IN VITRO ASSESSMENT OF POTENTIAL DRUG-DRUG INTERACTIONS FOLLOWING THE FDA AND EMA GUIDANCE

参照美国FDA和欧盟药品管理局的监管指南采用体外实验方法评估潜在的药物相互作用

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Outline

Introduction to drug-drug interactions (DDI)

Regulatory guidance

In vitro assessment of potential DDI

Prediction of potential DDI risk

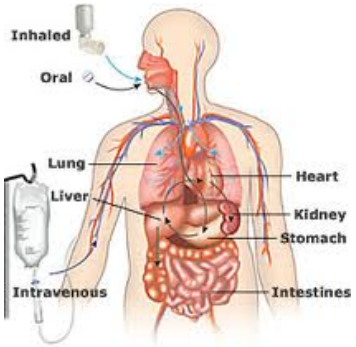
Abbreviations

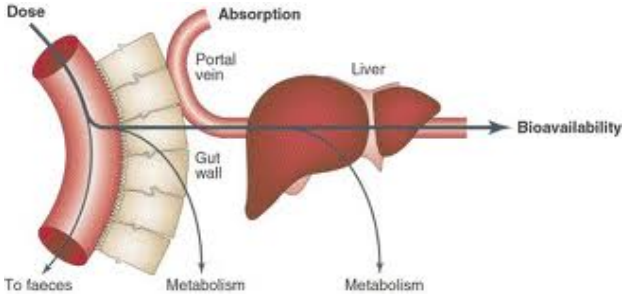
ADME	药物吸收分布代谢排泄
$C_{max.u}$	最高的血浆药物自由浓度
CYP	细胞色素P450酶
DDI	药物相互作用
EMA	欧盟药品管理局
HLM	人体肝脏微粒体
TDI	时间依赖性抑制
UGT	尿苷二磷酸葡萄醛酸转移酶

Pharmacokinetics (PK) Based DDIs

Data Source:
http://carnivoraforum.com

Fate of a Drug In Vivo (ADME)





ABSORPTION: Intestine (metabolism, passive and active transport)

DISTRIBUTION: All tissues and organs (passive and active transport)

METABOLISM: Liver (enzymes and active transport)

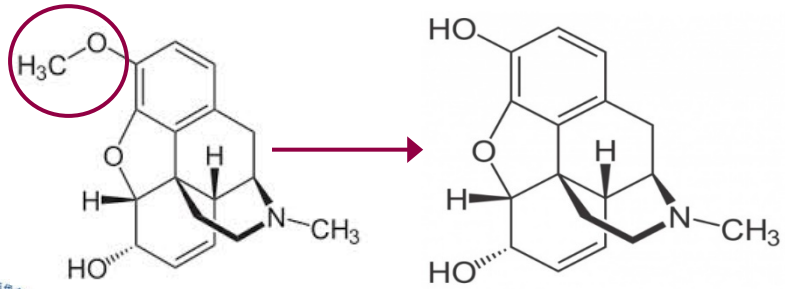
ELIMINATION: Kidney, liver, intestine (passive and active transport)

Two Key Players in ADME

1 2

METABOLIC ENZYMES

O-demethylation of codeine by CYP2D6



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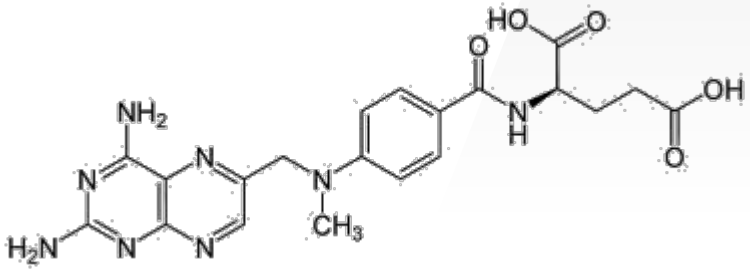
TRANSPORTERS

Active transport of methotrexate



DDI between DPC 333 and methotrexate
Luo et al, DMD, 35:835-840, 2007

Interactions with Enzymes/Transporters

SUBSTRATE

Oxycodone
CYP3A4 and CYP2D6

Metformin
OCT1 and OCT2

INHIBITOR

Quinidine
CYP2D6 and OCT1/2

Probenecid
OAT1/3 and MRP2

INDUCER

Phenobarbital
CYP2B6 and CYP3A4

Rifampicin
CYP3A4, CYP2Cs
and P-gp

Victim and Perpetrator

VICTIM DRUG

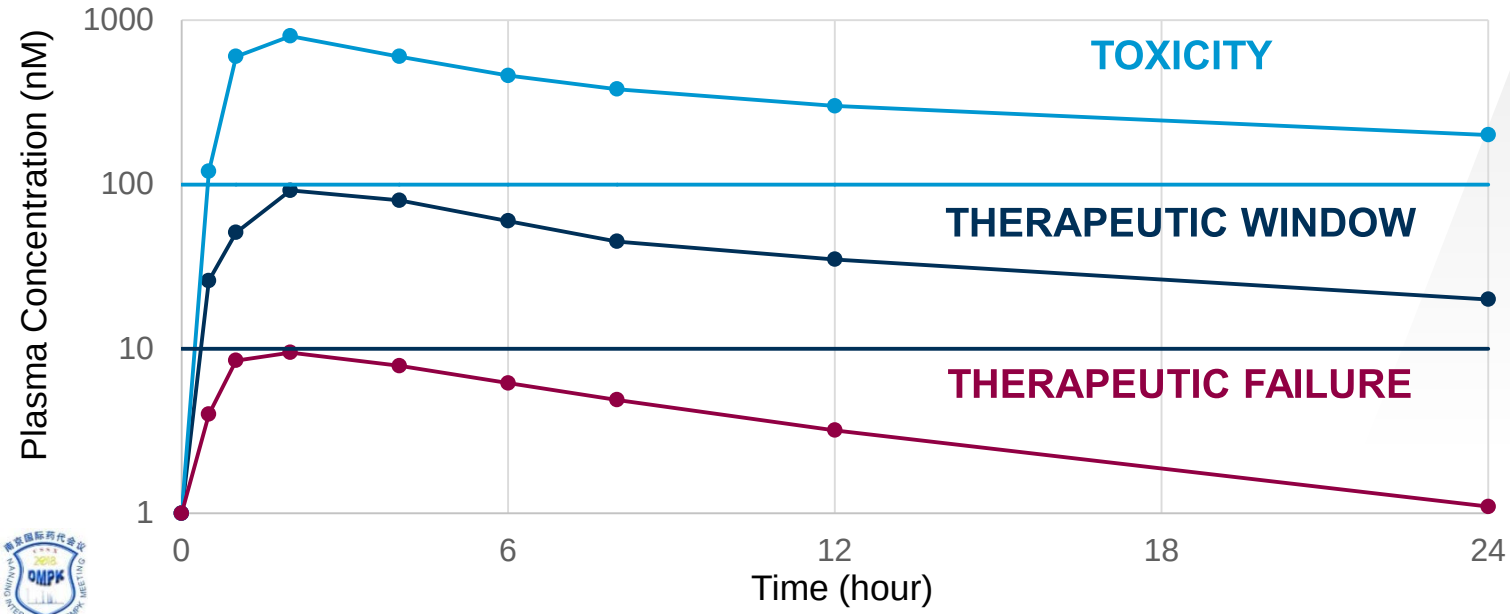
As a **substrate** of an enzyme/transporter, a drug becomes a victim of DDI when its plasma exposure is significantly altered by drug B which is administered concomitantly

PERPETRATOR DRUG

As an **inhibitor/inducer** of an enzyme/transporter, a drug becomes a perpetrator of DDI when it significantly affects the plasma exposure of drug A which administered concomitantly

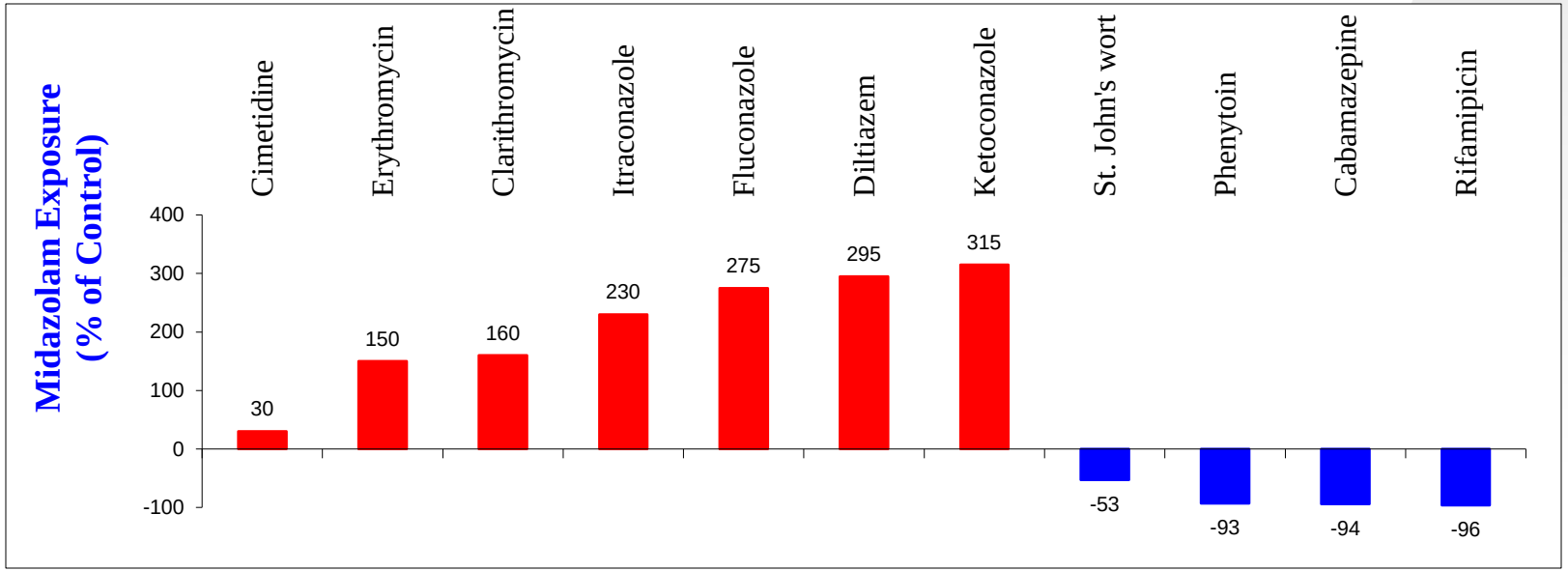
Adverse Consequences of DDIs

Inhibition → Higher exposure →toxicity
Induction → Lower exposure → therapeutic failure



Examples of Victim and Perpetrators

The plasma exposure (AUC) of midazolam can be markedly altered when it is administered concomitantly with CYP3A4 inhibitors or inducers



How and When to Assess DDIs

Drugs commonly used in combination in clinics

DDIs occasionally reported

Dozens of new drugs approved yearly

Regulatory guidance?

When to assess potential DDIs?

How to assess potential DDIs?

USA FDA DDI Guidance

DRAFT GUIDANCE FOR INDUSTRY:

Drug Interaction Studies—Study Design, Data Analysis, and Implications for Dosing and Labeling (**2006**)

GUIDANCE FOR INDUSTRY:

Drug Interaction Studies—Study Design, Data Analysis, and Implications for Dosing and Labeling Recommendations (**2012**)

DRAFT GUIDANCE IN VITRO METABOLISM

In Vitro Metabolism and Transporter-Mediated Drug-Drug Interaction Studies Guidance for Industry (**2017**)

EMA DDI Guidance

Draft Guideline on the Investigation of Drug Interactions (2010)

Draft Guideline on the Investigation of Drug Interactions (2012)

Regulatory Requirement

Interactions between an investigational new drug and other drugs should be defined during drug development, as part of an adequate assessment of the drug's safety and effectiveness (在药物开发间段, 定义新药和其它药物的相互作用, 应当是药物安全和有效评估的一部分)。

The objective of drug-drug interaction studies is to determine whether potential interactions between the investigational drug and other drugs exist and, if so, whether the potential for such interactions indicates the need for dosage adjustments, additional therapeutic monitoring, a contraindication to concomitant use, or other measures to mitigate risk (研究DDI的目的, 是鉴定新药和其它药物之间的DDI风险是否存在; 如果存在, 是否需要调整计量, 增加监控, 避免禁忌使用, 或采取其它措施来减低风险)。

—The FDA Draft Guidance (2012)

How to Assess Potential DDIs

Evaluating the DDI potential of an investigational new drug involves:

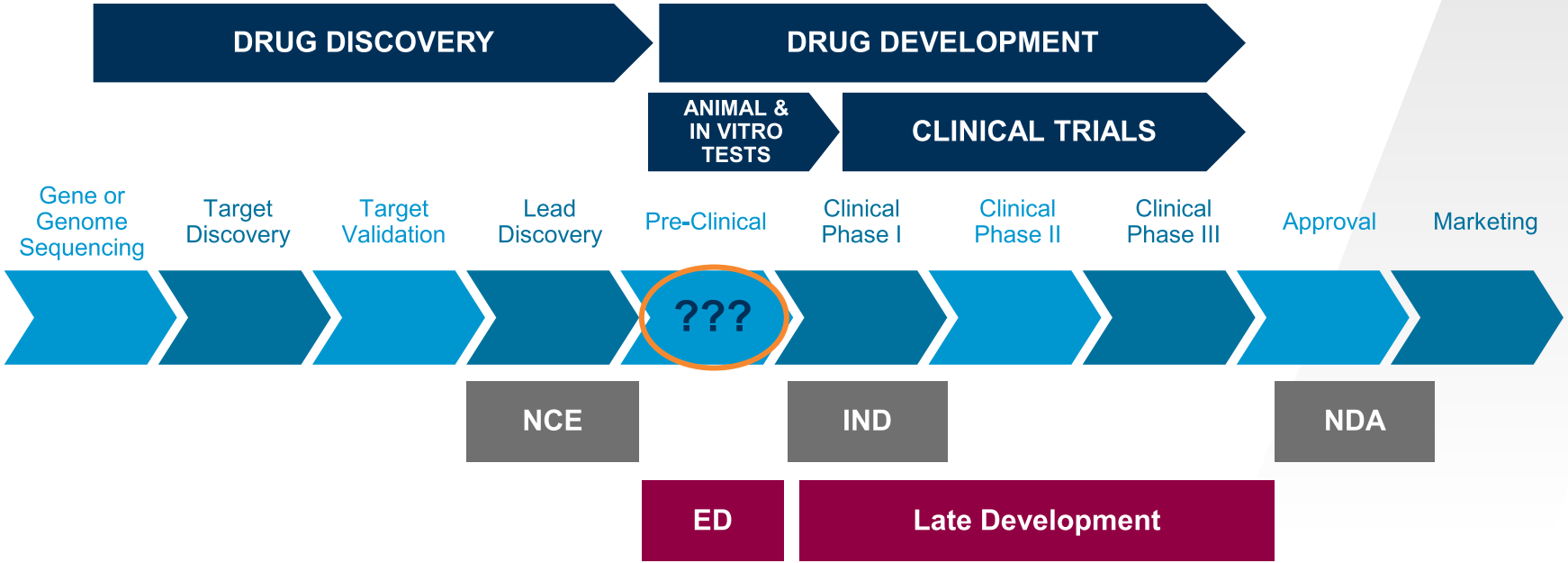
(1) identifying the principal routes of the drug's elimination;

(2) estimating the contribution of enzymes and transporters to the drug's disposition;

(3) characterizing the effect of the drug on enzymes and transporters

—The FDA Draft Guidance (2017)

When to Assess Potential DDIs



In Vitro DDI Assessment

Many possible DDIs

Clinical trials for DDIs: Time, cost, ethics

In Vitro DDI Assessment in early development:

1. Prior to first in man
2. Cost effective
3. Time efficient
4. Better understanding about mechanism
5. Direction to clinical trials

Clinical trial: A final confirmation

Scope of In Vitro DDI Assessment

Victims of metabolism-based DDIs

- An enzyme substrate, ≥25% contribution

Victims of transporter-based DDIs

- A transporter substrate, ≥25% contribution

Perpetrators of all kinds of DDIs

- An enzyme inhibitor or inducer
- A transporter inhibitor

Phenotyping Major CYP Enzymes

MAJOR CYP ENZYMES

Primary: 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4/5

Secondary: 2A6, 2J2, 4F2, & 2E1

Three Approaches

HLM ± chemical inhibitors or antibodies

Recombinant CYP enzymes

Metabolism correlations (not often)

Phenotyping Major UGT Enzymes

MAJOR UGT ENZYMES

1A1, 1A3, 1A4, 1A6, 1A9, 2B7, & 2B15

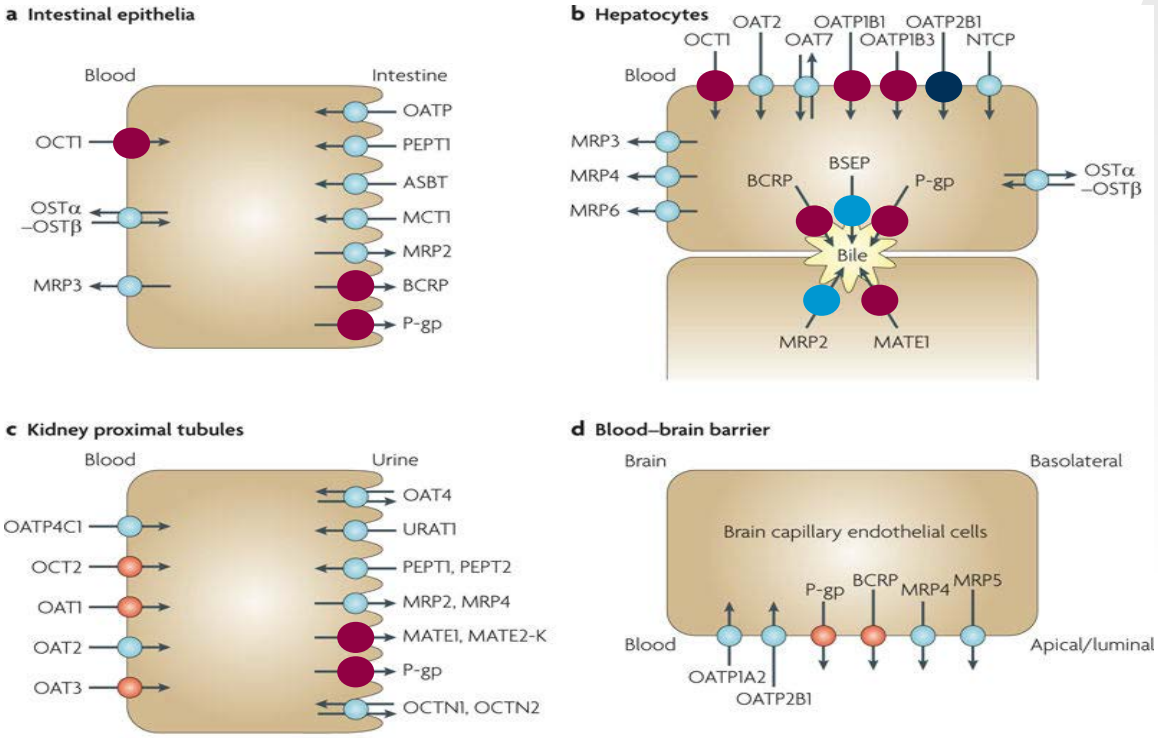
TWO Approaches

HLM ± inhibitors

Recombinant UGT enzymes

Drug Transporters in Play

BY BOTH FDA AND EMA
BY THE FDA ONLY
BY THE EMA ONLY



Phenotyping Major Transporters

MAJOR TRANSPORTERS

UPTAKE:
OAT1 & 3, OATP1B1, 1B3 & 2B1, OCT2 & OCT1

EFFLUX:
P-gp, BCRP, MATE1 & 2-K

SUBSTRATE CRITERIA

Fold uptake ≥2, reduced ≥50% by an inhibitor

Efflux ratio ≥2, reduced ≥50% by an inhibitor

EXPERIMENTAL MODELS

Cell-based models are more preferable

CYP Inhibition

MAJOR CYP ENZYMES
1A2, 2B6, 2C8, 2C9, 2C19, 2D6, & 3A4

TWO TYPES OF INHIBITION

REVERSIBLE INHIBITION

a **Preliminary assay:** IC50 determination

b **Definitive assay:** K_i determination if needed

TIME-DEPENDENT INHIBITION (TDI)

a **Preliminary assay:** TDI determination

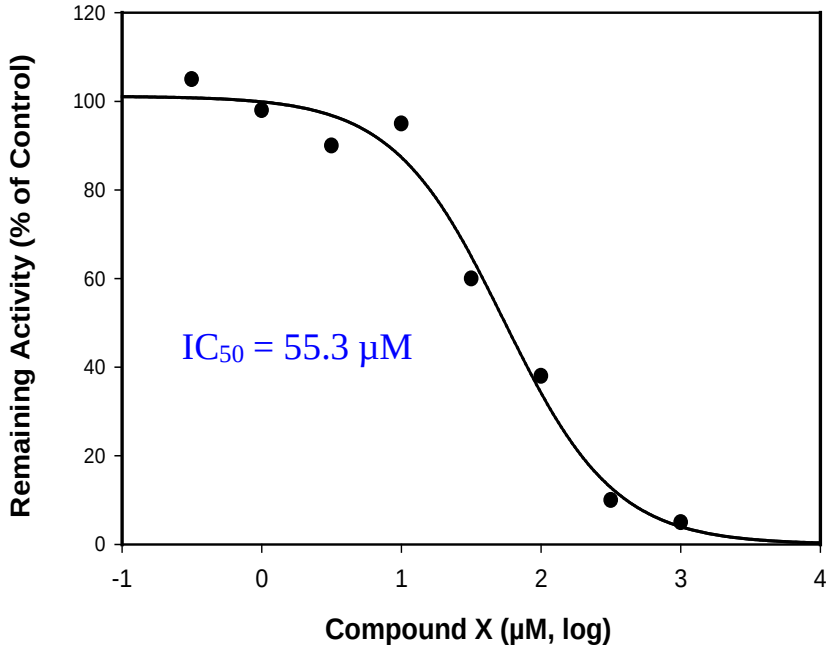
b **Definitive assay:** K_I & k_{inact}

Reversible CYP Inhibition Assays

CYP	Substrate (μM)	Enzyme Activity	Positive Inhibitor (μM)
CYP1A2	Phenacetin (110)	Phenacetin-*O*-deethylase	Fluvoxamine (0.5)
CYP2A6	Coumarin (1.6)	Coumarin 7-hydroxylase	Tranylcypromine (3)
CYP2B6	Bupropion (120)	Bupropion hydroxylase	Thiotepa (100)
CYP2C8	Amodiaquine (1.5)	Amodiaquine N-deethylase	Montelukast (0.1)
CYP2C9	Diclofenac (6)	Diclofenac 4'-hydroxylase	Sulfaphenazole (5)
CYP2C19	*S*-mephenytoin (50)	*S*-mephenytoin 4'-hydroxylase	Nootkatone (20)
CYP2D6	Bufuralol (11)	Bufuralol 1'-hydroxylase	Quinidine (0.3)
CYP2E1	Chlorzoxazone (170)	Chlorzoxazone 6-hydroxylase	DEDTC (700)
CYP3A4	Testosterone (65)	Testosterone 6ß-hydroxylase	Ketoconazole (0.2)
CYP3A4	Midazolam (1.5)	Midazolam 1'-hydroxylase	Ketoconazole (0.1)

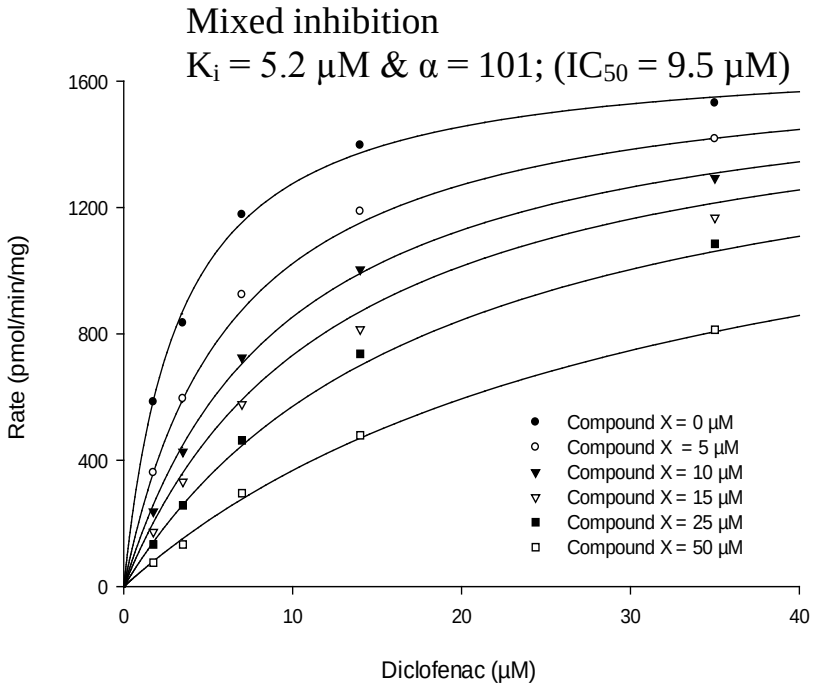
Pooled human hepatic microsomes (≥50 donors, mixed genders)
DEDTC: Diethyldithiocarbamate

Determination of IC50



- 1 Substrate conc. (~K_m)
- 7~8 Drug conc.
- Easy to be determined
- Partial inhibitory property
- An clue for K_i
- Quite variable
- Inhibition type unknown
- Not so valuable as K_i

Determination of Ki



- 5-6 Substrate conc.
- 5-8 Drug conc.
- Full inhibition property
- Inhibition type known
- A higher value for DDI
- Preliminary info needed
- Assay design challenge
- Calculation challenge

Time-Dependent CYP Inhibition

CYP	Substrate (μM)	Enzyme Activity	Positive Inhibitor (μM)
CYP1A2	Phenacetin (110)	Phenacetin-*O*-deethylase	Furafylline (1)
CYP2A6	Coumarin (1.6)	Coumarin 7-hydroxylase	8-MOP (10)
CYP2B6	Bupropion (120)	Bupropion hydroxylase	Ticlopidine (1.5)
CYP2C8	Amodiaquine (1.5)	Amodiaquine N-deethylase	GFG (10)
CYP2C9	Diclofenac (6)	Diclofenac 4'-hydroxylase	Tienilic acid (3)
CYP2C19	*S*-mephenytoin (50)	*S*-mephenytoin 4'-hydroxylase	S-fluoxetine (10)
CYP2D6	Bufuralol (11)	Bufuralol 1'-hydroxylase	MDMA (10)
CYP2E1	Chlorzoxazone (170)	Chlorzoxazone 6-hydroxylase	AMBA (40,000)
CYP3A4	Testosterone (65)	Testosterone 6ß-hydroxylase	Mifepristone (10)
CYP3A4	Midazolam (1.5)	Midazolam 1'-hydroxylase	Mifepristone (10)

Pooled human hepatic microsomes (≥50 donors)

AMAP, N-acetyle-m-aminophenol; GFG: Gemfibrozil 1-O-ß-Glucuronide;

MDMA: Methylenedioxymethamphetamine; 8-MOP, 8-Methoxypsoralen

UGT Inhibition

UGT	Substrate (μM)	Enzyme Activity	Positive Inhibitor (μM)
UGT1A1	Estradiol (10)	Estradiol-3-glucuronidation	Troglitazone (25)
UGT1A3	CDCA (15)	CDCA-24-*O*-glucuronidation	Schisantherin A (100)
UGT1A4	Trifluoperazine (5)	Trifluoperazine *N*-glucuronidation	Hecogenine (10)
UGT1A6	Naphthol (20)	Naphthol 1-glucuronidation	Demethylzeylasteral (10)
UGT1A9	Profolol (10)	Profolol glucuronidation	Niflumic acid (2.5)
UGT2B7	Morphine (500)	Morphine 3-glucuronidation	Mefenamic acid (50)
UGT2B15	*S*-Oxazepam (60)	*S*-Oxazepam glucuronidation	Niflumic acid (100)
UGT2B17	Testosterone (100)	Testosterone 17ß-*O*-glucuronidation	Quercetin (250)

Pooled human hepatic micrsomes (≥50 donors)

CDCA, Chenodeoxycholic acid

Transporter Inhibition

Transporter	Substrate (µM)	Positive Inhibitor (µM)
MATE1	^{14}C-Metformin (1)	Cimetidine (50)
MATE2-K	^{14}C-TEA (5)	Cimetidine (50)
OAT1	^{14}C-*P*-Aminohippurate (1)	Probenecid (200)
OAT3	^{3}H-Estrone-3-sulfate (1)	Probenecid (200)
OATP1B1	^{3}H-Estradiol-17ß-D-glucuronide (1)	Cyclosporine A (10)
OATP1B3	^{3}H-Cholecystokinin-8 (1)	Cyclosporine A (10)
OATP2B1	^{3}H-Estrone-3-sulfate (1)	Rifamycin SV (30)
OCT1	^{14}C-TEA (5)	Quinidine (256)
OCT2	^{14}C-Metformin (1)	Quinidine (256)
P-gp	^{3}H-Digoxin (1)	Zosuquidar (2)
BCRP	^{3}H-Estrone-3-sulfate (0.1)	Ko143 (1)

CYP Induction

Enzyme	Positive Inducer (μM)	mRNA	Substrate (μM)
CYP1A2	Omeprazole (50)	Yes	Phenacetin (100)
CYP2B6	Phenobarbital (1000)	Yes	Bupropion (500)
CYP3A4	Rifampicin (20)	Yes	Testosterone (250)
Negative control	Flumazenil (20)	All	All

- Primary cultures of human hepatocytes from 3 individual donors
- Determination of mRNA levels as well as activities
- Calculation of E_{max} and EC_{50}, if applicable
- Positive (RIS) and negative controls included

Criteria to be an CYP inducer

- mRNA level ≥4-fold or activity ≥2-fold over vehicle control AND
- ≥20% of positive control

Considerations in Assessment

The FDA and EMA DDI guidance

Validation of test systems

Concentrations used in vitro assessment

A single drug playing Multiple roles

A single drug being a victim and perpetrator

Fraction unbound (f_u) in plasma and HLM

Recommended Concentrations

CYP/TRANSPORTER INHIBITION

FDA
Liver & kidney: ≥50x $C_{max.u}$

GI: ≥0.1x dose/250 mL

EMA
Liver & kidney: ≥50x $C_{max.u}$

GI: ≥0.1x dose/250 mL

CYP INDUCTION

FDA
≥50x $C_{max.u}$ (or 10x C_{max})

Cytotoxicity & solubility

EMA
≥50x $C_{max.u}$

Cytotoxicity & solubility

CYP/TRANSPORTER SUBSTRATE

≤Km

CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4/5

<<Km

P-gp, BCRP, MATEs, OAT1/3, OATP1B1/3, 2B1, OCT1/2

A Single Drug Multiple Roles

	RITONAVIR	DPC 681
Substrate	CYP3A4	CYP3A4
Reversible inhibition	CYP3A4, P-gp, OATP	CYP3A4
Metabolism-based inactivation	CYP3A4	CYP3A4
Induction	CYP3A4, P-gp	CYP3A4

Luo et al., DMD, 30:795-804, 2002

Luo et al., DMD, 31: 1170-1175, 2003

Luo et al., Current Drug Metabolism, 5:485-505, 2004

Model-Based Assessments for DDI Potential

BASIC MODELS

Calculate R values (R1, R2, & R3)

Compare with the cutoff criteria

STATIC MECHANISTIC MODELS

Calculate AUCR

AUCR cutoffs (≥1.25 for inhibition & ≤0.8 for induction)

PBPK MODELS

Calculate exposure ratio

(Simcyp or GastroPlus)

Consider plausibility

Evaluate uncertainty

R_1 for Reversible CYP Inhibition

$$R_1 = 1 + (I_{max,u} / K_i) \quad \text{(significant if } R_1 \geq 1.02\text{)}$$

$$R_{1,gut} = 1 + (I_{gut} / K_i) \quad \text{(significant if } R_{1,gut} \geq 11\text{)}$$

R_1 or $R_{1,gut}$ is the predicted ratio of the victim drug's AUC in the presence and absence of an inhibitor for basic models of reversible inhibition

$I_{max,u}$ is the maximal unbound TA plasma conc.

I_{gut} is the intestinal luminal TA conc. (dose/250 mL)

K_i is the unbound inhibition constant determined in vitro

R_2 for TD CYP Inhibition

$$R_2 = (k_{obs} + k_{deg}) / k_{deg} \quad \text{(significant if } R_1 \geq 1.25\text{)}$$

$$\text{Where } k_{obs} = (k_{inact} \times 50 \times I_{max,u}) / (K_I + 50 \times I_{max,u})$$

R_2 is the predicted ratio of the victim drug's AUC in the presence and absence of an inhibitor for basic model of TDI

k_{obs} is the observed inactivation rate constant

k_{deg} is the apparent first-order degradation rate constant

K_I is the inhibition conc. Causing half-maximal inactivation

k_{inact} is the maximal inactivation rate constant

$I_{max,u}$ is the maximal unbound TA plasma conc.

R_3 for CYP Induction

$$R_3 = 1 / [1+ (d \times E_{max} \times I_{max,u}) / (EC_{50} + (10 \times I_{max,u}))]$$

R_3 is the predicted ratio of the victim drug's AUC in the presence and absence of an inducer for basic models of enzyme induction

d is the scaling factor and is assumed to be 1 unless supported by prior experience with system used

E_{max} is the maximum induction effect of the interacting drug

$I_{max.u}$ is the maximum unbound plasma conc. Of the interacting drug

EC_{50} is the concentration causing half-maximal effect determine in vitro

R for Transporter Inhibition

For P-gp & BCRP $R = I_{gut}/IC_{50} \geq 10$

For OATP1B1/3 $R = 1 + (f_{u,p} \times I_{in.max})/IC_{50} \geq 1.1$

$I_{in,max} = (I_{max} + (F_a F_g \times k_a \times Dose)/Q_h/R_B)$

For OAT/OCT $R = I_{max.u}/IC_{50} \geq 0.1$

For MATEs $R = I_{max.u}/IC_{50} \geq 0.02$

Static Mechanistic Models

$$AUCR = [1 / (A_g \times B_g \times C_g) \times F_g \times (1 - F_g)] * [1/ (A_h \times B_h \times C_h) \times f_m \times (1 - f_m)]$$

A: the effect of reversible inhibitions.

B: the effect of TDI.

C: the effect of induction.

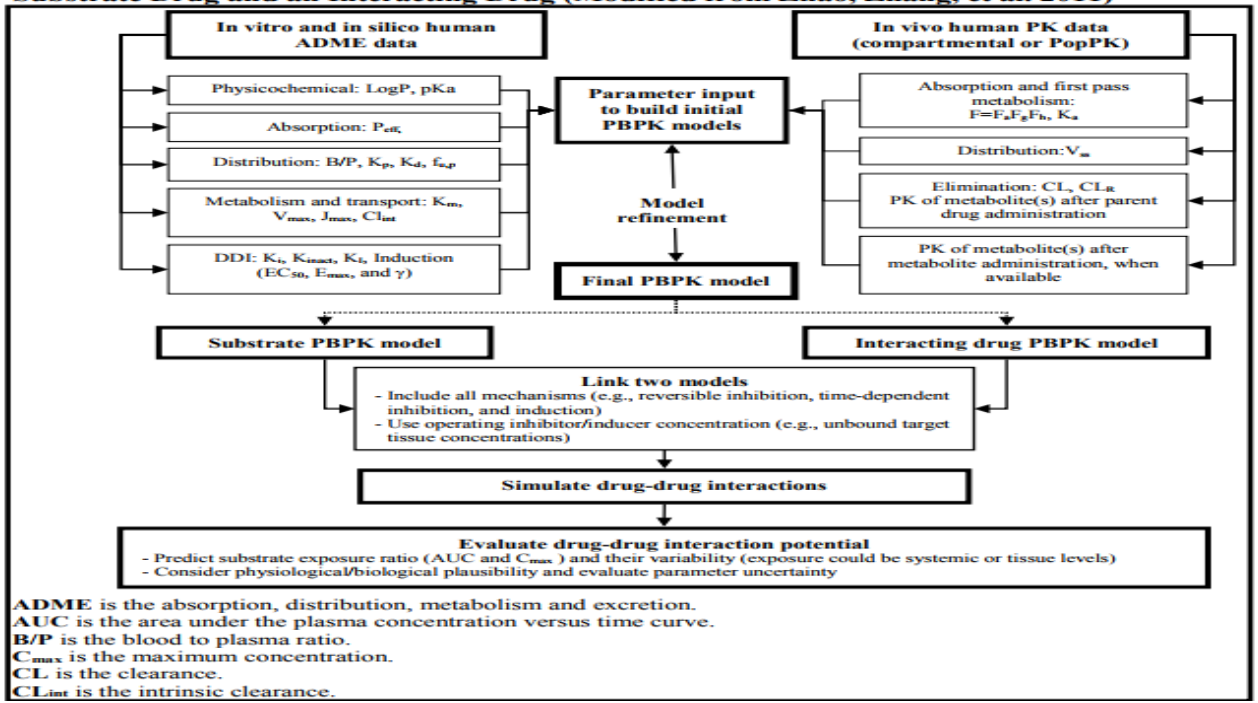
Fg: the fraction available after intestinal metabolism.

Fm: the fraction of systemic clearance of the substrate mediated by the CYP enzyme that is subject to inhibition/induction.

'h' denote liver; **'g'** denote gut.

PBPK Models

Figure 8. A PBPK Model-Based Framework to Explore the DDI Potential Between a Substrate Drug and an Interacting Drug (Modified from Zhao, Zhang, et al. 2011)*



ADME is the absorption, distribution, metabolism and excretion.
AUC is the area under the plasma concentration versus time curve.
B/P is the blood to plasma ratio.
C_{max} is the maximum concentration.
CL is the clearance.
CL_{int} is the intrinsic clearance.

Acknowledgements

Covance Laboratories Inc.

DMPK Management

In Vitro Group

Nanjing International DMPK Meeting

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Backup Slides

CYP Degradation Rate Constant

CYP Enzyme	$t_{1/2}$ Range (hr)	K_{deg} Range (hr^{-1})
CYP1A2	8-105	0.0066-0.0866
CYP2A6	19-37	0.0187-0.0365
CYP2B6	32	0.0217
CYP2C8	8-41	0.0169-0.0866
CYP2C9	104	0.00666
CYP2C19	7-50	0.0139-0.099
CYP2D6	50-70	0.0099-0.0139
CYP2E1	7-60	0.0116-0.099
CYP3A4	20-184	0.00377-0.0347
CYP3A5	15-70	0.0099-0.0462

K_{deg} values were calculated from Yang et al, Current Drug Metabolism, 384-393, 2008

Static Mechanistic Models

Figure 7: Equation to Calculate AUCR of the Substrate Drugs (AUC plus investigational drug/AUC minus investigational drug)

$$AUCR = \left(\frac{1}{[A_g \times B_g \times C_g] \times (1 - F_g) + F_g}\right) \times \left(\frac{1}{[A_h \times B_h \times C_h] \times f_m + (1 - f_m)}\right)$$

A is the effect of reversible inhibitions.
B is the effect of TDI.
C is the effect of induction.
F_g is the fraction available after intestinal metabolism.
f_m is the fraction of systemic clearance of the substrate mediated by the CYP enzyme that is subject to inhibition/induction.
Subscripts 'h' denote liver.
Subscripts 'g' denote gut.

Each value can be estimated with the following equations:

	Gut	Liver
Reversible inhibition	$A_g = \frac{1}{1 + \frac{[I]_g}{K_i}}$	$A_h = \frac{1}{1 + \frac{[I]_h}{K_i}}$
Time-dependent inhibition	$B_g = \frac{k_{deg,g}}{k_{deg,g} + \frac{[I]_g \times k_{inact}}{[I]_g + K_I}}$	$B_h = \frac{k_{deg,h}}{k_{deg,h} + \frac{[I]_h \times k_{inact}}{[I]_h + K_I}}$
Induction	$C_g = 1 + \frac{d \bullet E_{max} \bullet [I]_g}{[I]_g + EC_{50}}$	$C_h = 1 + \frac{d \bullet E_{max} \bullet [I]_h}{[I]_h + EC_{50}}$

$[I]_h = f_{u,p} \times (C_{max} + F_a \times k_a \times Dose/Q_h/R_B)$ (Ito, Iwatsubo, et al. 1998)
$[I]_g = F_a \times k_a \times Dose/Q_{en}$ (Rostami-Hodjegan and Tucker 2004)
$f_{u,p}$ is the unbound fraction in plasma. When it is difficult to measure accurately due to high protein binding (i.e., $f_{u,p}$ <0.01) in plasma, a value of 0.01 should be used for $f_{u,p}$.
C_{max} is the maximal total (free and bound) inhibitor concentration in the plasma at steady state.
F_a is the fraction absorbed after oral administration; a value of 1 should be used when the data are not available.
k_a is the first order absorption rate constant in vivo; a value of 0.1 min^{-1} (Ito, Iwatsubo, et al. 1998) can be used when the data are not available.
Q_{en} is the blood flow through enterocytes (e.g., 18 L/hr/70 kg (Yang, Jamei, et al. 2007a)).
Q_h is the hepatic blood flow (e.g., 97 L/hr/70 kg (Yang, Jamei, et al. 2007b)).
R_B is the blood-to-plasma concentration ratio.

南京怀古

梦戴雨花几多回，
身穿乌衣终不悔。
六朝金陵帝王家，
十里秦淮商女会。
英雄悲歌漫血色，
才子豪情少春晖。
回首千年弹指间，
地上荣耀若尘灰。

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