生物薬品と薬物の相互作用として以下の報告例がある.
・P450の発現レベルに影響を及ぼすことによるP450基質の代謝を修飾する例： IFNα-2bなどのサイトカインは，様々なP450分子種の転写レベルを低下させ酵素活性の低下を引き起こすことにより，当該P450分子種の基質薬の血中濃度を増加させる[57].
・サイトカインを介したP450分子種の酵素活性低下作用の抑制によるP450活性「正常化」の例：リウマチ患者に対するトシリズマブ投与によるシンバスタチンのAUC低下が挙げられる[58].
・P450又はトランスポーターの調節以外のメカニズムに基づく例：メトトレキサートの免疫抑制作用による，併用薬（治療用蛋白質）に対して形成される抗体の減少に伴うクリアランスの低下が挙げられる[59,60].

（１４） トランスポーターを介した薬物相互作用の評価に関する留意事項
①OATP に対する阻害における特殊な事例
OATP では，時間依存的な阻害が現れる場合があり，このような場合では，あらかじめトランスポーターを発現する細胞（発現系・ヒト肝細胞など）と阻害薬とを一定時間 preincubation 後に阻害実験を実施することにより，見かけの K_i 値が，preincubation なしでの通常の阻害実験から求められた K_i 値よりも低く見積もられることがある[61,62]．この見かけの K_i 値の方が，より *in vivo* での薬物相互作用の強度を反映する場合があることに留意が必要である．また，基質により阻害薬の K_i 値が異なるケースも報告されている[63]ため，阻害実験の際に，基質薬としては，臨床現場での併用が想定される薬物を用いた解析が有用である．さらに，蛋白結合形の薬物による阻害も考慮しないと阻害強度が説明できないケースも報告されており，蛋白結合形薬物濃度も含めた全薬物濃度に基づいた考察が必要になる場合もある[64].

②トランスポーターを介した内因性物質の変動
トランスポーターには，胆汁酸の肝輸送に寄与する Sodium-taurocholate cotransporting polypeptide (NTCP)や BSEP，ビリルビンないしはそのグルクロン酸抱合体の肝輸送に寄与する OATP 類や MRP2，クレアチニンや N-methylnicotinamide の腎排泄に関わる分泌に一部寄与する MATE 類などのように内因性物質の輸送に関わるトランスポーターがある[65-67]．これらトランスポーターの阻害により，内因性物質の血中濃度上昇や組織内蓄積が認められる場合がある．内因性物質の臨床検査値に変動が見られた場合には，肝毒性及び腎毒性だけでなく，トランスポーターの阻害もその原因になり得ることがあることに留意する必要がある．一方で，最近の報告では，BSEP の阻害強度が強い医薬品において，臨床での肝毒性発現のリスクが高い傾向がみられるとするものもあり，注意が必要である[68].

（１５） 投与期間と投与タイミングの重要性
CYP3A の阻害薬であると同時に CYP2C9 などの誘導薬でもあるリトナビルに代表されるように，代謝酵素の阻害薬であり誘導薬でもある場合，併用する時期により正味の相互作用が異なる可能性がある[69,70]．このような場合には，代謝酵素の発現量が新たな定常状態となるための十分な投与期間を設けると共に，必要に応じて，被験薬と併用薬の投与タイミングを変化させた臨床薬物相互作用試験を実施し，その影響を慎重に考察することが推奨される.
また，リファンピシンは，CYP3A をはじめとした薬物代謝酵素の強い誘導薬として知られているが，同時に OATP1B1 などのトランスポーターの阻害薬でもある[71,72]．したがって，リファンピシンによるトランスポーター阻害作用を検討する目的で併用投与試験を行う場合，被相互作用薬としての被験薬の濃度測定のためのサンプリングはリファンピシンの単回投与直後に行うのが最適である．一方，強い酵素誘導薬としてのリファンピシンによる影響を明確にして他の誘導薬の作用を推定することが目的である場合，リファンピシンの OATP1B1 阻害作用により酵素誘導作用が過小評価されることがあるため，リファンピシン最終投与の翌日に被験薬のサンプリングを行うのが最適である.

（１６） 代謝酵素の基質薬の選択
被験薬と併用される薬物の中に，治療域の狭い基質が含まれる場合には特に注意が必要である．治療域の狭い基質薬は，P450阻害薬との併用によって C_{max} やAUCがわずかに増加するだけで，重篤な安全性の懸念が生じるおそれがある薬物である．治療域の狭い基質薬の典型例としては，ワルファリン（濃度が若干増加しただけで，重大出血を引き起こすおそれがある），torsade de pointesを引き起こすおそれがある薬物，ほとんどの細胞障害性抗腫瘍薬，及びアミノグリコシド系抗生物質などが挙げられる．こ

れら治療域の狭い基質薬との併用が想定される場合には，安全性の観点に立って臨床薬物相互作用試験の必要性，並びに基質薬の投与量や投与期間を検討するべきである．

臨床薬物相互作用試験に使用される指標薬のいくつかは，2種類以上のP450又はトランスポーターの基質である場合があるため，選択的基質ではないことに注意する．例として，オメプラゾールはCYP2C19の基質であるが，CYP3Aによっても代謝される．CYP2C19阻害（誘導）を評価するためにオメプラゾールを基質として使用する場合は，未変化体と共に代謝物（CYP2C19を介するヒドロキシオメプラゾール及びCYP3Aを介するオメプラゾールスルホン）を測定することが推奨される[73]．また，レパグリニドはCYP2C8の指標薬として用いられるが，OATP1B1の基質でもあるため，同トランスポーターを阻害する薬物との相互作用試験の結果の解釈には注意が必要である．

（１７）代謝酵素とトランスポーターの両方が関わる薬物相互作用の事例

複数の酵素/トランスポーターを阻害又は誘導する場合の例としては，CYP3A 及び P-gp を共に阻害するイトラコナゾールや共に誘導するリファンピシンがある．この際，CYP3A 及び P-gp の両者に対して必ずしも同等の阻害能や誘導能を示すとは限らない．したがって，CYP3A の基質，P-gp の基質，又は CYP3A と P-gp の両者の基質である被験薬との薬物相互作用試験のために阻害薬を選択する際は，CYP3A 及び P-gp に対する阻害作用の違いを考慮する[37]．なお，リファンピシンは複数の P450 及びトランスポーターの誘導薬であることが立証されており，取り込みトランスポーターOATP1B1 の阻害薬でもあることに留意する（留意事項（15）参照）．

また，複数の薬物を同時併用することで，代謝酵素とトランスポーターの両者が阻害され，より複雑な影響が現れた例としては，イトラコナゾール及びゲムフィブロジルの同時投与によるレパグリニドの AUC が大きく変化した場合がある．これは，酵素（CYP3A）に対するイトラコナゾールの阻害作用，及びトランスポーター（OATP1B1）及び酵素（CYP2C8）に対する，ゲムフィブロジルとその代謝物による阻害作用の総合的な作用と考えられる[74]．

（１８）カクテル基質試験による評価

通常，カクテル試験は一般的な臨床薬物相互作用試験と同様に，*in vitro* で示された作用を検討するために行われるが，酵素（及びトランスポーター）に対する多種多様な代謝物の阻害能及び誘導能を評価することを目的として，*in vitro* 試験の代わりに行ってもよい．

試験において使用する基質は，特定の酵素（及びトランスポーター）に対する選択的阻害薬を用いた薬物相互作用試験あるいは薬理遺伝学的試験などにおいて，その特異性が証明されている必要がある．カクテル基質試験における使用用量の妥当性は，お互いに相互作用を及ぼさないことが臨床において示されていることが望ましいが，評価対象の酵素（及びトランスポーター）に対する K_m 値と循環血中の C_{max} や消化管における推定濃度を比較して，十分低い濃度であれば基質間の相互作用が無いとみなすことができる．

（１９）遺伝子多型を考慮した薬物相互作用の評価

CYP2C19 は主として *CYP2C19*2* 及び *CYP2C19*3* 多型により東アジア人で活性欠損者の頻度が高く，CYP2D6 は東アジア人で活性欠損者は少ないが，活性が大きく減じる遺伝子多型である *CYP2D6*10* の頻度が高い[32]．このため，これらの分子種がクリアランスの主要経路である被験薬については，東アジア人を対象とした試験と東アジア人以外を対象とした試験の結果を比較考察する場合に遺伝子多型に注意が必要である．特に，CYP2C19 の活性欠損者において薬物相互作用の程度が大きいと予想され，臨床的に問題となる可能性がある場合には遺伝子多型を考慮した薬物相互作用の検討を目的とした臨床試験を追加することが有用である．遺伝子多型を考慮した臨床試験の実施に際しては，活性欠損者の血中濃度は高値となることが予想され，被験者の安全性に最大限配慮する．また，薬物相互作用に影響を及ぼす可能性を，*in vitro* 試験の成績等に基づき，モデリングとシミュレーションにより検討することも有用である．

遺伝子多型を考慮すべき薬物相互作用の例として以下がある．

CYP2C19 で主に代謝されるボリコナゾールは，CYP2C19 の活性欠損者では，代替経路である CYP3A の阻害薬の併用で顕著に全身曝露が増大する[75]．CYP2D6 で主に代謝されるトルテロジンは，CYP2D6 の活

性欠損者では，代替経路であるCYP3Aの阻害薬の併用で全身曝露が顕著に増大する[76]．

CYP3A5，UGT1A1，OATP1B1（*SLCO1B1*），BCRP（*ABCG2*）などの分子種でも，遺伝子多型によりクリアランスが変化することが知られている[32,33,77]．CYP3A5で頻度の高い遺伝子多型として，酵素発現の消失をもたらす*CYP3A5*3*が知られている．CYP3A5は，一般にCYP3A4と基質認識性が類似しているが，一部の阻害薬ではCYP3A4とCYP3A5の阻害定数が異なることが報告されている．したがって，CYP3A4の阻害が強くCYP3A5の阻害が弱い場合では，*CYP3A5*3*を有する被験者はCYP3A基質薬のクリアランスが大きく低下することに留意する必要がある．また，日本人では，酵素活性の低下を示す*UGT1A1*6*，*UGT1A1*28*，及び輸送機能の低下が示唆される*SLCO1B1* c.521T>C，*ABCG2* c.421C>Aの頻度が比較的高いため注意を要する．

11. 用語一覧

1) 基質：本ガイドラインでは，一般に代謝を受ける薬物あるいはトランスポーターにより輸送される薬物.
2) 分布容積：分布容積が小さいとは，ほぼ細胞外液量あるいはそれ以下の値（ヒトで約0.25 L/kg以下），分布容積が大きいときはヒトで約0.8 L/kg以上とする.
3) 併用薬：複数の薬物を使用する場合，それぞれを広義の併用薬と呼ぶ．なお，狭義の意味では，基礎療法に用いられている薬物に更に追加して使用される薬物を併用薬と呼ぶ.
4) 相互作用薬：薬物動態学的相互作用においては，併用することにより，他の薬物の体内動態に影響を与える薬物．例えば代謝に関しては，代謝酵素を阻害するものと誘導するものなどがある.
5) 被相互作用薬：薬物動態学的相互作用においては，併用薬物により，その体内動態が影響を受ける薬物．例えば代謝に関しては，代謝酵素が阻害されその薬物の代謝が低下するものと酵素誘導により代謝が亢進するものなどがある.
6) 被験薬：本ガイドラインでは，併用薬に薬物相互作用を与えるか，又は併用薬から影響を受けるかについての可能性が検討される医薬品あるいは開発中の薬物.
7) 指標薬：薬物動態に関与する酵素，トランスポーター又は血漿蛋白質に対する特異性が高いことが複数の臨床試験で確認されており，薬物動態の変動を示す指標となる薬物．定量が可能な薬物で，臨床試験で使用される薬物の場合は安全性が高いことが必要である.
8) 単代謝酵素薬物：主として一つの代謝酵素により代謝される薬物．当該代謝酵素の活性変動による薬物相互作用を受けた場合に総代謝クリアランスの変動が大きく，その場合のリスクが高い.
9) 多代謝酵素薬物：複数の代謝酵素により代謝される薬物．一般に，薬物相互作用による代謝酵素活性変動を受けた場合に総代謝クリアランスの変動が小さく，よりリスクが低い.
10) トランスポーター：生体膜を横切り，薬物を細胞の内外へ輸送する担体.
11) 選択的阻害薬，選択的基質薬：ある代謝酵素又はトランスポーターに対してのみ，比較的強い阻害作用を有する薬物，又は比較的選択的に代謝又は輸送を受ける薬物.
12) 典型阻害薬，典型基質薬（表6-4，6-5）：あるトランスポーターの阻害に良く用いられるが，複数の代謝酵素又はトランスポーターを阻害する場合があり，典型基質は複数の代謝酵素又はトランスポーターの基質となる場合があるため，必ずしも選択的阻害薬又は選択的基質薬とはならない.
13) 強い阻害薬，中程度の阻害薬，弱い阻害薬：「相互作用を受けやすい基質薬」のAUCを，5倍以上に上昇（CL/Fが1/5未満に減少）させると考えられる医薬品などを「強い阻害薬」，2倍以上5倍未満に上昇（CL/Fが1/2未満1/5以上に減少）させると考えられる医薬品などを「中程度の阻害薬」，1.25倍以上2倍未満に上昇（CL/Fが1/1.25未満1/2以上に減少）させると考えられる医薬品などを「弱い阻害薬」とする（7.6項の記載を参照）.
14) 強い誘導薬，中程度の誘導薬，弱い誘導薬：「相互作用を受けやすい基質薬」のAUCを1/5以下に減少（CL/Fが5倍より大きく上昇）させると考えられる医薬品などを「強い誘導薬」，1/2以下1/5より大きく減少（CL/Fが2倍以上5倍未満に上昇）させると考えられる医薬品などを「中程度の誘導薬」，1/1.25以下1/2より大きく減少（CL/Fが1.25倍以上2倍未満に上昇）させると考えられる医薬品などを「弱い誘導薬」とする（7.7項の記載を参照）.
15) 相互作用を受けやすい基質薬，相互作用の受けやすさが中程度の基質薬：「強い阻害薬」の併用によりAUCが5倍以上に上昇（CL/Fが1/5未満に減少）する基質薬を「相互作用を受けやすい基質薬」，「強い阻害薬」との併用によりAUCが2倍以上5倍未満に上昇（CL/Fが1/5以上1/2未満に減少）する基質薬を「相互作用の受けやすさが中程度の基質薬」とする（7.8項の記載を参照）.

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III. 研究成果の刊行に関する一覧表と別刷

研究成果の刊行に関する一覧表

書籍

著者氏名	論文名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
Maeda K, Sugiyama Y.	Prediction of Hepatic Transporter-Mediated Drug-Drug Interaction from In vitro Data.	Sugiyama Y and Steffansen B	Transporters in Drug Development	Springer	New York	2013	121-153
伊藤清美、大野泰雄、久米俊行、斎藤嘉朗、鈴木洋史、永井尚美、樋坂章博、前田和哉	薬物相互作用ガイドラインの作成	渡邉裕司	GCP ハンドブック	じほう	東京	2014	28-41

雑誌

著者氏名	論文名	雑誌名	巻	ページ	刊行年
大野泰雄	薬物相互作用ガイドライン改訂の背景と検討方針.	ファルマシア			印刷中
永井尚美	薬物相互作用に関する指針の改定について.	ファルマシア			印刷中

Chapter 6
Prediction of Hepatic Transporter-Mediated Drug–Drug Interaction from In Vitro Data

Kazuya Maeda and Yuichi Sugiyama

Abstract The importance of transporter-mediated drug–drug interaction (TP-DDI) has been rapidly recognized by the recent publication of its clinical evidences and subsequent updated regulatory guidance (guideline). The methods of TP-DDI prediction are roughly divided into two approaches; static model and dynamic model. Static model with theoretically maximum unbound concentration is useful to sensitively catch the signal of DDIs, but predicted DDI risk should always be overestimated. Dynamic model fully considers the time courses of the plasma and tissue concentrations of both substrate and inhibitor drugs by the physiologically based pharmacokinetic (PBPK) model, thus accurate estimation of DDI risk can be achieved. However, the universal methods to set up model parameters based on the in vitro results with scaling factors remain to be discussed. This chapter is mainly focused on the basic theory and recent progress of the methods for TP-DDI predictions.

Abbreviations

AUC	Area under the concentration-time curve
BCRP	Breast cancer resistance protein
BSP	Bromosulfophthalein
CYP	Cytochrome P450

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DDI	Drug–drug interaction
$E_2$17βG	Estradiol-17β-D-glucuronide
EMA	European Medicines Agency
FDA	Food and Drug Administration
MRP	Multidrug resistance-associated protein
NTCP	Sodium taurocholate cotransporting polypeptide
OATP	Organic anion transporting polypeptide
PBPK	Physiologically based pharmacokinetic
PET	Positron emission tomography
P-gp	P-glycoprotein
TP-DDI	Transporter-mediated drug–drug interaction

6.1 Introduction

The number of clinical drug–drug interaction (DDI) studies involving drug transporters has increased rapidly in recent years, and the ability to predict transporter-mediated DDIs (TP-DDIs) is needed in the process of drug development. The liver is one of the most important organs for the detoxification of drugs. The liver expresses many kinds of metabolic enzymes and uptake/efflux transporters, and DDIs with hepatic enzymes or transporters often lead to a change in the plasma concentration and subsequently the pharmacological and toxicological effects of drugs. The US Food and Drug Administration (FDA) DDI draft guidance and European Medicines Agency (EMA) DDI guideline note that organic anion transporting polypeptide (OATP) 1B1 and OATP1B3 in the liver are important transporters for the hepatic uptake of various organic anions and that pharmaceutical companies must investigate whether new chemical entities are substrates or inhibitors of OATP1B1 and OATP1B3. In previous clinical reports, the plasma concentrations of several OATP substrates were increased significantly by coadministration of OATP-inhibitor drugs such as cyclosporine A and rifampicin (Fig. 6.1). By contrast, several biliary efflux transporters such as P-glycoprotein (P-gp), multidrug resistance-associated protein 2 (MRP2), and breast cancer resistance protein (BCRP) can recognize a broad range of compounds as substrates. The inhibition of these efflux transporters may lead to an increase in the hepatic concentration of substrate drugs, but not their plasma concentration, suggesting that such DDIs may be difficult to be detected.

This chapter briefly reviews the theoretical background and experimental methods for DDI prediction and recent progress in DDI prediction strategies.

6.2 Basic Theory of the Quantitative Prediction of Transporter-Mediated DDIs

When the kinetic property of transporter function follows traditional Michaelis–Menten kinetics, the intrinsic transport clearance of substrates via target transporters (CL_{int}) can be described as follows:

$$CL_{int} = \frac{V_{max}}{K_m + C_u} \tag{6.1}$$

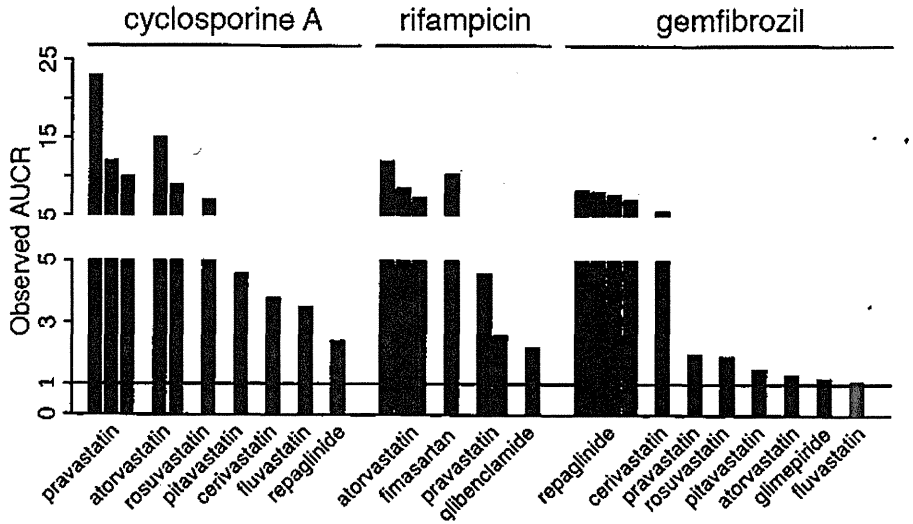

Fig. 6.1 Effects of coadministration of cyclosporine A, rifampicin, and gemfibrozil on the plasma AUCs of OATP substrate drugs (statins, sartans, and antidiabetic drugs) (cited from Yoshida et al. 2013). *Y-axis indicates the ratio of plasma AUC of substrates in the presence of inhibitors to that in their absence*

V_{max}, K_m, and C_u represent the maximum transport velocity, Michaelis–Menten constant, and concentration of protein-unbound compounds, respectively, which are thought to be recognized by transporters as substrates. If the unbound concentration of a substrate is far below the K_m value, (6.1) can be converted into (6.2).

$$CL_{int} = \frac{V_{max}}{K_m} \tag{6.2}$$

Assuming that an inhibitor drug inhibits the transporter in a competitive or non-competitive manner, the intrinsic transport clearance of a substrate in the presence of an inhibitor drug can be described by the following equation:

$$CL_{int}(+\text{inhibitor}) = \frac{V_{max}}{K_m \cdot \left(1 + \frac{I_u}{K_i}\right)} \tag{6.3}$$

$$\frac{CL_{int}(+\text{inhibitor})}{CL_{int}(-\text{inhibitor})} = \frac{1}{1 + \frac{I_u}{K_i}} \tag{6.4}$$

Thus, according to (6.4), the decrease in the transport function of a target transporter can be estimated quantitatively by two parameters, I_u and K_i, which are defined as the protein-unbound concentration of an inhibitor at the vicinity of the target transporter and the inhibition constant, respectively. Because the $1+I_u/K_i$ value is key to predicting the change in intrinsic clearance, we sometimes call it the "*R* value."

To consider the impact of decreased function of a single target transporter by DDIs on the in vivo pharmacokinetics of substrate drugs, one must consider the following points based on the pharmacokinetic theory.

6.2.1 *Relative Contribution of a Target Transporter to the Overall Membrane Transport*

Several transporters are expressed on the same side (basal or apical) of the plasma membrane of polarized cells, and their substrate specificities often overlap each other (e.g., OATP1B1 and OATP1B3 in the liver). Thus, multiple transporters can often mediate the membrane transport of a single compound in the same direction (uptake or efflux). If a compound is lipophilic enough to pass partially through the plasma membrane by passive diffusion, intrinsic membrane transport clearance ($PS_{transport}$) is defined as the sum of the intrinsic clearance of passive permeation through the plasma membrane ($CL_{passive}$) and active transport mediated by transporter *i* ($CL_{TP,i}$) as follows:

$$PS_{transport} = CL_{passive} + CL_{TP,1} + CL_{TP,2} + \cdots + CL_{TP,i} \tag{6.5}$$

If the function of transporter 1 is inhibited only by inhibitor drugs, the fold-change in the $PS_{transport}$ depends largely on the fraction of intrinsic transport clearance mediated by transporter 1 in the $PS_{transport}$ ($f_{m,1}$).

$$\frac{PS_{transport}(+\text{inhibitor})}{PS_{transport}(-\text{inhibitor})} = \frac{\dfrac{CL_{TP,1}}{1+\dfrac{I_u}{K_i}} + (CL_{passive} + CL_{TP,2} + \cdots + CL_{TP,i})}{CL_{TP,1} + (CL_{passive} + CL_{TP,2} + \cdots + CL_{TP,i})} = \frac{f_{m,1}}{1+\dfrac{I_u}{K_i}} + (1 - f_{m,1}) \tag{6.6}$$

When a target transporter is inhibited completely by an inhibitor drug, $PS_{transport}$ decreases to $(1-f_{m,1})$ at a maximum, and thus estimation of the relative contribution of each transporter to the overall membrane transport of a substrate drug in the normal condition requires knowing the lower limit of the decreased intrinsic clearance in the presence of potent inhibitors of the target transporter. Moreover, inhibitor drugs sometimes simultaneously inhibit multiple transporters with different inhibition potencies. In this case, (6.6) is modified as follows:

$$\frac{PS_{transport}(+\text{inhibitor})}{PS_{transport}(-\text{inhibitor})} = \sum_{p=1}^{k} \frac{f_{m,p}}{1+\dfrac{I_u}{K_{i,p}}} + \left(1 - \sum_{p=1}^{k} f_{m,p}\right) \tag{6.7}$$

Thus, knowing the f_m and K_i values of inhibitor drugs for each target transporter is needed for the precise prediction of the change in $PS_{transport}$.

6.2.2 *Rate-Limiting Step of the Overall Intrinsic Organ Clearance*

In the "traditional" assumption, if a drug is metabolized extensively, its intrinsic organ clearance is thought to be determined by metabolic clearance. Several reports have indicated that the hepatic intrinsic clearance of a drug can be predicted by a simple scale-up of in vitro intrinsic metabolic clearance with human liver microsomes. However, there are several new drugs that are substrates of both metabolic enzymes and transporters. For example, atorvastatin is eliminated in the liver by extensive metabolism by cytochrome P450 (CYP) 3A4, whereas fluvastatin, torsemide, glibenclamide, and nateglinide are metabolized mainly by CYP2C9. On the other hand, these drugs are also substrates of hepatic uptake transporters, the OATPs. In this case, the detoxification efficacy of these drugs in the liver is determined by the functions of the uptake and efflux transporters as well as the metabolic enzymes, and the traditional assumption can no longer be applied for the prediction of the intrinsic clearance of transporter substrates (Shitara et al. 2006; 2013; Yoshida et al. 2013). In the "extended" pharmacokinetic theory, hepatic intrinsic clearance of transporter substrates ($CL_{int,all}$) should be determined by the metabolic intrinsic clearance (CL_{met}), intrinsic clearance of hepatic uptake (PS_{inf}), sinusoidal efflux (PS_{eff}), and biliary excretion in an unchanged form (PS_{ex}), as in the following equation (Fig. 6.2):

$$CL_{int,all} = PS_{inf} \frac{PS_{ex} + CL_{met}}{PS_{eff} + PS_{ex} + CL_{met}} \tag{6.8}$$

According to (6.8), if the PS_{eff} is much smaller than the sum of PS_{ex} and CL_{met}, $CL_{int,all}$ can approximate the PS_{inf} value.

$$CL_{int,all} \sim PS_{inf} \tag{6.9}$$

On the other hand, if the PS_{eff} is much larger than the sum of PS_{ex} and CL_{met}, $CL_{int,all}$ can be described by (6.10).

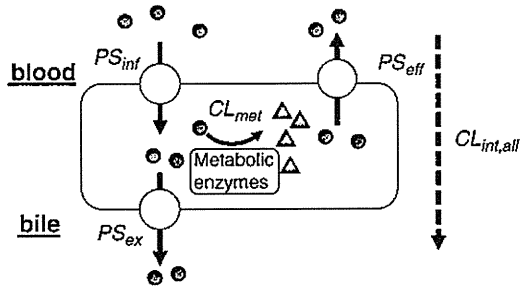

Fig. 6.2 The intrinsic processes making up overall hepatic intrinsic clearance ($CL_{int,all}$). PS_{inf} intrinsic clearance for hepatic influx transport, PS_{eff} intrinsic clearance for sinusoidal efflux transport, PS_{ex} intrinsic clearance for biliary efflux transport in an unchanged form, CL_{met} intrinsic clearance for metabolism

$$CL_{int,all} \sim PS_{inf} \cdot \frac{PS_{ex} + CL_{met}}{PS_{eff}} \tag{6.10}$$

If a drug can pass rapidly through the plasma membrane mainly by passive diffusion and PS_{ex} is negligible, PS_{eff} is very large compared with PS_{ex} and CL_{met} and is equal to PS_{inf}, and (6.6) can be converted as follows:

$$CL_{int,all} \sim CL_{met} \tag{6.11}$$

Thus, under such conditions, the aforementioned "traditional" assumption, in which metabolic intrinsic clearance solely dominates the overall intrinsic hepatic clearance, can be applied for the prediction of intrinsic clearance of drugs from in vitro metabolism assay using liver microsome.

Watanabe et al. showed that the in vivo intrinsic hepatic clearance of four kinds of statins (pravastatin, pitavastatin, atorvastatin, and fluvastatin), two of which are eliminated from the body by extensive CYP-mediated metabolism, is similar to the uptake intrinsic clearance estimated using the multiple-indicator dilution method in rats and an in vitro uptake assay using cryopreserved human hepatocytes (Watanabe et al. 2010). By contrast, the metabolic intrinsic clearance obtained from an in vitro metabolism assay using rat or human liver microsomes considerably underestimated the in vivo intrinsic hepatic clearance. A clinical microdosing study also indicated that the rate-limiting step of the hepatic clearance of atorvastatin is the hepatic uptake process mediated by OATP transporters in vivo in humans (Maeda et al. 2011). This was based on the observations that the plasma area under the concentration-time curve (AUC) of atorvastatin was increased markedly by orally administered rifampicin, a typical OATP-selective inhibitor, but not by intravenously administered itraconazole, a typical CYP3A4-selective inhibitor, although the AUC of the major hydroxy metabolites of atorvastatin decreased significantly.

We sometimes define "β value" as an indicator representing whether the rate-limiting step of hepatic intrinsic clearance is likely to be an uptake process according to the following equations:

$$\beta = \frac{PS_{ex} + CL_{met}}{PS_{eff} + PS_{ex} + CL_{met}} \tag{6.12}$$

$$CL_{int,all} = PS_{inf} \cdot \beta \tag{6.13}$$

If the β value is close to 1, $CL_{int,all}$ can approximate PS_{inf} as in (6.9), whereas if the β value is far less than 1, $CL_{int,all}$ can be described as in (6.10).

Let us consider the impact of a DDI at each transport process on the overall intrinsic hepatic clearance based on the "extended" pharmacokinetic concept. If uptake transporters are inhibited by a coadministered drug, the reduction in the uptake intrinsic clearance (PS_{inf}) always directly affects the decrease in the overall intrinsic hepatic clearance ($CL_{int,all}$) regardless of the β value. On the other hand, if biliary excretion transporters or metabolic enzymes and uptake transporters are inhibited simultaneously by coadministered drugs, when the β value is close to 1, even in the presence of an inhibitor drug, $CL_{int,all}$ should not be changed according to (6.14).

$$\frac{CL_{int,all}(+\text{inhibitor})}{CL_{int,all}(-\text{inhibitor})} \sim \frac{PS_{inf} \Big/ \left(1 + \dfrac{I_u}{K_{i,inf}}\right)}{PS_{inf}} = \frac{1}{R_{inf}} \tag{6.14}$$

However, when the β value is much smaller than 1, the ratio of $CL_{int,all}$ in the presence of an inhibitor relative to that in its absence is described by (6.15) or (6.16) if the compound is eliminated from the body by extensive metabolism or by biliary excretion in an unchanged form, respectively.

$$\frac{CL_{int,all}(+\text{inhibitor})}{CL_{int,all}(-\text{inhibitor})} \sim \frac{PS_{inf} \Big/ \left(1 + \dfrac{I_u}{K_{i,inf}}\right) \dfrac{CL_{met} \Big/ \left(1 + \dfrac{I_u}{K_{i,met}}\right)}{PS_{eff}}}{PS_{inf} \dfrac{CL_{met}}{PS_{eff}}} = \frac{1}{R_{inf}} \frac{1}{R_{met}} \tag{6.15}$$

$$\frac{CL_{int,all}(+\text{inhibitor})}{CL_{int,all}(-\text{inhibitor})} \sim \frac{PS_{inf} \Big/ \left(1 + \dfrac{I_u}{K_{i,inf}}\right) \dfrac{PS_{ex} \Big/ \left(1 + \dfrac{I_u}{K_{i,ex}}\right)}{PS_{eff}}}{PS_{inf} \dfrac{PS_{ex}}{PS_{eff}}} = \frac{1}{R_{inf}} \frac{1}{R_{ex}} \tag{6.16}$$

Thus, the reduction in the overall intrinsic hepatic clearance is estimated by the product of the decreased fraction of uptake clearance and that of metabolic clearance or biliary excretion clearance.

6.2.3 *Impact of the Change in the Intrinsic Clearance on Organ Clearance and In Vivo Pharmacokinetics of Substrate Drugs*

Based on the pharmacokinetic theory, after oral administration of a drug, the blood AUC (AUC_B) is calculated by the following equation:

$$AUC_B = \frac{F_a F_g \cdot F_h \cdot \text{Dose}}{CL_{tot}} \tag{6.17}$$

F_aF_g, F_h, and CL_{tot} represent the fraction of a drug reaching the portal vein from the intestinal lumen while avoiding intestinal metabolism for an orally administered dose (intestinal availability), the fraction of a drug reaching the systemic circulation while avoiding first-pass hepatic metabolism (hepatic availability), and total clearance, respectively. Total clearance is described simply as the sum of organ clearance (in the liver, kidney, etc.). Organ clearance, defined as the rate of elimination of a drug divided by its blood concentration, is dominated by the tissue intrinsic clearance, blood flow rate in tissues, and protein-unbound fraction of a drug in the blood. Several models have been created to relate the intrinsic clearance to organ clearance. Among them, the "well-stirred" model is used most frequently because of its simple mathematical handling. In this model, rapid and complete mixing (hence its name) of a drug coming from the blood circulation and blood in the tissue occurs, and the blood concentration of a drug at the exit of tissue is assumed to be equal to that in the tissue. Under such an assumption, hepatic clearance (CL_h) can be expressed as in (6.18).

$$CL_h = \frac{Q_h \cdot f_B \cdot CL_{int,h}}{Q_h + f_B \cdot CL_{int,h}} \tag{6.18}$$

Q_h, f_B, and $CL_{int,h}$ represent the hepatic blood flow rate, protein-unbound fraction of a drug in the blood, and the hepatic intrinsic clearance of a drug, respectively.

When Q_h is much smaller than $f_B CL_{int,h}$, (6.18) is approximated by (6.19), and hepatic clearance is determined solely by hepatic blood flow rate.

$$CL_h \sim Q_h \tag{6.19}$$

In this case, when the intrinsic hepatic clearance is decreased by DDIs, hepatic clearance is not changed if $Q_h \ll f_B CL_{int,h}$ is still maintained in the presence of inhibitor drugs. On the other hand, when Q_h is much larger than $f_B CL_{int,h}$, (6.18) is approximated by (6.20), and hepatic clearance is affected by the change in intrinsic hepatic clearance.

$$CL_h \sim f_B \cdot CL_{int,h} \tag{6.20}$$

When a drug is administered orally and eliminated from the liver alone, the blood AUC can be converted into (6.21) based on (6.17) and (6.18).

$$AUC_B = \frac{F_a F_g \cdot F_h \cdot \text{Dose}}{CL_{tot}} = \frac{F_a F_g \cdot \dfrac{Q_h}{Q_h + f_B\, CL_{int,h}} \cdot \text{Dose}}{\dfrac{Q_h \cdot f_B \cdot CL_{int,h}}{Q_h + f_B \cdot CL_{int,h}}} = \frac{F_a F_g \cdot \text{Dose}}{f_B \cdot CL_{int,h}} \tag{6.21}$$

Thus, regardless of the rate-limiting step of hepatic clearance (Q_h or $f_B CL_{int,h}$), the AUC ratio (+inhibitor/−inhibitor) is inversely proportional to the ratio of hepatic intrinsic clearance (6.22).

$$\frac{AUC_B(\text{+inhibitor})}{AUC_B(\text{−inhibitor})} = \frac{CL_{int}(\text{−inhibitor})}{CL_{int}(\text{+inhibitor})} \tag{6.22}$$

6.3 In Vitro Experimental Methods to Estimate the Kinetic Parameters Used for the Prediction of Transporter-Mediated DDIs

To predict precisely the extent of transporter-mediated DDIs, several key parameters such as the K_i value of an inhibitor for the target transporter and the relative contribution of each transporter to the overall membrane permeation of a substrate (f_m value) should be estimated. A wide variety of in vitro experimental tools are now available to estimate the kinetic parameters describing the transport properties of drugs. This section briefly reviews the current in vitro experimental systems and methods.

6.3.1 *Determination of K_i Values of Inhibitors for Uptake and Efflux Transporters*

As described above, the K_i value is one of the most critical parameters to quantitatively estimate the alteration of intrinsic clearance by transporter-mediated DDIs. In general, the K_i value can be obtained by observing the uptake clearance of substrates mediated by a single isoform of transporters in the presence of various

concentrations of inhibitors and fitting the theoretical curve calculated from (6.4) to the observed data. The IC_{50} value, which is defined as the inhibitor concentration that decreases the function of a transporter by half, is sometimes used in the literature instead of the K_i value. The relationship between the IC_{50} and K_i is expressed in (6.23), assuming competitive inhibition.

$$K_i = \frac{IC_{50}}{1 + \frac{S}{K_m}} \tag{6.23}$$

S and K_m represent the substrate concentration used in the inhibition assay and the Michaelis–Menten constant of a substrate, respectively. Because the IC_{50} value becomes higher as the substrate concentration increases, the risk of a clinical DDI is possibly underestimated by the calculation of the R value using IC_{50} instead of K_i when the IC_{50} value is determined with a higher concentration of substrates compared with the clinical unbound concentration of a substrate drug at the target site. From (6.23), if the substrate concentration is much lower than the K_m value, the IC_{50} value is regarded as equal to the K_i value.

Several experimental systems, such as immortalized cell lines that stably express the transporter and transporter cRNA-injected *Xenopus* oocytes, can be used to characterize an uptake transporter. Human cryopreserved hepatocytes can be purchased from various commercial sources, and suspended hepatocytes are also used in the characterization of hepatic uptake of compounds, but the apparent K_i value can be obtained only from an in vitro inhibition assay with hepatocytes because inhibitor drugs sometimes inhibit multiple transporters that can also recognize typical substrates with different K_i values. The function of efflux transporters is usually evaluated by measuring the ATP-dependent uptake of compounds into inside-out membrane vesicles that overexpress efflux transporters or canalicular membrane vesicles (CMVs) obtained from liver samples, or the directional transcellular transport of substrates in single- or double-transfected polarized cell lines, or in sandwich-cultured hepatocytes. When using cell lines, the K_i value for an efflux transporter should be measured with regard to the intracellular unbound concentration of an inhibitor. In practical applications, the apparent K_i value is often estimated based on the medium concentration in the compartment to which an inhibitor is added initially. However, if the intracellular protein-unbound concentration of an inhibitor is not the same as its (protein-unbound) medium concentration because of its active transport, the apparent K_i value with regard to the medium concentration is not identical to the real K_i value for efflux transporters, and the ratio of these K_i values should correspond to the ratio of the unbound concentration of an inhibitor inside and outside the cells ($K_{p,uu}$ value) (Shitara et al. 2013).

In the routine high-throughput assay in the process of drug development, the K_i values of inhibitors are sometimes estimated using the same typical substrate for the target transporter, and the K_i values are used to predict the risk of DDIs with other substrate drugs. However, previous reports indicated that some transporters have more than two substrate-binding sites, and thus inhibition potency of an inhibitor sometimes largely depends on the substrates used. For example, Noe et al. demonstrated that 200 μM gemfibrozil can potently inhibit the OATP1B1-mediated uptake of taurocholate and statins, but not that of estrone-3-sulfate and troglitazone sulfate (Noe et al. 2007). Soars et al. compared the IC_{50} values of eight drugs for OATP1B1-mediated uptake of three typical substrates, pitavastatin, estradiol-17β-glucuronide ($E_2$17βG), and estrone-3-sulfate (Soars et al. 2012). The overall trend in the rank order of IC_{50} values was $E_2$17βG ≤ pitavastatin < estrone-3-sulfate. Thus, it is recommended to use the real combination of substrate and inhibitor to estimate the K_i value when predicting a specific case of DDI, although for the first high-throughput screening, $E_2$17βG might be useful as a sensitive substrate for OATP1B1 inhibition. Moreover, some drugs have been reported to increase the transporter-mediated transport, possibly because of their binding to the allosteric site of the transporters. Several compounds have been shown to simulate the uptake into MRP2-expressing Sf9 membrane vesicles and the transcellular transport of an MDCKII monolayer expressing MRP2 (Zelcer et al. 2003). In particular, 1 mM sulfanitran increased the MRP2-mediated transport of $E_2$17βG almost 30 times. For OATP1B1 and OATP1B3, several nonsteroidal anti-inflammatory drugs, such as diclofenac and ibuprofen, significantly increased the uptake of pravastatin, but not that of bromosulfophthalein (BSP) (Kindla et al. 2011). At present, the significance of these phenomena in in vivo DDIs has not been characterized.

Interestingly, the time-dependent inhibition of OATP transporters has been observed in in vitro experiments. Shitara et al. showed that in vivo hepatic uptake of BSP determined by the liver uptake index method was significantly decreased 3 days after administration of cyclosporine A in rats and that the uptake of BSP in rat hepatocytes was also decreased after preincubation with cyclosporine A, despite its removal from the medium during the BSP uptake assay (Fig. 6.3a) (Shitara et al. 2009). Amundsen et al. also confirmed this preincubation effect in OATP1B1-expressing HEK293 cells and have shown that the apparent K_i value of cyclosporine A for the uptake of atorvastatin after a 1 h preincubation with cyclosporine A was 1/22 of that after its coincubation (Fig. 6.3b) (Amundsen et al. 2010). We note that such a phenomenon can also be applied to all the OATP-inhibitor drugs because the K_i value obtained from a conventional inhibition assay may be overestimated, which leads to the underestimation of the risk of DDIs.

6.3.2 *Determination of the Relative Contribution of Each Transporter to the Overall Membrane Permeation of a Substrate (f_m Value)*

As mentioned above, the f_m value is important for determining the lower limit of the decreased intrinsic clearance of membrane permeation when a target transporter is potently inhibited by inhibitors. As for CYP-mediated metabolism, the methods to

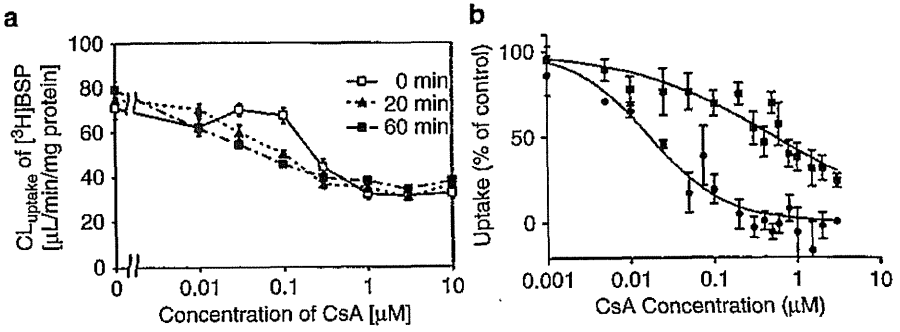

Fig. 6.3 Effect of preincubation of cyclosporine A on its K_i value for the transport of OATP substrates. (**a**) The inhibitory effect of cyclosporine A on the uptake of BSP in rat hepatocytes after preincubation with cyclosporine A (cited from Shitara et al. 2009). Hepatocytes were exposed to different concentrations of cyclosporine A for 0 (*open squares*), 20 (*closed triangles*), or 60 min (*closed squares*), subsequently followed by inhibition studies with the same concentrations of cyclosporine A. (**b**) Inhibition of OATP1B1-mediated uptake of atorvastatin acid (0.5 μM) into OATP1B1-expressing HEK293 cells by preincubation (*closed circles*) or coincubation (*closed squares*) of cyclosporine A (cited from Amundsen et al. 2010)

determine the contribution of each CYP isoform to the overall hepatic metabolism of substrates have been established by the use of isoform-specific metabolism of substrates and a specific inhibitor for each CYP isoform used with human liver microsomes or human hepatocytes. Similar methods can also be applied to transporter-mediated membrane permeation. The first approach is to use the relative activity factor (RAF) method, which was established originally in the field of metabolic enzymes by Crespi and Penman (1997). In this method, the uptake clearances of specific substrates for each transporter are measured in transporter expression systems and hepatocytes, and their ratio (hepatocytes/expression systems) for transporter i is defined as "$R_{act,i}$." Once the uptake clearance of a test compound in cells expressing transporter i ($CL_{test,i}$) is determined, the uptake clearance of a test compound mediated by transporter i in hepatocytes can be estimated by the product of the $R_{act,i}$ and $CL_{test,i}$ values. Assuming that the hepatic uptake clearance of a test compound ($CL_{hep,test}$) can be explained by the functions of transporter $1-i$, the following equation should be true:

$$CL_{hep,test} = \sum_i R_{act,i} \times CL_{test,i} \qquad (6.24)$$

Kouzuki et al. originally proposed a method using reference compounds of rat Oatp1a1 ($E_2$17βG) and sodium taurocholate cotransporting polypeptide (Ntcp) (taurocholate) to determine their contributions to the hepatic uptake of bile acids and organic anions in rats, although these compounds are no longer used for selective substrates of these transporters because many other transporters have been identified since the original publication (Kouzuki et al. 1998; Kouzuki et al. 1999). Hirano et al. applied this concept to human hepatocytes to estimate the relative contribution of OATP1B1 and OATP1B3 to the hepatic uptake of $E_2$17βG and pitavastatin in cryopreserved human hepatocytes by the use of estrone-3-sulfate as an OATP1B1-selective substrate and cholecystokinin octapeptide as an OATP1B3-selective substrate (Hirano et al. 2004). They showed that the hepatic uptake of both compounds is mediated mainly by OATP1B1. Their observed uptake clearances in human hepatocytes were similar to the sum of their estimated clearances mediated by OATP1B1 and OATP1B3. They also confirmed their results using two different approaches. One was the direct estimation of the ratio of the expression levels of OATP1B1, 1B3, and 2B1 in human hepatocytes to that in the expression systems by comparing the band density in Western blot analysis and then estimating their contributions using the ratio instead of the $R_{act,i}$ value (Hirano et al. 2006). The absolute protein amounts of transporters in human liver samples can now be estimated directly by the quantification of peptide fragments digested with trypsin. This method provides a more accurate estimation of the relative expression levels compared with that from the band density in Western blot analysis (Li et al. 2009; Ohtsuki et al. 2011).

The other approach is to estimate the inhibitable portion of the uptake of test compounds in human hepatocytes in the presence of a specific inhibitor for each transporter (Ishiguro et al. 2006). Estrone-3-sulfate can be used as an inhibitor of OATP1B1, but not OATP1B3. A previous report indicated that the uptake of pitavastatin and $E_2$17βG was potently inhibited by estrone-3-sulfate in human hepatocytes, whereas the uptake of telmisartan was not inhibited, which suggests that telmisartan is a selective substrate for OATP1B3 in human liver (Ishiguro et al. 2006). Some specific inhibitors of the efflux transporters have also been proposed. For example, Ko143 preferentially inhibits BCRP-mediated transport, whereas PSC833 and LY335979 inhibit P-gp-mediated transport more potently than they inhibit transport via other efflux transporters (Allen et al. 2002; Dantzig et al. 1996; Kusunoki et al. 1998). However, when applied to cell systems, most of these selective inhibitors also inhibit the cellular uptake process, and the efflux clearance must be investigated separately to evaluate the inhibitory effects of inhibitors on efflux transporters accurately (Oostendorp et al. 2009).

Gene-silencing techniques such as antisense, ribozyme, and RNA interference are also powerful tools to determine the transport activity of a specific protein. Hagenbuch et al. investigated the effect of coinjection of transporter (Ntcp or Oatp1a1)-specific antisense oligonucleotide on the uptake of BSP and taurocholate in *Xenopus* oocytes injected with total rat liver mRNA (Hagenbuch et al. 1996). The expression level of a target transporter was reduced specifically, and the authors concluded that Na^+-dependent and Na^+-independent uptakes of taurocholate were almost fully accounted for by Ntcp and Oatp1a1, respectively, whereas only half of the BSP uptake could be explained by Oatp1a1. Nakai et al. took the different approach to estimate the contribution of OATP1B1 to the hepatic uptake of pravastatin and $E_2$17βG in humans (Nakai et al. 2001). Oocytes microinjected with human liver poly (A) mRNA showed Na^+-independent uptake of pravastatin and $E_2$17βG, and the simultaneous injection of OATP1B1 antisense oligonucleotides completely abolished this uptake, suggesting that OATP1B1 is a major transporter for their uptake. However, one should also consider their underlying assumption that the