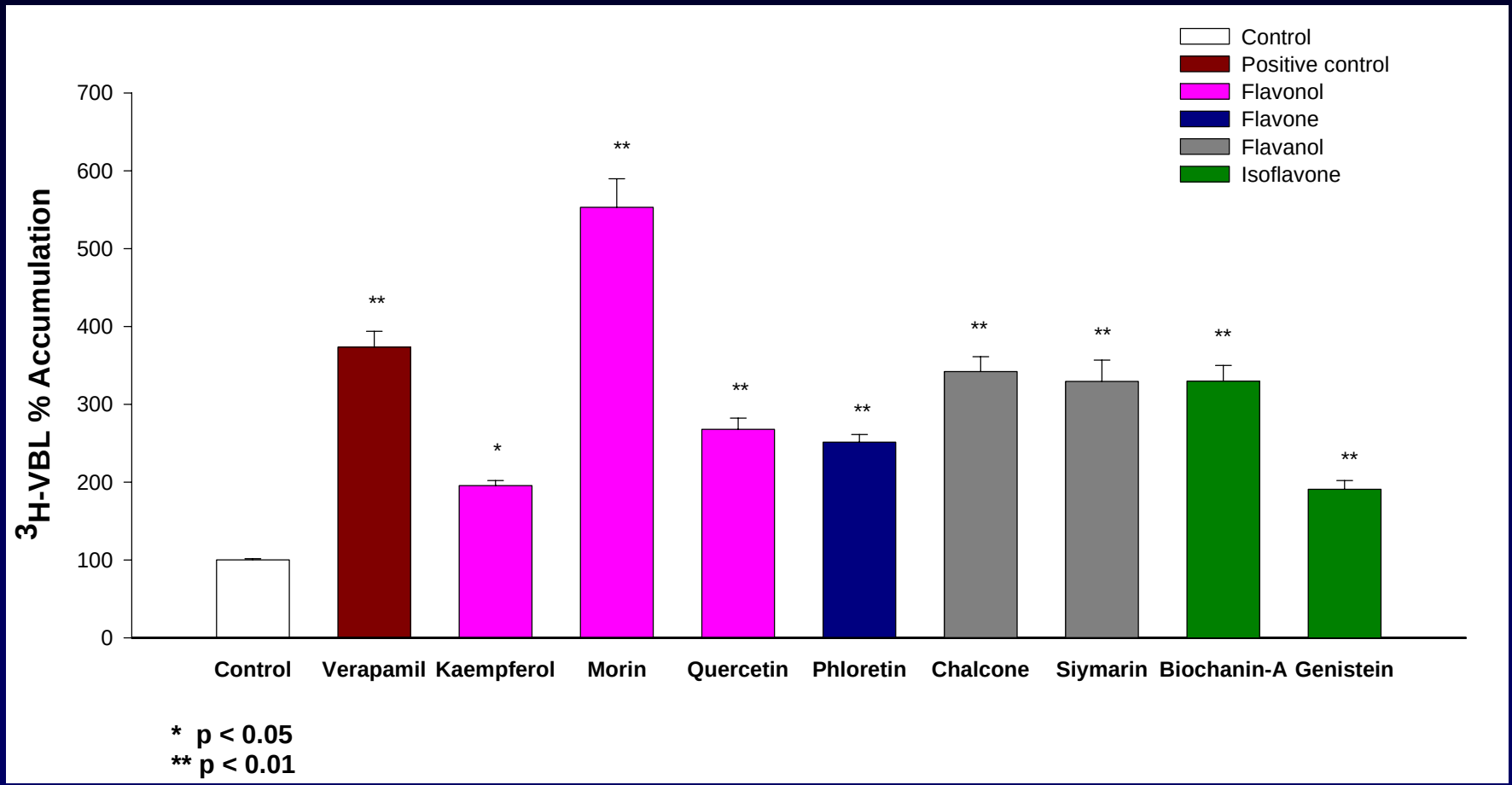
- Endogenous substrates: leukotriene C_4, glutathione disulfide, steroid glucuronides (17β-estradiol 17-β-D-glucuronide)
- Exogenous substrates: daunomycin, vinca alkaloid (vinblastine), methotrexate, fluorouracil, chlorambucil, calcein, drug conjugates

Involvement of GSH in MRP1-mediated transport



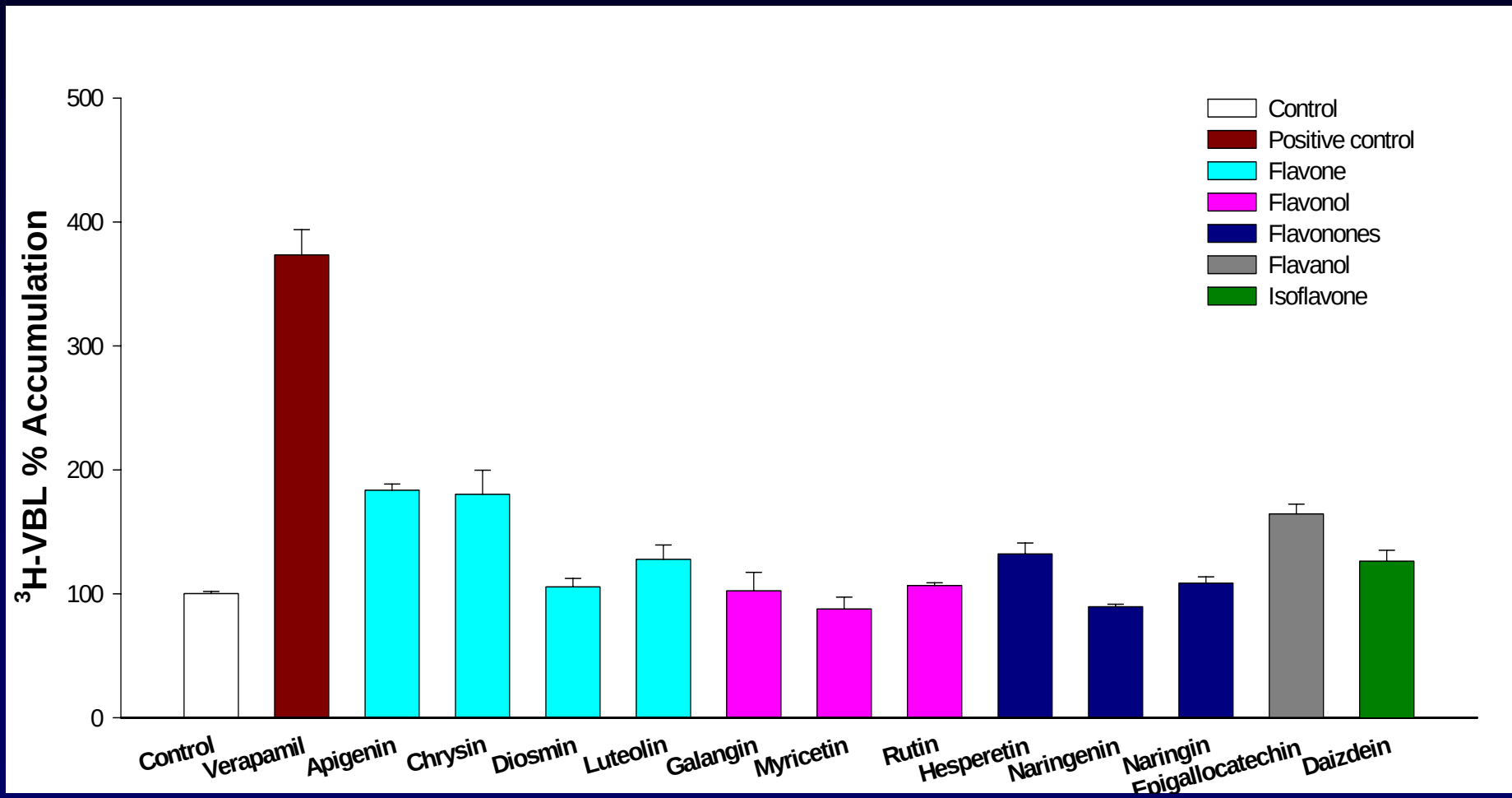
Qingcheng Mao

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



Inhibitory constants for ^{3}H-LTC4 transport in MRP1 membrane vesicles

FLAVONOID	Ki (μM)
myricetin	13.3 ± 2.7 (SD)
quercetin	8.1 ± 1.7
naringenin (+ GSH)	20.8 ± 6.4
kaempferol	2.4 ± 1.6
apigenin (+GSH)	4.9 ± 0.7

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:

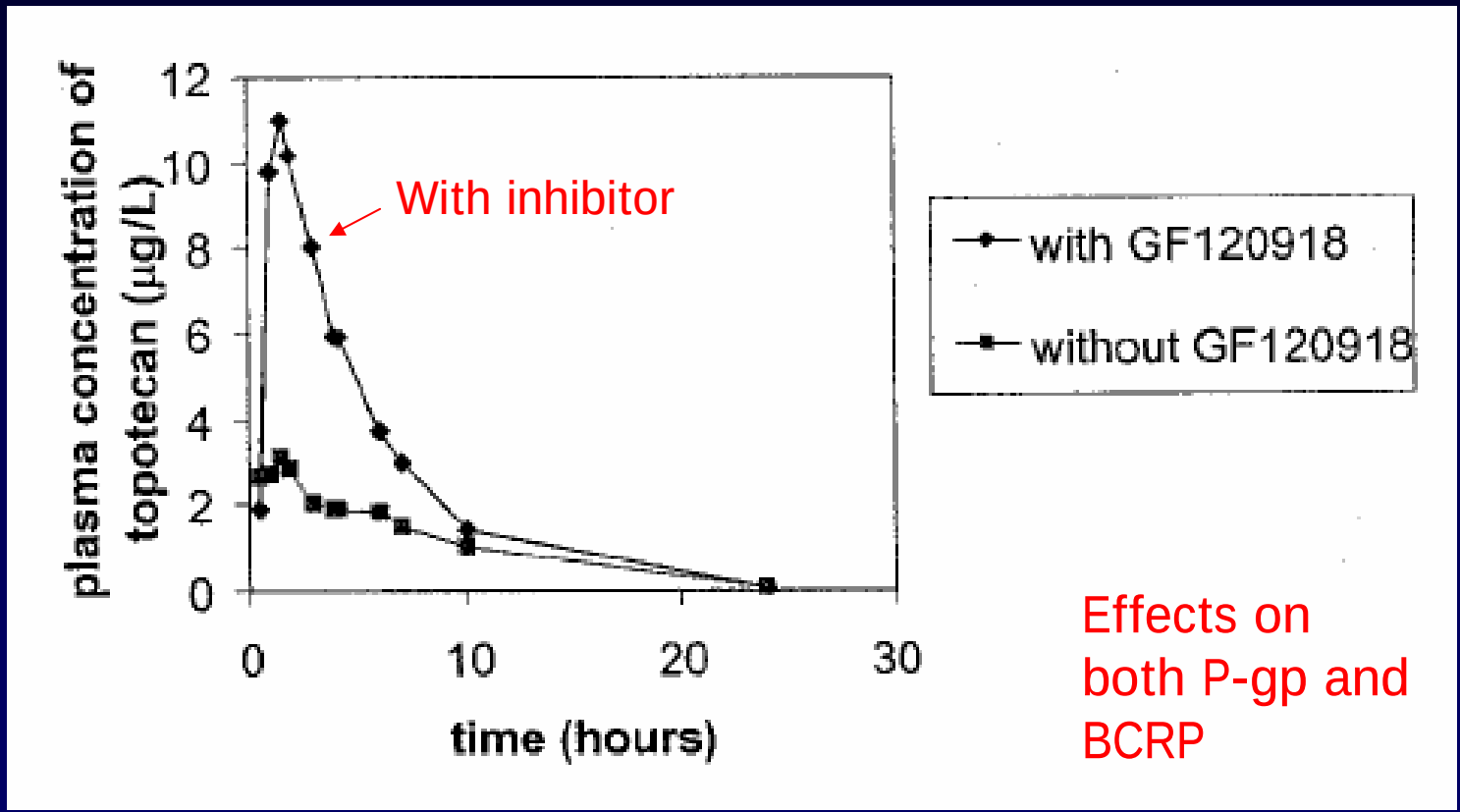
- placenta
- intestine (expression level is higher than P-glycoprotein)
- liver canalicular membrane
- brain microvessels

An important determinant for drug disposition

Steinbach et al. (2002) Leukemia 16: 1443-1447
Diestra et al (2002) J. Pathol. 198: 213-219
Jonker et al. (2000) J Natl Cancer Inst 92:1651-1656
Cooray et al (2002) Neuroreport 13:2059-2063
Maliepaard et al (2001) Cancer Res 61:3458-3464

BCRP Clinical Implications

- **Apparent oral bioavailability : 40.0% ⇒ 97.1%**

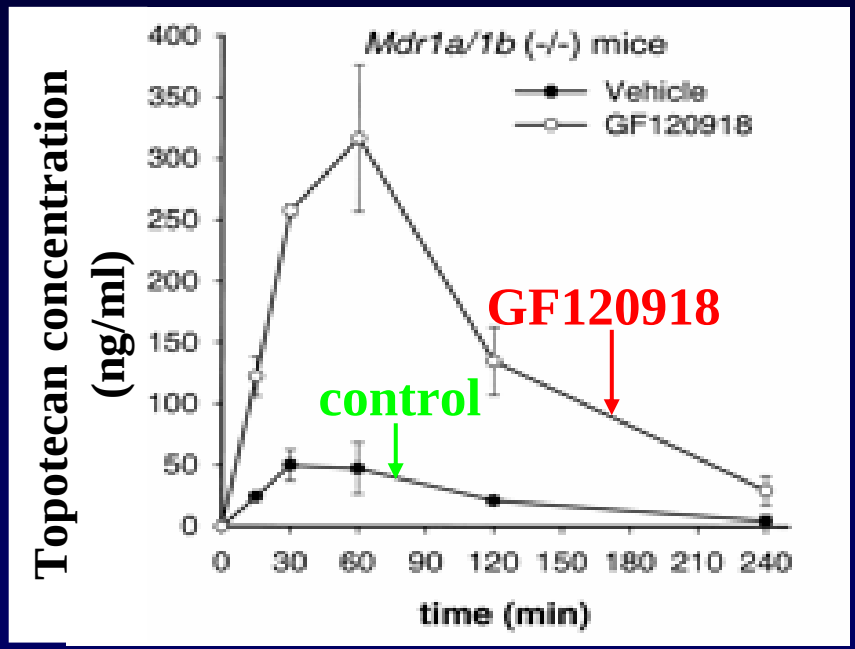


Kruijtzer, C. M. et al., *J. Clin. Oncol.* 20, 2943–2950, 2002

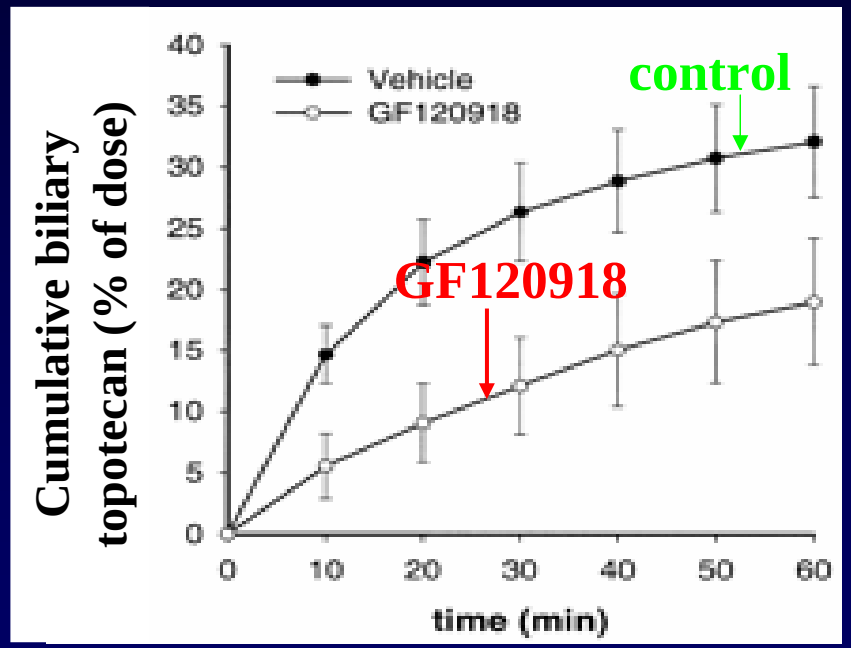
Breast Cancer Resistance Protein (BCRP)

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

Plasma concentration of topotecan



Biliary excretion of topotecan



Jonker et al. (2000) J Natl Cancer Inst 92:1651-1656

Investigated Flavonoids

A total of 20 naturally occurring flavonoids were studied.

Flavones:

apigenin
chrysin
luteolin

Isoflavones:

biochanin A
daidzein
genistein

Flavanones:

hesperetin
naringenin
silybin
silymarin
epigallocatechin (EGC)
epigallocatechin gallate (EGCG)
naringin

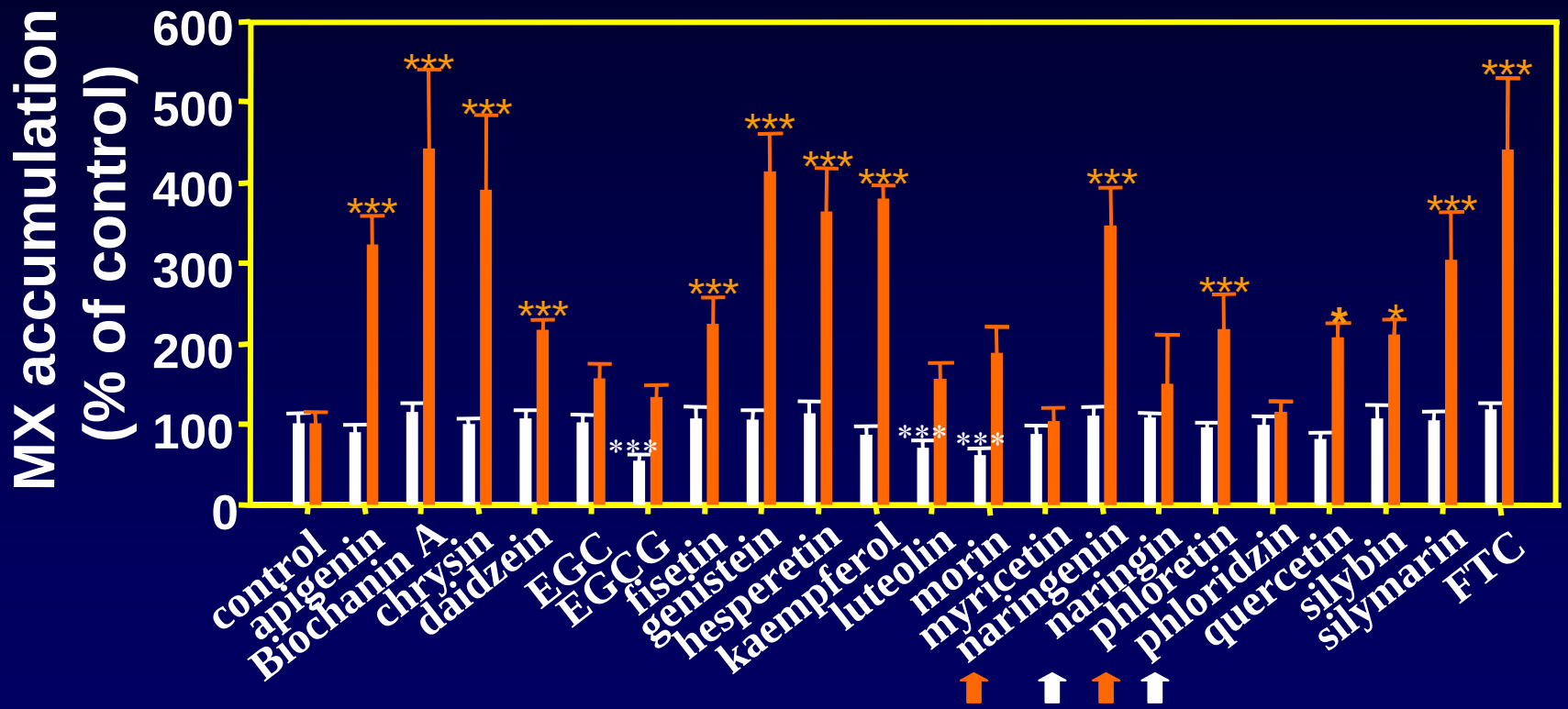
Flavonols:

fisetin
kaempferol
morin
myricetin
quercetin

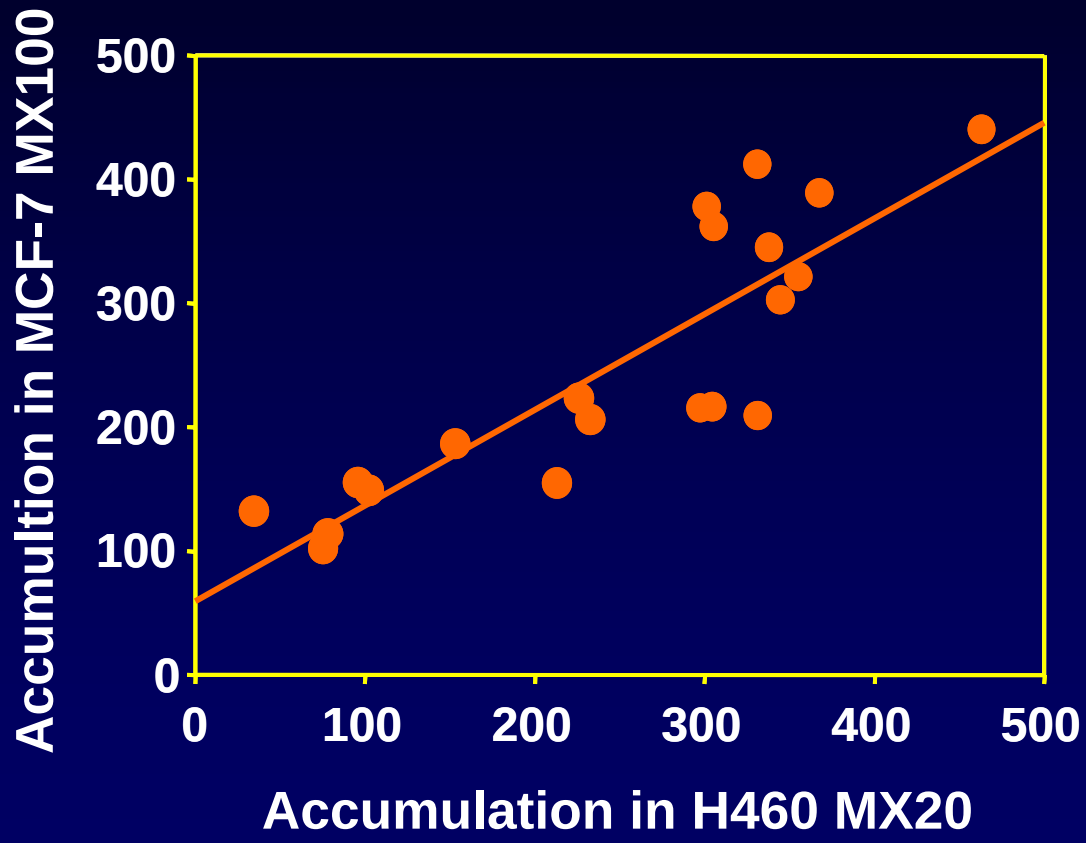
Chalcones:

phloretin
phloridzin

Mitoxantrone Accumulation In MCF-7 Cells



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

	MCF-7/sensitive		MCF-7 MX100			
compounds	*10 μM*	*50 μM*	*2.5 μM*	*5 μM*	*10 μM*	*50 μM*
control	5.30 ± 2.22			199 ± 19.3		
apigenin	3.66 ± 0.52	3.44 ± 0.35		219 ± 10.0	10.5 ±7.13***	1.73 ± 1.42***
BA	2.40 ±0.27***	1.86 ±0.35***	107 ± 17.6***	30.9 ± 5.18***	9.23 ± 2.07***	2.19 ± 1.04***
chrysin		0.95 ± 0.46***	18.8 ± 0.06***	6.25 ± 2.13***	3.35 ± 1.70***	1.13 ± 1.11***
genistein		6.98 ± 0.81		148 ± 23.2	29.3 ± 6.76***	2.29 ± 0.86***
kaempferol		6.10 ± 0.96		196 ± 20.9	228 ± 13.8	0.95 ± 0.19***
hesperetin				88.6 ± 20.8***	11.6 ± 0.83***	1.23 ± 0.16***
naringenin				189 ± 11.6	163 ± 29.5	1.23 ± 0.16***
silymarin				99.1 ± 50.2***	104 ± 35.5***	53.0 ± 7.27***
FTC	2.30 ± 0.29***				1.79 ± 1.52***	

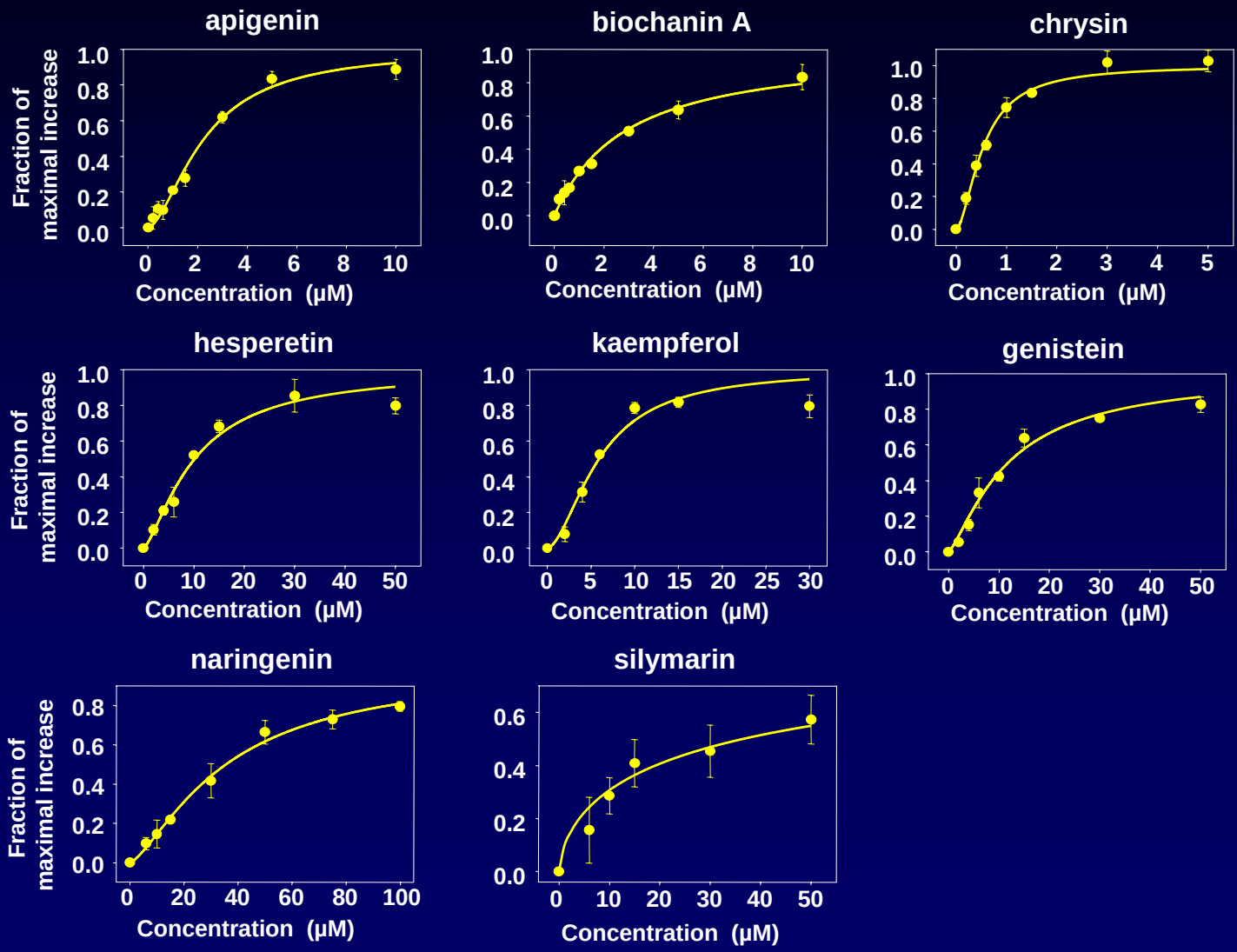
N = 1 experiment performed in quadruplicate in MCF-7/sensitive cells

N = 3 independent experiments performed in triplicate in MCF-7 MX100 cells

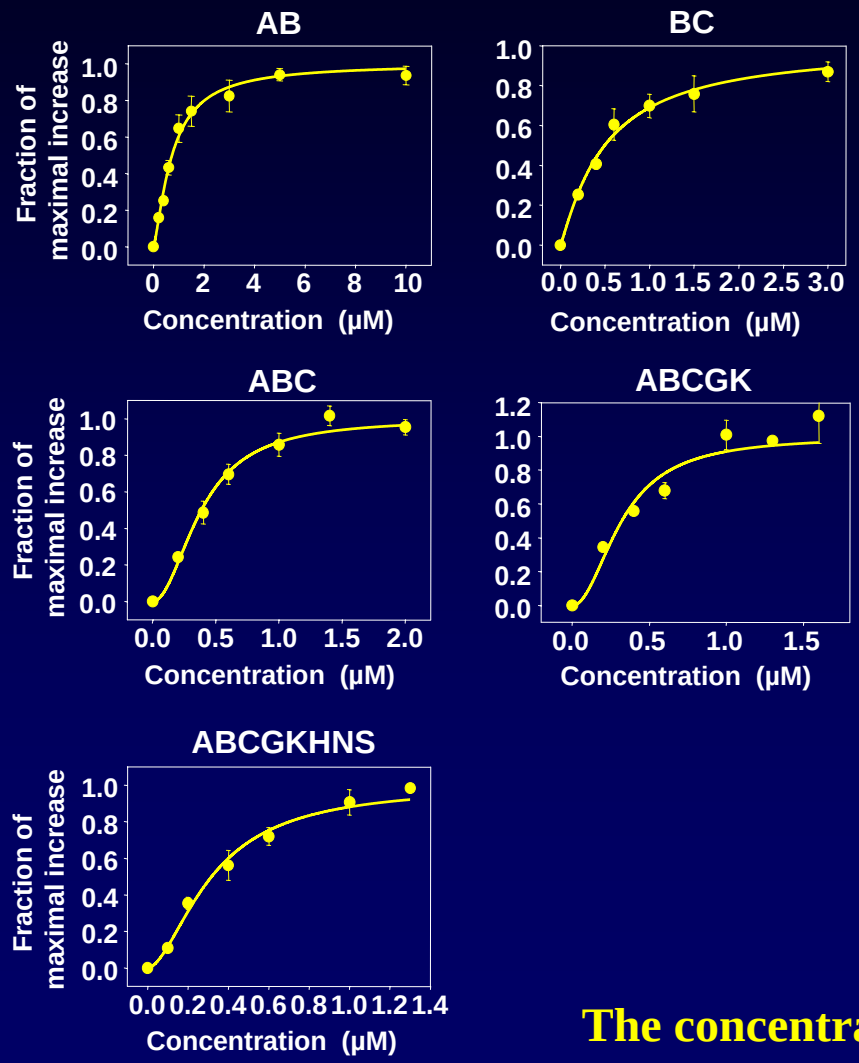
Combined Effects of Flavonoids on BCRP-mediated Transport

- 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



Dose-Response Profiles for Flavonoid Combinations



AB: apigenin + biochanin A

BC: biochanin A + chrysin

ABC: apigenin + biochanin A + chrysin

ABCGK: apigenin + biochanin A + chrysin + genistein + kaempferol

ABCGKHNS: all the eight flavonoids investigated

The concentration values indicate the concentrations for each individual flavonoid in the combination

EC_{50}, EC_{30}, EC_{70} for Increasing MX Accumulation

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

- **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

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

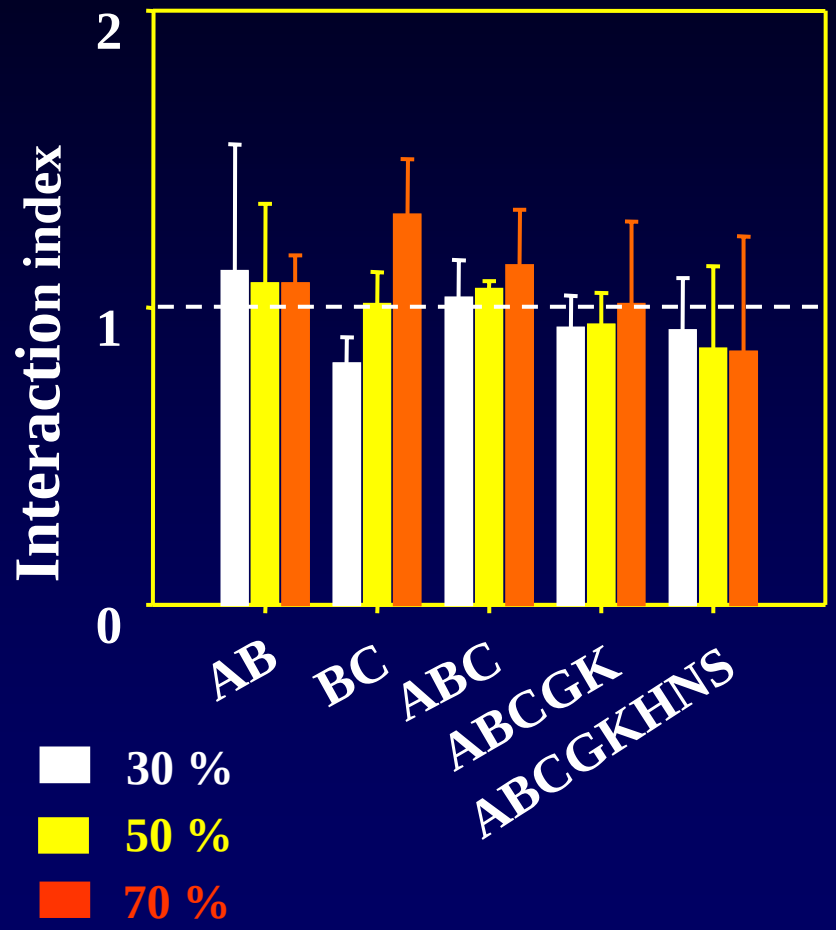
$EC_{x,I}$: the concentration of the constituent "i" to produce x effect;

$D_{x,I}$: 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

Zhang et al. (2004) Pharm Res, 21: 1263-73

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.

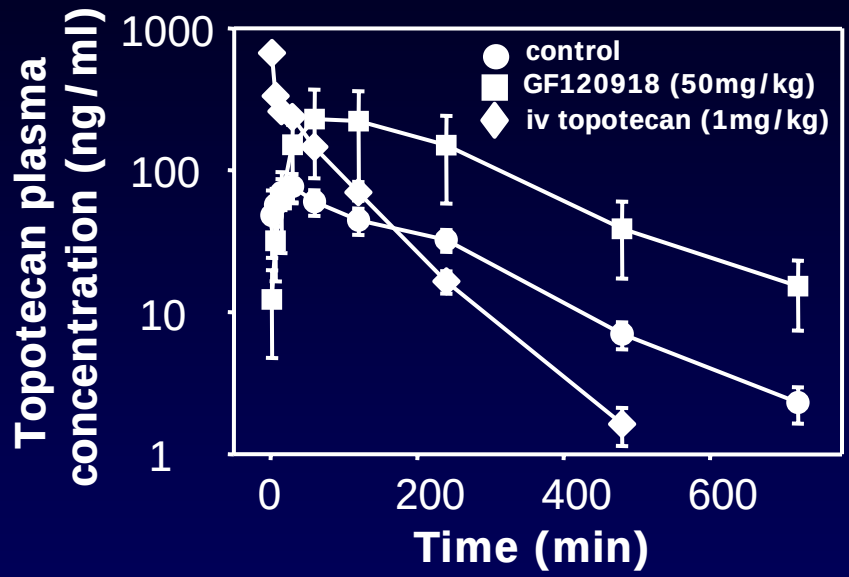
Effects of flavonoids on topotecan pharmacokinetics in vivo

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



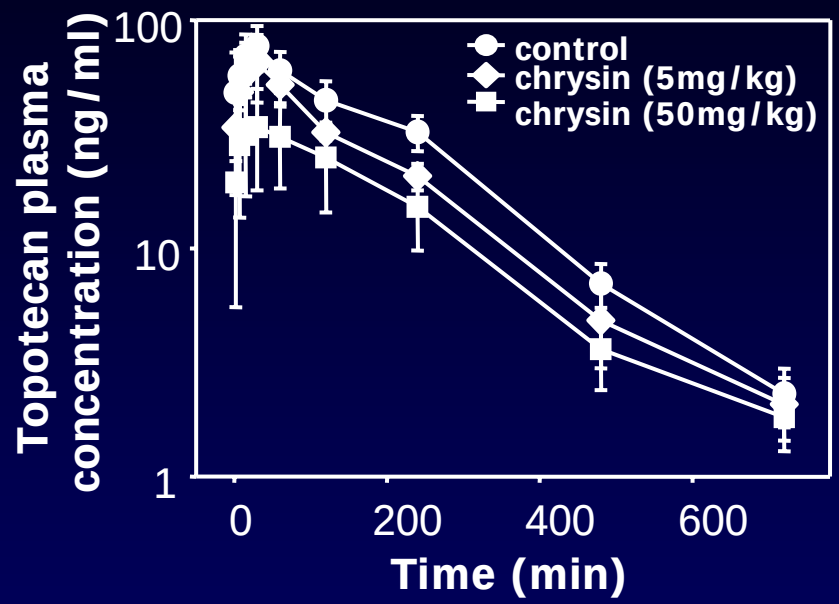
Parameters	Control (n=7)	GF120918 (50mg/kg) (n=4)
AUC_{0-360} (ng/ml•min)	1.74 ± 0.86 (×10^4)	7.65 ± 3.78 (×10^4)**
$AUC_{0-\infty}$ (ng/ml·min)	1.80 ± 0.89 (×10^4)	7.91 ± 356 (×10^4)**
Tmax (min)	52 ± 85.3	75 ± 30
Cmax (ng/ml)	86.4 ± 42.9	257 ± 154*
terminal $T_{1/2}$ (min)	127 ± 20.0	167 ± 65.1
F (%)	29.7± 14.8	130 ± 58.8**

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)

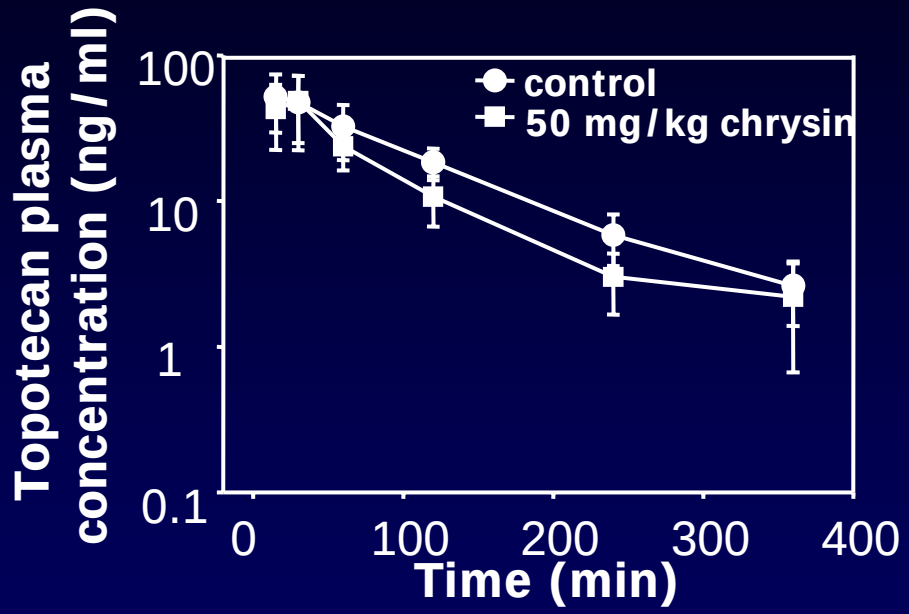


Parameters	Control (n=7)	Chrysin (5mg/kg) (n=3)	chrysin (50mg/kg) (n=6)
AUC_{0-360} (ng/ml•min)	1.74 ± 0.86 (×10^4)	1.29 ± 0.24 (×10^4)	0.88 ± 0.83 (×10^4)
$AUC_{0-\infty}$ (ng/ml·min)	1.80 ± 0.89 (×10^4)	1.34 ± 0.27 (×10^4)	0.93 ± 0.85 (×10^4)
Tmax (min)	52 ± 85.3	35.0 ± 22.9	75.0 ± 82.2
Cmax (ng/ml)	86.4 ± 42.9	68.3 ±32.2	36.0 ± 37.9
terminal T1/2 (min)	127 ± 20.0	139 ± 40.4	173 ± 47.7
F (%)	29.7± 14.8	22.1 ± 4.47	15.3 ± 14.1

Effect of Chrysin on Topotecan PK in mdr*1a*/*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)



Parameters	Control (n=4)	Chrysin (50mg/kg) (n=4)
AUC_{0-360} (ng/ml·min)	4.56 ± 3.95 (×10^3)	4.17 ± 2.93 (×10^3)
$AUC_{0-\infty}$ (ng/ml·min)	5.01 ± 3.96 (×10^3)	4.65 ± 2.98 (×10^3)
Tmax (min)	45.0 ± 46.4	33.7 ± 18.9
Cmax (ng/ml)	45.2 ± 47.3	50.8 ± 45.8
terminal T1/2 (min)	63.6 ± 42.7	90.6 ± 20.5

Possible Reasons for the *In vitro* and *In vivo* Discrepancy

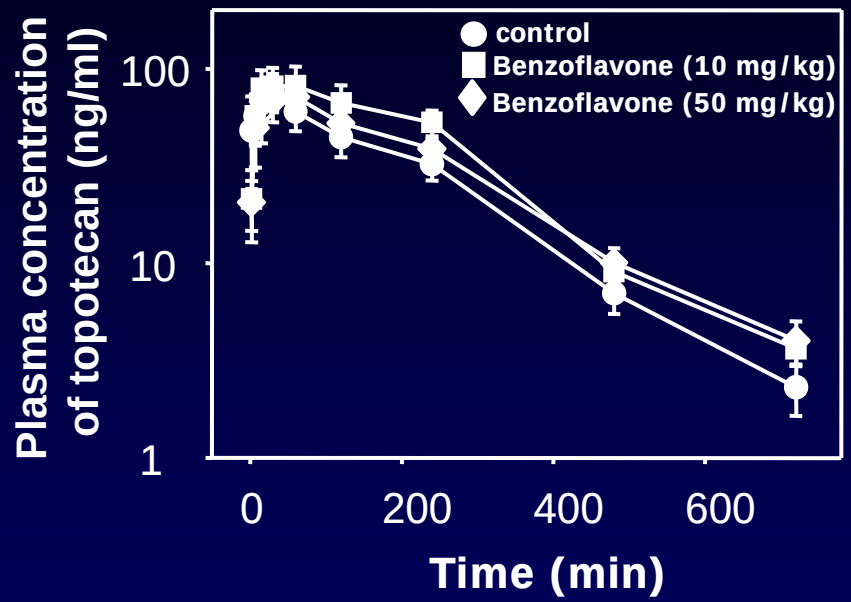
- **Metabolism**
- **Substrate dependence**
- **Species difference**
- **Inhibition of topotecan uptake transporter**

Effect of 7,8-benzoflavone (BF) on Topotecan PK in SD Rats

EC_{50} 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)



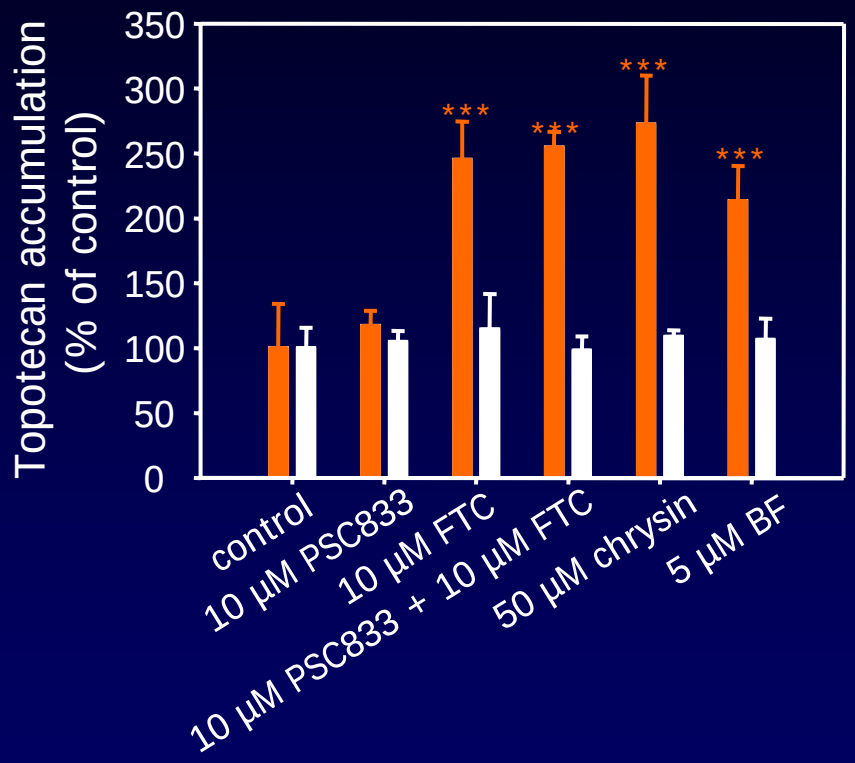
Parameters	Control (n=7)	benzoflavone (10 mg/kg) (n=9)	benzoflavone (50mg/kg) (n=8)
AUC_{0-360} (ng/ml·min)	1.74 ± 0.86 (×10^4)	2.04 ± 0.48 (×10^4)	2.50 ± 1.14 (×10^4)
$AUC_{0-\infty}$ (ng/ml·min)	1.80 ± 0.89 (×10^4)	2.15 ± 0.43 (×10^4)	2.57 ± 1.15 (×10^4)
Tmax (min)	52.0 ± 85.3	82.4 ± 91.4	107 ± 91.2
Cmax (ng/ml)	86.4 ± 42.9	98.0 ± 45.7	101 ± 50.9
terminal T1/2 (min)	127 ± 20.0	150 ± 63.6	127 ± 37.3
F (%)	29.7± 14.8	35.5 ± 7.33	42.5 ± 7.09

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



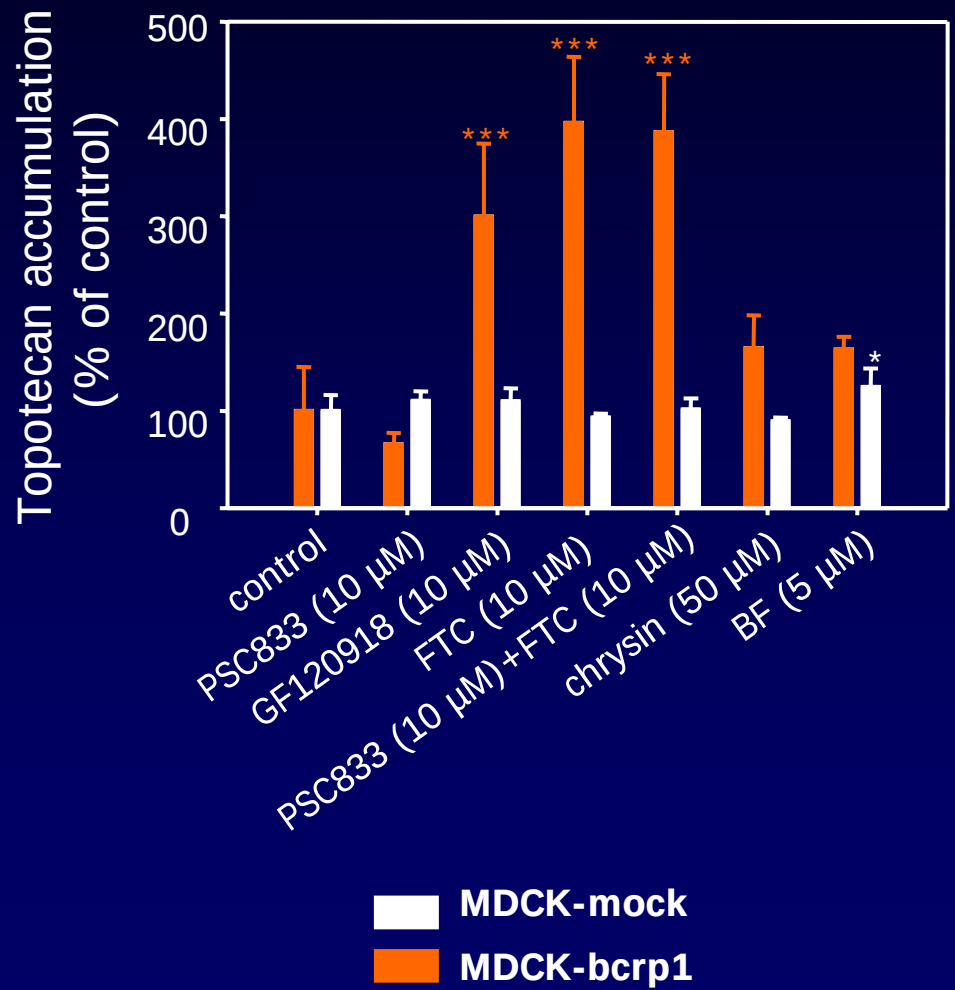
Chrysin and BF can inhibit BCRP-mediated efflux of topotecan

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

Flavonoid Interactions with ABC 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.