28. ビグアナイド系化合物により惹起される乳酸アシドーシスへの有機カチオントランスポーター（Oct1）の関与

東京大学大学院薬学系研究科

楠原洋之　王　徳勝

加藤将夫　杉山雄一

The Netherlands Cancer Institute

Alfred H. Schinkel

Role of Hepatic Organic Cation Transporter 1 (Oct1) in the Lactic Acidosis Caused by Biguanide Compounds

Hiroyuki Kusuhara[1], De-Sheng Wang[1], Yukio Kato[1], Alfred H. Schinkel[2] and Yuichi Sugiyama[1]

[1]Graduate School of Pharmaceutical Sciences, the University of Tokyo
[2]The Netherlands Cancer Institute

はじめに

糖尿病は生活習慣病ともいわれ，インスリンの感受性に応じてtype 1，type 2の二つに分類される。そのうちtype 2の患者数は最も多く，発病率の9割以上を占め，アメリカ国立衛生研究所（NIH）はtype 2の患者数は2025年までに全世界で約1億3千万から3億人に達すると予想している。type 2糖尿病患者のうち一部の人は食事制限および運動により症状を緩和できるが，多くの患者が経口糖尿病治療薬に頼らざるをえない状況にある。経口糖尿病治療薬のうち，スルフォニルウレア系化合物とビグアナイド系化合物は歴史が長く，本研究で取り上げたビグアナイド系化合物としてはメトフォルミンとブフォルミンが現在臨床の場で用いられている。ビグアナイド系化合物は歴史が長いものの，フェンフォルミンが臨床上重篤な副作用である乳酸アシドーシスを引き起こすため，1970年代から使用は中止されている。ビグアナイド系化合物の作用機序が明らかにされたこと，ならびにインスリンの分泌を誘導するスルフォニルウレアとはまったく違う薬理作用が評価され，1995年FDAによるメトフォルミンが承認されて以来，再評価が進められている。

ビグアナイド系化合物で最も危惧されている副作用である乳酸アシドーシスは，主に腎機能低下の患者においてよく発症することが近年の研究によって明らかにされている[1]。これはビグアナイド系化合物が，主として腎臓から消失することと一致す

る[2,3]。腎機能の低下により，ビグアナイド系化合物の血中滞留性が増加し，副作用を惹起する血中濃度にまで達したものと考えられる。われわれは，ビグアナイド系化合物が有機カチオントランスポーター（Oct1）の基質となることを見出した[4]。Oct1は12回の膜貫通領域からなるトランスポーターで，tetraehtylammoniumのように水溶性の有機カチオンを基質とする[5~8]。Oct1はラットでは肝臓・腎臓に，ヒトでは肝臓のみに発現している[5~8]。われわれはオランダのSchinkelらのグループが作製したOct1ノックアウトマウス[9]を用いてメトフォルミンの体内動態を検討し，Oct1ノックアウトマウスではメトフォルミンの肝取り込みがほとんど細胞外容積で説明できる程度にまで低下していることを報告している[4]。本研究では，このノックアウトマウスを用いて，ビグアナイド系化合物の乳酸アシドーシスにおける肝臓の重要性を検討した。

I 実験方法

Sprague-Dawleyラット（雄性，8週齢，Charles River Japanより購入）に，メトフォルミン（25, 50, 100, 175, 250 mg/hr/kg），ブフォルミン（2.5, 5, 12.5, 25, 50 mg/hr/kg），フェンフォルミン（1, 2.5, 5, 12.5, 25 mg/hr/kg）を定速静注（8.0 mL/hr/kg）した。ビグアニド系化合物の血漿中濃度は，HPLCを用いて決定した。HPLCの条件は，下記のとおりである。

HPLC systemはL-7100 pumpとL-7400 UV monitor（Hitachi, Tokyo, Japan）を，カラムは300×3.9 mm I. D. C_{18}μBondapak（粒径10μm, Waters, Milford, MA）を用いた。移動相の組成は0.01 M phosphate buffer（pH6.5）：acetonitrile＝30：70で，流速1 mL/minで測定した。ビグアニド系化合物の検出には，236 nmでの吸収を用いた。

Oct1（−/−）ならびにOct1（＋/＋）FVB mice（雄性，12〜16週齢）を実験に用いた。メトフォルミンを，大腿静脈より定速静注（150 mg/hr/kg, 8.0 mL/hr/kg，生理食塩水）により，マウスに投与した。投与後，90, 150, 210分の血漿中のメトフォルミンならびに血中乳酸濃度を決定した。投与210分後にマウスを屠殺し，肝臓中・筋肉中のメトフォルミン濃度を決定した。また，血中乳酸濃度の定量は，Sigma Diagnosticsのキットを用いて，付属のプロトコールに従って定量した。血漿中，肝臓・筋肉中のメトフォルミン濃度は，HPLC法を用いて測定した。

II 結果と考察

ラットにビグアニド系化合物を投与したところ，いずれの化合物においても投与量に応じて，経時的に血漿乳酸値が増加した。フェンフォルミンは最も低濃度で，血中乳酸値の増加をもたらした。投与開始後4時間までの血中乳酸値の増加のAUCを，定常状態のビグアニド系化合物の血漿中非結合型薬物濃度を基にEC_{50}値を算出した。その値は，フェンフォルミン，ブフォルミン，メトフォルミンで，それぞれ4.97±0.87, 119±18, 734±168 μMであった。フェンフォルミンとメトフォルミンでは，その値に約150倍の差がみられた。

マウスにメトフォルミンを定速静注により投与したところ，野生型では投与後150分以後，急激に血中乳酸値は増加し，210分後での血中乳酸値は2.56±0.22 mg/mLであったのに対して，Oct1（−/−）マウスでは1.02±0.11 mg/mLであった。また，投与開始210分後のメトフォルミンの血漿中濃度，肝臓中濃度，筋肉中濃度を比較すると，野生型とOct1（−/−）マウスでそれぞれ，102±4, 96.8±17.8 μg/mL, 417±178, 49.3±10.4 μg/g liver, 153±18, 107±17 μg/g muscleであり，血漿中濃度，筋肉中濃度では野生型，Oct1（−/−）マウスで差がみられないものの，肝臓中濃度は大きく低下していた。この結果に基づくと，ビグアニド系化合物による乳酸アシドーシスは，メトフォルミンの肝臓内濃度が重要であることを示唆している。肝取り込み過程は，肝臓内濃度を決定する重要な要因の一つである。ビグアニド系化合物の肝取り込み過程は，Oct1により決定されることから，Oct1による輸送活性が，乳酸アシドーシスを生じるファクターの一つとなる。Oct1をCHO細胞に発現させた系を用いて輸送活性を測定すると，フェンフォルミン，ブフォルミン，メトフォルミンの輸送の固有能力（V_{max}/K_m）は，9.6, 5.8, 3.7 μL/min/mg proteinであり[4]，*in vivo*での乳酸アシドーシスのincidenceの順と一致している。

おわりに

ビグアニド系化合物は，ミトコンドリアでの complex I を阻害することが報告されている[10,11]。この呼吸鎖の阻害結果，肝臓での糖新生を抑制することがビグアニド系化合物の薬効メカニズムの一つとして考えられている。一方，乳酸アシドーシスと呼吸鎖の抑制との関連は明確ではなく，今後の課題である。

本研究成果に基づくと，肝臓内濃度が乳酸アシドーシスの incidence を決定する要因の一つであることが明らかになった。乳酸アシドーシスを避けるという点では，Oct1 による輸送活性が低いビグアニド系化合物を開発することが望ましい。今後，さらに呼吸鎖の抑制と乳酸アシドーシスとの関連，併せて糖新生との関連を総合的に評価し，より安全なビグアニド系化合物開発のための評価系を確立していくことが重要であると考えている。

文　献

1) Davidson MB, Peters AL. An overview of metformin in the treatment of type 2 diabetes mellitus. Am J Med 1997；102：99-110.
2) Sirtori CR, et al. Disposition of metformin (*N*, *N*-dimethylbiguanide) in man. Clin Pharmacol Ther 1978；24：683-93.
3) Tucker GT, et al. Metformin kinetics in healthy subjects and in patients with diabetes mellitus. Br J Clin Pharmacol 1981；12：235-46.
4) Wang D-S, et al. Involvement of organic cation transporter 1 in the hepatic and intestinal distribution of metformin. J Pharmacol Exp Ther. In press.
5) Koepsell H. Organic cation transporters in intestine, kidney, liver, and brain. Annu Rev Physiol 1998；60：243-66.
6) Inui KI, et al. Cellular and molecular aspects of drug transport in the kidney. Kidney Int 2000；58：944-58.
7) Dresser MJ, et al. Transporters involved in the elimination of drugs in the kidney：organic anion transporters and organic cation transporters. J Pharm Sci 2001；90：397-421.
8) Russel FG, et al. Molecular aspects of renal anionic drug transport. Annu Rev Physiol 2002；64：563-94.
9) Jonker JW, et al. Reduced hepatic uptake and intestinal excretion of organic cations in mice with a targeted disruption of the organic cation transporter 1 (Oct1 [Slc22a1]) gene. Mol Cell Biol 2001；21：5471-7.
10) El-Mir MY, et al. Dimethylbiguanide inhibits cell respiration via an indirect effect targeted on the respiratory chain complex I. J Biol Chem 2000；275：223-8.
11) Owen MR, et al. Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain. Biochem J 2000；348：607-14.

*　　*　　*

Jpn Pharmacol Ther（薬理と治療）vol. 30 supplement 2002

29. ヒトOATP2とMRP2を同時発現させたダブルトランスフェクタントの評価

—肝臓におけるCerivastatinの経細胞輸送特性の定量的評価に向けて—

東京大学大学院薬学系研究科　製剤設計学教室

松島総一郎　前田和哉　佐々木誠

鈴木洋史　杉山雄一

昭和大学薬学部　臨床分子薬品学教室

設楽悦久

Estimation of a Double-transfectanted MDCK II Monolayer Co-expressing Human OATP2 and MRP2

—In Order to Quantitatively Estimate the Property of Transcellular Transport of Cerivastatin in Human Liver—

Soichiro Matsushima[1], Kazuya Maeda[1], Yoshihisa Shitara[2], Makoto Sasaki[1], Hiroshi Suzuki[1] and Yuichi Sugiyama[1]

[1]Department of Biopharmaceutics, Graduate School of Pharmaceutical Sciences, University of Tokyo
[2]Department of Clinical and Molecular Pharmacokinetics/Pharmacodynamics Faculty of Pharmaceutical Sciences, Showa University

はじめに

肝からの薬物の排泄は，血管側膜を介した取り込み，および胆管側膜を介した排泄の両過程に支配されており，ヒトにおいてもそれぞれの過程において種々のトランスポーターが関与していることが知られている[1,2]。特に有機アニオン系化合物の輸送に関して，局在や広範な基質選択性を考慮すると，取り込み過程にはorganic anion transporting polypeptide 2(Oatp2/Oatp-C/LST-1, *Gene symbol SLC21A6*)[3~5]，排泄過程にはmultidrug resistance associated protein 2 (Mrp2, *Gene symbol ABCC2*)[6]が重要な役割を果たしていることが示唆される。これらのトランスポーターの基質特異性は一部異なるものの大部分はオーバーラップしており，有機アニオン系化合物の血管側から胆管側への極性輸送に関与していることが示唆されている。

そこで，当教室の佐々木らは，この血管側から胆

管側への極性輸送を再現しうるモデルとして，極性を有するMDCKII（Madin-Darby canine kidney II）細胞のbasal側にOatp2，apical側にMrp2を同時に発現させたダブルトランスフェクタントを構築し，双方の代表的基質であるestradiol-17β-glucuronideやHMG-CoA還元酵素阻害剤であるpravastatinなどがbasalからapicalへの方向性のある経細胞輸送が観察されることを報告した[7,8]。

一方，pravastatinは，非常に効率の良い腸肝循環をうけることで高い肝臓への滞留性を保持しているが，有機アニオンである本薬物の小腸および肝臓の膜透過過程において，近年トランスポーターの関与が示唆されてきている[4,7,9]。他のstatinsもコレステロールの主要合成組織である肝臓に選択的に作用することが望まれるが，cerivastatinはpravastatinに比べ脂溶性が高いが，肝臓への移行性は非常に高い[10]。このことからcerivastatinはpravastatinと同様，腸肝循環をうける薬物であると考えられ，ダブルトランスフェクタントを用いて経細胞輸送を測定することはcerivastatinの輸送を担う肝臓でのトランスポーターの関与を理解するうえで重要である。

そこで，本研究では，肝細胞における輸送にかかわるトランスポーターが未同定であるcerivastatinの経細胞輸送をダブルトランスフェクタントを用いて測定するとともに，cerivastatinの経細胞輸送の飽和性が取り込み，または排泄のトランスポーターの飽和性の影響をどのように受けるのかについて検討した。

I 方　　法

transwell上に単層培養したダブルトランスフェクタントを介した薬物のbasal側からapical側およびapical側からbasal側へのフラックス，さらにbasal側からcell内への取り込みについて以下のように検討した。細胞をKrebs-Henseleit buffer中で37℃，20分間preincubationした。[^{14}C]-cerivastatin（または，非標識体cerivastatinを加えたもの）をapicalまたはbasal側のmedium中に加えることにより開始し，一定時間後に，化合物を加えた側の反対側のmediumを回収し放射活性を測定した。さらに実験終了後，氷冷したbufferでwashすることでトランスポーターの機能を低下させ，細胞を0.2 M NaOHで可溶化し，細胞内に取り込まれたcerivastatinの放射活性を測定した。control細胞としてinsertを含まない発現vectorのみを導入した細胞，またOatp2単独発現細胞，Mrp2単独発現細胞を用いた。速度論的パラメータ（K_m，V_{max}）の算出においては，非線形最小二乗法プログラムMULTIを用いて解析した[11]。

II 結果および考察

1 cerivastatinの経細胞輸送の経時変化

現時点で輸送にかかわるトランスポーターが未同定であるcerivastatin（0.5μM）の経細胞輸送をcontrol細胞，Oatp2単独発現系，Mrp2単独発現系，およびOatp2/Mrp2共発現系を用いて測定した。control細胞，Oatp2単独発現系，Mrp2単独発現系では，basal側からapical側へのフラックスはapical側からbasal側へのフラックスとほぼ同程度であったが，Oatp2/Mrp2共発現系においてはbasal側からapical側へのフラックスはapical側からbasal側へのフラックスに比べ約3倍であり，明確な方向性ある輸送が観察された。

このことから，cerivastatinはOatp2，Mrp2の両トランスポーターの基質になることが示唆され，両トランスポーターによる輸送が肝クリアランスの少なくとも一部を決めていることが示唆された。肝臓における取り込み，排泄側にはOatp2，Mrp2以外にも種々のトランスポーターが発現しており，一般的にトランスポーターの基質認識性が比較的広範であることを考慮すると，cerivastatinが他のトランスポーターの基質になることも考えられる。したがって，肝クリアランスを完全に予測するためには，各種トランスポーターの寄与率を決定することが必要であり，今後の検討課題である。

2 cerivastatinの経細胞輸送の飽和性についての検討

ダブルトランスフェクタントにおけるcerivastatinの経細胞輸送の飽和性が，取り込みおよび排泄の各輸送過程の飽和性の影響をどのように受けるかについて検討した。まず，Oatp2/Mrp2共発現系およびOatp2単独発現系を用いて経細胞輸送の飽和性について速度論解析を行ったところ，両発現系とも飽和

性の輸送が観察され，基質との親和性を示す K_m 値，最大輸送速度を示す V_{max} 値はそれぞれ共発現系において 5.93（μM），38.22（pmol/min/mg protein），単独発現系において 4.32（μM），17.32（pmol/min/mg protein）となった。共発現系は単独発現系に比べ輸送能力が大きいものの，基質との親和性についてはどちらの発現系においても差はみられなかった。さらにそのときの細胞内濃度を測定し，細胞内濃度基準で apical 側へのクリアランスを濃度依存的に算出した。最大の細胞内総濃度は約 300（μM）となったが，cerivastain は蛋白結合率が非常に高いことが知られており[9]，細胞内における蛋白結合率 90％と仮定すると，細胞内非結合型濃度は 30（μM）と計算されるが，この濃度に達するまでクリアランスの変化は観察されず，Mrp2 の輸送については少なくともこの濃度範囲においては飽和が起きていないことが示唆された。

次に Oatp2 単独発現系および control 細胞を用いて，取り込み過程における飽和性について速度論解析を行ったところ，Oatp2 単独発現系において飽和性が観察され，K_m 値，V_{max} 値はそれぞれ 4.19（μM），17.28（pmol/min/mg protein）となった。これらすべての結果から考察すると，経細胞輸送の飽和性は主に取り込み過程における飽和性の影響を受けており，cerivastatin の経細胞輸送においては，このダブルトランスフェクタントにおいては取り込み過程が律速段階であることが示唆された。ラットにおいては，取り込み過程が肝臓における pravastatin の排泄の律速段階であることを報告しており[12]，今後ダブルトランスフェクタントにおけるおのおののトランスポーターの発現量とヒト肝臓における発現量の比較を通じて，ヒト肝臓における経細胞輸送の飽和性に関する考察についても検討していく予定である。

おわりに

本研究では，ダブルトランスフェクタントを用いることで，cerivastatin が Oatp2，Mrp2 の両トランスポーターの基質になることが示され，これらが，少なくとも肝クリアランスの一部を担っていることを示唆する結果を得た。また各素過程における速度論解析を行った結果，経細胞輸送の飽和性から考えると，本実験系における輸送の律速段階は取り込み過程であることが示唆された。この *in vitro* 実験系から得られた結果を *in vivo* における肝クリアランスの定量的な予測につなげるためには，ダブルトランスフェクタントとヒト肝細胞におけるトランスポーターの発現量の比較や他のトランスポーター，また細胞内における代謝酵素や抱合酵素の寄与についての定量的考察が必要である。発現量の比較に関しては，ある種の数学モデルを構築して間接的に各トランスポーターの輸送を補正する方法論のほか，ダブルトランスフェクタント自身の発現量調節を行うことで，直接 *in vivo* の状態を作ることも可能であると考え，現在検討を進めている。また，寄与率の決定に関しては，発現量による外挿や，各トランスポーターの特異的阻害剤の適用が考えうる。

現在，経細胞輸送を mimic した系としては，このダブルトランスフェクタントのほか，Keppler らによって，Oatp8/Mrp2 のダブルトランスフェクタントが構築されている[13]が，理想的には，各臓器における経細胞輸送を再構築した系を作ることも可能であり，寄与率から重要と思われる分子を複数発現させたマルチ発現系のセットをあらかじめ用意しておくことで，容易により直接的に経細胞輸送を予測することも可能になると考えており，今後，検討していく予定である。

【謝辞】 標識体の cerivastatin を御供与いただきましたバイエル薬品㈱の皆様にこの場をお借りして深謝いたします。

文　献

1) Kullak-Ublick GA, Ismair MG, Stieger B, Landmann L, Huber R, Pizzagalli F, et al. Organic anion-transporting polypeptide B (OATP-B) and its functional comparison with three other OATPs of human liver. Gastroenterology 2001；120：525-33.
2) Suzuki H, Sugiyama Y. Excretion of GSSG and Glutathione Conjugates mediated by MRP1 and cMOAT/MRP2. Seminars in liver disease 1998；18：359-76.
3) Abe T, Kakyo M, Tokui T, Nakagomi R, Nishio T, Nomura H, et al. Identification of a novel gene family encoding human liver-specific organic anion transporter LST-1. J Biol Chem 1999；274：17159-63.
4) Hsiang B, Zhu Y, Wang Z, Wu Y, Sasseville V, Yang WP, et al. A novel human hepatic organic anion transporting

polypeptide (OATP2). J Biol Chem 1999 ; 274 : 37161-8.
5) Konig J, Cui Y, Nies AT, Keppler D. A novel human organic anion transporting polypeptide localized to the basolateral hepatocyte membrane. Am J Physiol Gastrointest Liver Physiol 2000 ; 278 : G156-64.
6) Cui Y, Konig J, Buchholz JK, Spring H, Leier I, Keppler D. Drug resistance and ATP-dependent conjugate transport mediated by the apical multidrug resistance protein, MRP2, permanently expressed in human and canine cells. Mol Pharmacol 1999 ; 55 : 929-37.
7) Sasaki M, Suzuki H, Ito K, Abe T, Sugiyama Y. Transcellular transport of organic anions across a double-transfected madin-darby canine kidney II cell monolayer expressing both human organic anion-transporting polypeptide (OATP2/SLC21A6) and multidrug resistance-associated protein 2 (MRP2/ABCC2). J Biol Chem 2002 ; 277 : 6497-503.
8) 佐々木誠，鈴木洋史，阿部高明，杉山雄一．MDCK 細胞を用いた取り込みおよび排出輸送担体（OATP2, MRP2）共発現系の構築．薬理と治療 2001 ; 29(Suppl. 2) : S237-8.
9) Nakai D, Nakagomi R, Furuta Y, Tokui T, Abe T, Ikeda T, et al. Human liver-specific organic anion transporter, LST-1, mediates uptake of pravastatin by human hepatocytes. J Pharmacol Exp Ther 2001 ; 297 : 861-7.
10) Muck W. Clinical pharmacokinetics of cerivastatin. Clin Pharmacokinet 2000 ; 39 : 99-116.
11) Yamaoka K, Tanigawara Y, Nakagawa T, Uno T. A pharmacokinetic analysis program (multi) for microcomputer. J Pharmacobiodyn 1981 ; 4 : 879-85.
12) Yamazaki M, Akiyama S, Nishigaki R, Sugiyama Y. Uptake is the rate-limiting step in the overall hepatic elimination of pravastatin at steady-state in rats. Pharm Res 1996 ; 13 : 1559-64.
13) Cui Y, Konig J, Keppler D. Vectorial transport by double-transfected cells expressing the human uptake transporter SLC21A8 and the apical export pump ABCC2. Mol Pharmacol 2001 ; 60 : 934-43.

*　　*　　*

ELSEVIER

Toxicology 181–182 (2002) 287–290

TOXICOLOGY

www.elsevier.com/locate/toxicol

Toxicological implications of hepatobiliary transporters

Yukio Kato*, Hiroshi Suzuki, Yuichi Sugiyama

Department of Biopharmaceutics, Graduate School of Pharmaceutical Sciences, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

Abstract

Recent progress in molecular biological research has revealed that many types of transporters are expressed in the liver. Such xenobiotic transporters have a wide range of substrate specificity, which allows them to act as a barrier to protect the body from potentially harmful xenobiotics. Most of them mediate active transport of the substrates, leading to concentrative uptake and excretion into the hepatocytes and bile. Transport properties such as these may play a role in the toxicity of certain types of substrates. For example, anticancer agents such as methotrexate and irinotecan are efficiently excreted into the bile and such excretion has been suggested to account for their toxic gastrointestinal side-effects. A similar hypothesis has also been proposed for NSAIDs. Considering their tissue-specific expression, these transporters also appear to be a promising target for the delivery of small molecules, while simultaneously minimizing their side-effects. For example, transporters involved in the oral absorption, hepatic uptake, and biliary excretion of pravastatin, an HMG-CoA reductase inhibitor, ensure its enterohepatic circulation giving it an important pharmacokinetic advantage since its pharmacological target is the liver. Of the major ACE inhibitors currently available, temocaprilat is a substrate of hepatic transporters. Since the major elimination route for other ACE inhibitors is via urinary excretion, their plasma concentrations exhibit a high degree of inter-patient variability over a wide range of renal function whereas the concentrations of temocaprilat exhibit a much lower degree of inter-patient variability due to its elimination into the bile. Thus, the hepatobiliary transporters are involved in some aspects of toxicology and, therefore, a rational strategy for regulating the recognition by such transporters may be required for optimum drug design. This presentation will discuss the possible role of hepatic transporters from a toxicological point of view.

Keywords: Toxicokinetics; Transporter; Oatp; cMOAT/MRP2

1. Hepatobiliary transporters involved in systemic elimination

The possible implications from a toxicological point of view, as far as hepatobiliary transporters are concerned, are listed in Table 1. Hepatobiliary transporters are mainly involved in the systemic elimination of certain types of therapeutic agents and, therefore, play a role in their pharmacokinetics and/or toxicokinetics. For example, highly efficient hepatic uptake and subsequent biliary excretion has been found for several types of small peptides such as endothelin antagonists, somatostatin analogs and renin inhibitors, resulting in a lower bioavailability and stability in the circula-

* Corresponding author. Tel.: +81-3-5841-4772; fax: +81-3-5841-4766

E-mail address: ykato@p.kanazawa-u.ac.jp (Y. Kato).

0300-483X/02/$ - see front matter © 2002 Published by Elsevier Science Ireland Ltd.
PII: S0300-483X(02)00458-4

Table 1
Toxicological implications of hepatobiliary transporters

Hepatobiliary transporters are mainly involved in the systemic elimination of certain types of therapeutic agents and determine their oral bioavailability and/or systemic exposure in individual patients
The exposure of certain types of therapeutic agents to the liver is determined by the transport efficiency both in the sinusoidal and canalicular membranes. Sometimes this may be a pharmacokinetic advantage for a drug, the target site of which is the liver, but it may also be a pharmacokinetic disadvantage in terms of hepatic accumulation causing hepatotoxicity
Concentrative excretion into the bile of a certain types of therapeutic agents may be related to their gastrointestinal toxicity

tion (Cathapermal et al., 1991; Ziegler et al., 1994). Organic anion-specific transporters are involved in each membrane transport process. To develop the longer-acting ET-receptor antagonists required to treat chronic diseases, one possible strategy involves minimizing the recognition by hepatobiliary transporters. It has been demonstrated that insertion of a cationic moiety into the endothelin antagonist, BQ-123, leads to an increase in its systemic stability due to the reduction in transport efficiency across the bile canalicular membrane, mainly mediated by canalicular multispecific organic anion transporter (cMOAT/MRP2) (Akhteruzzaman et al., 1999; Kato et al., 1999).

Multiple elimination pathways may be one useful pharmacokinetic option for therapeutic agents, reducing the inter-patient variability in clinical efficacy, and hepatobiliary transporters may be an appropriate target for introducing such multiplicity into their systemic elimination route. Temocaprilat is actively transported across both sinusoidal and canalicular membranes, with organic anion transporting polypeptide (Oatp1) and cMOAT/MRP2, respectively, being involved (Ishizuka et al., 1998). Therefore, in contrast to the other ACE inhibitors, most of which are mainly eliminated via urinary excretion, it has been reported that the plasma concentration profile of temocaprilat exhibits a relatively lower degree of inter-patient variability over a wide range of renal function due to its elimination into the bile (Puchler et al., 1997).

2. Hepatobiliary transporters playing a role in hepatic efficacy and toxicity

The steady-state hepatic concentration of therapeutic agents can be described by the ratio of influx clearance to the sum of the clearance via sinusoidal efflux, canalicular efflux and intracellular metabolism. Therefore, the biological activity can be affected by the transport efficiency in each membrane process. Pravastatin, an HMG-CoA reductase inhibitor, is one example where hepatobiliary transporters are used to target the liver and reduce side-effects. Transporters are involved in the oral absorption, hepatic uptake, and biliary excretion of this compound (Yamazaki et al., 1996). Since the target of this HMG-CoA reductase inhibitor is the liver, efficient enterohepatic circulation may be a pharmacokinetic advantage.

Long-term methotrexate (MTX) therapy is associated with side-effects including hepatotoxicy (Bannwarth et al., 1996). Such toxicity is believed to stem from long-term accumulation of MTX in the liver leading to its polyglutamation, and there is a potential need for new derivatives, which are less likely to accumulate. Therefore, knowledge of the factors contributing to the tissue accumulation will provide a basis for the rational design and synthesis of such novel antifolate compounds. cMOAT/MRP2 is mainly involved in the canalicular transport of MTX (Han et al., 2001a). cMOAT/MRP2 has a wide range of substrate specificity and little information is currently available concerning the important structural features need for recognition by these transporters. We examined the recognition of MTX and 24 novel antifolate compounds by cMOAT/MRP2 to identify the most important parameters for cMOAT/MRP2 recognition (Han et al., 2001b). The affinity for cMOAT/MRP2 closely correlates with the octanol/water partition coefficient (c log P), and a linear combination of polar and nonpolar surface areas, suggesting that recognition by cMOAT/MRP2 depends on a balance of dynamic surface properties between the polar and nonpolar regions of MTX analogs. The so-called 'molecular weight threshold' for the cMOAT/MRP2 affinity of these compounds can be explained by their physico-

chemical parameters, especially the nonpolar surface areas of these compounds.

3. Hepatobiliary transporters playing a role in gastrointestinal toxicy

The concentrative excretion into the bile of a certain types of therapeutic agents may be related to their gastrointestinal toxicity. One such example is the gastrointestinal toxicity caused by NSAIDs. Such toxicity correlates with the amount excreted into the bile in several animal species, suggesting a relationship between toxicity and biliary excretion.

Anticancer agents, including MTX and irinotecan (CPT-11), are also known to cause gastrointestinal toxicity. We previously found that the active metabolite of irinotecan, SN-38, and its glucuronide (SN38-Glu) are mainly excreted into the bile via cMOAT/MRP2 both in rats and humans (Chu et al., 1997a,b, 1998; Niinuma et al., 1999) whereas the major excretion mechanism for CPT-11 involves P-glycoprotein. It should be noted that the inter-CMV variability in the transport activity of CPT-11 is less than twofold among five CMV samples while there is over a 40-fold inter-CMV difference in the transport of SN-38. This shows that the biliary excretion of SN-38 exhibits a large interindividual variability. During clinical use, CPT-11 produces severe, but unpredictable, gastrointestinal toxicity, namely diarrhea, in certain patients. Although the action of the active metabolite, SN-38, on gastrointestinal cells is believed to be responsible for this toxicity, its exact mechanism is still unknown. Several hypothesis for CPT-11-induced diarrhea, including biliary excretion of SN-38 and subsequent uptake by GI epithelial cells and biliary excretion of SN38-Glu and subsequent deconjugation by microflora, have been proposed. Our finding of a large inter-CMV difference in SN-38 uptake suggests that such interindividual variability in SN-38 biliary excretion may, at least partially, account for such unpredictable diarrhea found in patients.

The importance of biliary excretion in gastrointestinal toxicity has been suggested by our recent finding that the administration of an inhibitor of this excretion, probenecid, reduced the late-onset diarrhea caused by repeated administration of CPT-11 in rats. Coadministration of probenecid with a reduced intravenous dose of CPT-11 resulted in almost complete abolition of the biliary excretion of CPT-11 and its metabolites, dramatically reduced intestinal SN-38 concentrations and severe diarrhea, but systemic exposure to SN-38 and myerosuppression comparable with that obtained by administration of CPT-11 alone. These results suggest that the biliary excretion of the parent compound and/or metabolites play a role in the chronic toxicity caused by CPT-11 administration.

4. Hepatobiliary transporters playing a role in drug interactions

Transporter-mediated disposition of therapeutic agents may be a disadvantage in terms of the possible appearance of drug interactions. So far, little information is available about drug interactions via hepatobiliary transporters although interaction involving the hepatic uptake process has been implied, based on a pharmacokinetic analysis of a HMG-CoA reductase inhibitor and cyclosporin A in humans (Muck et al., 1999). We have proposed a rational strategy for quantitatively predicting such drug interactions via hepatobiliary transporters from in vitro experiments involving isolated hepatocytes and canalicular membrane vesicles (Ueda et al., 2001). By using these systems, respectively, the interaction between MTX and probenecid in vivo can be quantitatively predicted. In addition, interactions involving the net biliary excretion from the systemic circulation into the bile can also be quantitatively estimated by the two systems in order to avoid false negative predictions (Ueda et al., 2001).

Assessment of possible interactions via the transporters may be important for therapeutic agents in cases where their membrane transport is mainly mediated by such transporters. We have screened the inhibition potential, as far as cMOAT/MRP2 is concerned, for a variety of types of therapeutic agents used in clinical situations. Among such agents, only probenecid may have a partial inhibitory effect in clinical situations.

References

Akhteruzzaman, S., Kato, Y., Hisaka, A., Sugiyama, Y., 1999. Primary active transport of peptidic endothelin antagonists by rat hepatic canalicular membrane. J. Pharmacol. Exp. Ther. 288, 575–581.

Cathapermal, S.M., Foegh, M.L., Rau, C.S., Ramwell, P.W., 1991. Disposition and tissue distribution of angiopeptin in the rat. Drug Metab. Dispos. 19, 735–739.

Bannwarth, B., Pehourcq, F., Schaeverbeke, T., Dehais, J., 1996. Clinical pharmacokinetics of low-dose pulse methotrexate in rheumatoid arthritis. Clin. Pharmacokinet. 30, 194–210.

Chu, X., Kato, Y., Niinuma, K., Sudo, K., Hakusui, H., Sugiyama, Y., 1997a. Multispecific organic anion transporter is responsible for the biliary excretion of the camptothecin derivative irinotecan and its metabolites in rats. J. Pharm. Exp. Ther. 281 (1), 304–314.

Chu, X., Kato, Y., Sugiyama, Y., 1997b. Multiplicity of biliary excretion mechanisms for irinotecan, CPT-11, and its metabolites in rats. Cancer Res. 57, 1934–1938.

Chu, X., Kato, Y., Ueda, K., Suzuki, H., Niinuma, K., Tyson, C.A., Weizer, V., Dabbs, J.E., Froehlich, R., Green, C.E., Sugiyama, Y., 1998. Biliary excretion mechanism of CPT-11 and its metabolites in humans: involvement of primary active transporters. Cancer Res. 58, 5137–5143.

Han, Y., Kato, Y., Watanabe, Y., Terao, K., Asoh, Y., Sugiyama, Y., 2001a. Carrier-mediated hepatobiliary transport of a novel antifolate, *N*-[4-[(2,4-diamminopteridine-6-yl)methyl]-3,4-dihydro-2H-1,4-benzothiazin-7-yl]carbonyl-L-homoglutamic acid, in rats. Drug Metab. Dispos. 29, 394–400.

Han, Y., Kato, Y., Haramura, M., Ohta, M., Matsuoka, H., Sugiyama, Y., 2001b. Physicochemical parameters responsible for the affinity of methotrexate analogs to rat canalicular multispecific organic anion transporter (cMOAT/MRP2). Pharm. Res. 18, 579–586.

Ishizuka, H., Konno, K., Naganuma, H., Nishimura, K., Kouzuki, H., Suzuki, H., Stieger, B., Meier, P.J., Sugiyama, Y., 1998. Transport of temocaprilat into rat: role of organic anion transporting polypeptide. J. Pharmacol. Exp. Ther. 287, 37–42.

Kato, Y., Akhteruzzaman, S., Hisaka, A., Sugiyama, Y., 1999. Hepatobiliary transport governs overall elimination of peptidic endothelin antagonists in rats. J. Pharmacol. Exp. Ther. 288, 568–574.

Muck, W., Mai, I., Fritsche, L., Ochmann, K., Rohde, G., Unger, S., Johne, A., Bauer, S., Budde, K., Roots, I., Neumayer, H.H., Kuhlmann, J., 1999. Increase in cerivastatin systemic exposure after single and multiple dosing in cyclosporine-treated kidney transplant recipients. Clin. Pharmacol. Ther. 65, 251–261.

Niinuma, K., Kato, Y., Suzuki, H., Tyson, C.A., Weizer, V., Dabbs, J.E., Froehlich, R., Green, C.E., Sugiyama, Y., 1999. Primary active transport of organic anions on bile canalicular membrane in humans. Am. J. Physiol. 276, G1153–G1164.

Puchler, K., Eckl, K.M., Fritsche, L., Renneisen, K., Neumayer, H.H., Sierakowski, B., Lavrijssen, A.T., Thomsen, T., Roots, I., 1997. Pharmacokinetics of temocapril and temocaprilat after 14 once daily oral doses of temocapril in hypertensive patients with varying degrees of renal impairment. Br. J. Clin. Pharmacol. 44, 531–536.

Ueda, K., Kato, Y., Komatsu, K., Sugiyama, Y., 2001. Inhibition of the biliary excretion of methotrexate by probenecid in rats: quantitative prediction of the interaction from in vitro data. J. Pharmacol. Exp. Ther., 297.

Yamazaki, M., Suzuki, H., Sugiyama, Y., 1996. Advances in carrier-mediated hepatic uptake and biliary excretion of xenobiotics. Pharm. Res. 13, 497–513.

Ziegler, K., Kolac, C., Ising, W., 1994. ATP-dependent transport of the linear renin-inhibiting peptide EMD-51921 by canalicular plasma membrane vesicles of rat liver: evidence of drug-stimulatable ATP-hydrolysis. Biochim. Biophys. Acta 1196, 209–217.

Drug Metabol. Pharmacokin. 17 (2): 93-108 (2002).

Review

Drug Transporters: Their Role and Importance in the Selection and Development of New Drugs

Naomi MIZUNO[2] and Yuichi SUGIYAMA[1]

[1]*Graduate School of Pharmaceutical Sciences, The University of Tokyo, Bunkyo-ku, Tokyo, Japan and* [2]*Pharmacokinetics Laboratory, Mitsubishi Pharma, Co., Kisarazu-shi, Chiba, Japan*

Summary: Drug transporters expressed in various tissues play a significant role in drug disposition. By regulating the function of such transporters, it may be possible to eventually develop drugs with ideal pharmacokinetic profiles. In this article, we summarize the significant role played by drug transporters in drug disposition, focusing particularly on their potential use during the drug development process. The ability to manipulate transporter function offers the opportunity of being able to deliver a drug to the target organ, avoiding distribution to other organs (thereby reducing the chance of toxic side-effects), controlling the elimination process, and/or improving oral bioavailability. During drug development, it would be very useful to be able to select a lead compound that may or may not interact with transporters, depending on whether such an interaction is desirable. The use of specific inhibitors of transporters is also an attractive approach to controlling drug disposition, leading to improved efficacy. Currently, optimizing the pharmacokinetic properties of a drug during the early stages of its development is widely accepted as being of great importance. High-throughput screening systems using transporter gene transfected cells or computational (*in silico*) approaches are efficient tools for assessing transport activity during the early stage of drug development. In addition, drug-drug interactions involving drug transporters and functional genetic polymorphisms of drug transporters are also described. It would also be extremely valuable to be able to quantitatively predict inter-individual pharmacokinetic differences caused by transporter polymorphisms or pharmacokinetic changes caused by drug-drug interactions involving transporters during drug development.

Key words: Transporter; drug discovery; drug development

Introduction

Drug transporters expressed in various tissues, such as the intestine, brain, liver, and kidney, play key roles in the absorption, distribution and excretion of drugs and are one of the determinant factors governing drug disposition (**Fig. 1**).[1-5] Recently, a number of important transporters have been cloned and significant progress has been made in characterizing the cellular properties and functions of these transporters.[6-8] Transporters have been classified as either primary, secondary or tertiary active transporters. Secondary and tertiary active transporters are driven by an exchange of intracellular ions while the driving force for primary transporters, like ATP-binding cassette transporters, is ATP hydrolysis.[9] Furthermore, each gene family of transporters is composed of a multiplicity of members. The Human Gene Nomenclature Committee has classified transporters by standardized names such as the solute carrier superfamily (*SLC*) and ATP-binding cassette transporters (*ABC*) (http://www/gene.ucl.ac.ul/nomenclature/genefamily. shtml). These standardized names, accompanied by conventional names, are both given in this article. The tissue distribution and elimination route of some drugs is determined by the degree of expression of each transporter subtype in each tissue and its corresponding substrate affinity. Thus, regulating the function of transporters should allow the efficient development of drugs with ideal pharmacokinetic profiles. In recent years, high-throughput screening methods have resulted in the rapid discovery of new chemical entities and also reduced the costs of drug development in the pharmaceutical industry. Many pharmaceutical companies are now carrying out high-throughput pharmacokinetic-oriented screening and are optimizing the pharmacokinetic properties during the early stages of drug development to make the entire process much more efficient.[10,11] As drug development involving the

Received; December 20, 2001, Accepted; February 15, 2002
To whom correspondence should be addressed: Yuichi SUGIYAMA, *Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan. Tel. +81-3-5841-4770, Fax. +81-3-5841-4766, E-mail: sugiyama@mol.f.u-tokyo.ac.jp*

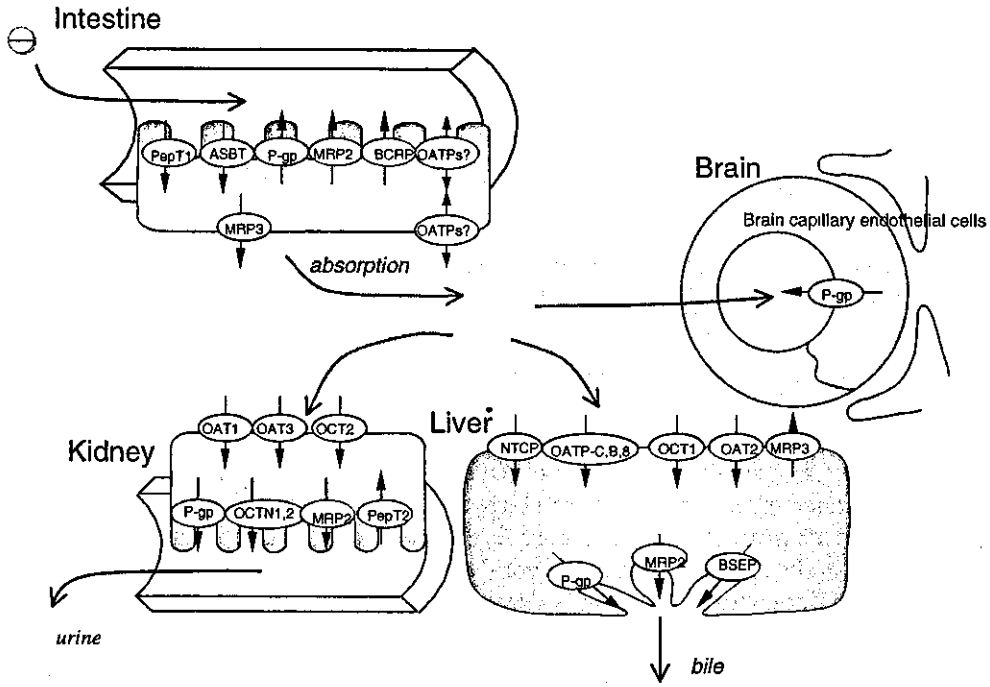


Fig. 1. Major drug transporters in humans.

Abbrevations

		[symbol]
MDR1/P-gp	multidrug resistant gene/P-glycoprotein	*ABCB1*
BSEP/SPGP	bile salt export pump/sister P-glycoprotein	*ABCB11*
MRP1	multidrug resistance associated protein 1	*ABCC1*
MRP2	multidrug resistance associated protein 2	*ABCC2*
MRP3	multidrug resistance associated protein 3	*ABCC3*
BCRP	breast cancer resistance protein	*ABCG2*
NTCP	sodium taurocholate cotransporting peptide	*SLC10A1*
ASBT	apical sodium-dependent bile acid transporter	*SLC10A2*
PEPT1	oligopeptide transporter 1	*SLC15A1*
PEPT2	oligopeptide transporter 2	*SLC15A2*
OATP-C/OATP 2/LST-1	organic anion transporting peptide-C/organic anion transporting peptide 2/liver-specific organic anion transporter-1	*SLC21A6*
OATP8	organic anion transporting peptide 8	*SLC21A8*
OATP-B	organic anion transporting peptide-B	*SLC21A9*
OCT1	organic cation transporter 1	*SLC22A1*
OCT2	organic cation transporter 2	*SLC22A2*
OCT3	organic cation transporter 3	*SLC22A3*
OCTN1	novel organic cation transporter 1	*SLC22A4*
OCTN2	novel organic cation transporter 2	*SLC22A5*
OAT1	organic anion transporter 1	*SLC22A6*
OAT2	organic anion transporter 2	*SLC22A7*
OAT3	organic anion transporter 3	*SLC22A8*

use of transport mechanisms increases, the need for an effective *in vitro* screening system for transporters will also increase. Accordingly, methods allowing the rational prediction and extrapolation of *in vivo* drug disposition from *in vitro* data are also essential. The drug-drug interactions involving drug transporters and genetic polymorphisms of drug transporters have been described.[12,13] The changes in pharmacokinetics due to genetic polymorphisms and drug-drug interactions can often directly affect the therapeutic safety and efficacy of many important drugs. In order to obtain detailed information about these inter-individual differences, the contribution made by transporters to drug absorption, distribution and excretion needs to be taken into account throughout the drug discovery and development process.

In this article, we summarize the key role played by drug transporters in drug disposition, focusing particularly on their potential use during the drug discovery and development stages. We introduce the strategy of

Table 1. Effect of BCRP inhibitor GF120918 on pharmacokinetics of topotecan in mdr1a/1b(−/−) mice (From Ref. 33)

		Vehicle treated	GF120918 treated	Ratio
Oral administration of topotecan (1mg/kg)[a]				
AUC	(mg·hr/L)	96	596	×6.2
Intravenous administration of topotecan (1 mg/kg)[a]				
AUC	(mg·hr/L)	200	406	×2.0
Biliary excretion[b]	(%)	14.7	5.5	×0.4
Intestinal content of [^{14}C] topotecan-derived radioactivity[c]				
Intestinal	(%)	31.8	10.2	×0.3
Plasma	(ng/mL)	40	102	×2.6

[a] Mdr1a/1b(−/−) mice were given an oral dose of GF120918 (50 mg/kg) or vehicle 15 minutes before an oral or intravenous dose of topotecan (1 mg/kg).
[b] Cumulative biliary excretion of unchanged topotecan for 11 minutes.
[c] GF120918 (50 mg/kg) was administered orally; 15 minutes later, [^{14}C] topotecan (1 mg/kg) was administered intravenously; 60 minutes after administration of [^{14}C] topotecan, the intestinal content of [^{14}C] was determined.

drug development using transporters, transporter screening systems, methods for estimating the contribution of transporters to drug disposition and the prediction of *in vivo* drug disposition from *in vitro* data. As the detailed characterization of transporters and their nomenclature are not described in this review, the readers is requested to refer to other reviews for further information.[8,12,14-16]

Role of Transporters in Drug Absorption

Various transporters expressed in the small intestine are involved in the absorption of nutrients or endogenous compounds.[13] The use of influx transporters expressed in gut, such as oligopeptide transporters (PEPT1/*SLC15A1*), apical sodium-dependent bile acid transporters (ASBT/*SLC10A2*) or organic anion transporting polypeptide-B (OATP-B/*SLC21A9*), will help improve drug absorption (**Fig. 1**).[17-21]

Primary active efflux transporters, such as P-glycoprotein (P-gp) encoded by multidrug resistance gene (MDR1/*ABCB1*), multidrug resistance associated protein 2 (MRP2/*ABCC2*) or the breast cancer resistance protein (BCRP/*ABCG2*), are expressed on the brush border membrane of enterocytes and excrete their substrates into the lumen, resulting in a potential limitation of net absorption (**Fig. 1**). P-gp contributes to the absorption of many drugs because of its broad substrate specificity.[22,23] The intestinal P-gp content correlates with the AUC after oral administration of digoxin, a P-gp substrate (**Fig. 2**).[24] This result suggests that P-gp in the epithelium of the gut wall determines the plasma concentration of orally administered digoxin. Another report has also demonstrated a good correlation between tacrolimus plasma concentrations after oral administration and the intestinal MDR1 expression level.[25] It is possible that, in addition to P-gp, MRP and BCRP are responsible for extrusion from enterocytes, thereby affecting the absorption of certain kinds of drugs. MRP2 transports glutathione conjugates,

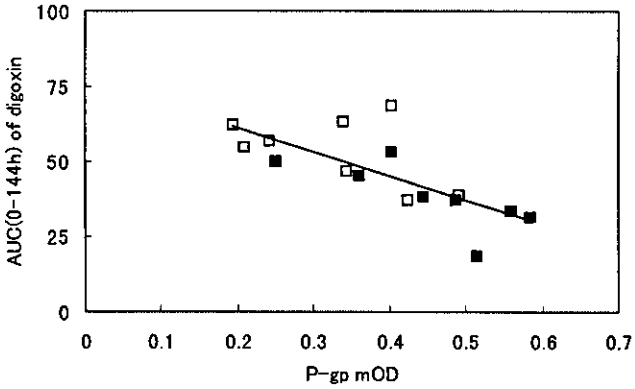


Fig. 2. Correlation between the AUC of orally administered digoxin (1 mg) and the expression of duodenal P-gp (n = 16). Open squares = without rifampin; closed squares = with rifampin (600 mg). (From Ref. 24)

glucuronides and non-conjugated organic anions and, so, it may play a functional role in the secretion of many drugs and/or their metabolites from the small intestine.[26-29] ME3299 is an ester-type prodrug designed to increase the oral bioavailability of pharmacologically active carboxylate drugs (a IIb/IIIa receptor antagonist). However, ME3299 exhibits low oral bioavailability (~10% in rat) of the active agent because most of the carboxylates produced from prodrugs in enterocytes are actively excreted into the lumen.[30,31] Furthermore, it is suggested that BCRP plays a role in the secretion of clinically important drugs such as topotecan.[32,33] The BCRP inhibitor, GF120918, increases the bioavailability of topotecan dramatically in P-gp-deficient mice (**Table 1**).[33] Using P-gp-deficient mice, the effect of P-gp on topotecan absorption can be avoided. GF120918 also reduces the plasma clearance and hepatobiliary excretion of topotecan and increases (re-) uptake by the small intestine. In principle, the inhibition of intestinal efflux transporters is a useful way to improve the oral

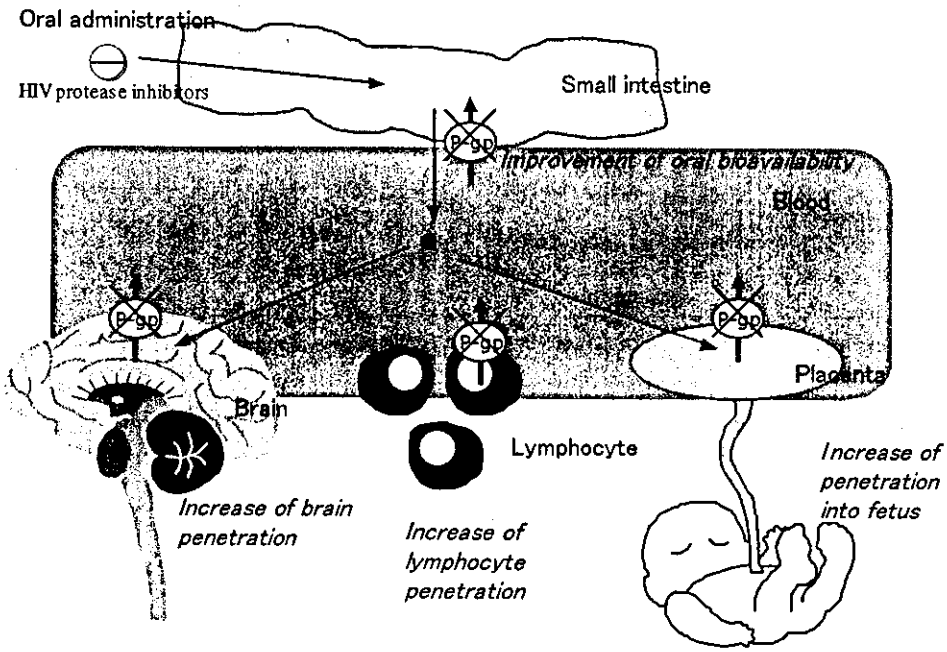


Fig. 3. Possible effect of P-gp blockade on the pharmacokinetics of HIV protease-inhibitors.

bioavailability of a co-administered drug.[34]

The Role of Transporters in Tissue Distribution

Drug Delivery to Target Tissues using Transporters: The transporters expressed in selective organs are promising targets for drug delivery. The most comprehensively documented case is pravastatin where transporters are involved in its oral absorption, hepatic uptake, and biliary excretion.[21,35,36] Since the target of this HMG-CoA reductase inhibitor is the liver, efficient enterohepatic circulation is a useful pharmacokinetic feature, maximizing its pharmacological effect while minimizing its side-effects. Although the mechanism of this pharmacokinetic property of pravastatin was identified after its development, attempts to design molecules like pravastatin during the drug discovery stage will be required in the future. It has been found that successful targeting of anti-cancer drugs can be achieved using PEPT1 expressed in tumors.[37,38] Because PEPT1 is expressed in the small intestine too, it may also be possible to increase the absorption of anti-cancer drugs.

The efflux transporters in target tissues may be responsible for poor delivery to target tissues. P-gp is expressed extensively in brain capillary endothelial cells or tumor cells and prevents a number of drugs from entering these tissues.[39-41] Therefore, the strategy of screening compounds not transported by efflux transporters such as P-gp may be useful for improving the efficacy of anti-cancer drugs or drugs acting on the central nervous system. Preventing efflux transport by the use of specific inhibitors is also an attractive approach to improve the distribution of drugs to target organs.[42] Since over-expression of P-gp or MRP on the surface of tumor cells causes multidrug resistance, the use of a chemomodulator to inhibit efflux transport has been tried in an attempt to overcome this resistance.[43-48] Several inhibitors of P-gp have been discovered and are currently undergoing clinical trials.[49-52] However, P-gp modulators may increase the brain penetration of anti-cancer drugs by blocking brain P-gp, leading to CNS side-effects. Furthermore, blockade of P-gp is expected to improve the efficacy of HIV protease-inhibitors (HIV-PI) (**Fig. 3**).[53] P-gp limits the therapeutic efficacy of HIV-PI, which are transported by P-gp.[54] P-gp is not only present in lymphocytes, one of the main targets of HIV, but also in HIV pharmacological sanctuary sites, such as the brain or testis, and limits access of HIV-PI to these tissues.[55] One serious problem is that placental P-gp limits the ability of HIV-PI to reach the fetus in pregnant HIV-positive patients.[56,57] Moreover, P-gp in the small intestine limits the oral bioavailability of HIV-PI.[58] Thus, it is expected that co-administration of highly efficient P-gp inhibitors will improve HIV-PI penetration into these sanctuaries sites and lead to increased oral bioavailability (**Fig. 3**). In actual fact, the low oral bioavailability of saquinavir has been dramatically increased by co-administration of ritonavir, which inhibits not only cytochrome P450 3A (CYP3A) but also P-gp.[59-61] Detailed clinical studies are expected to establish whether such a strategy will indeed improve HIV-PI efficacy, and whether it will be safe enough for routine use.

Avoiding Toxicity using Transporters: Reducing brain penetration by means of efflux transporters is

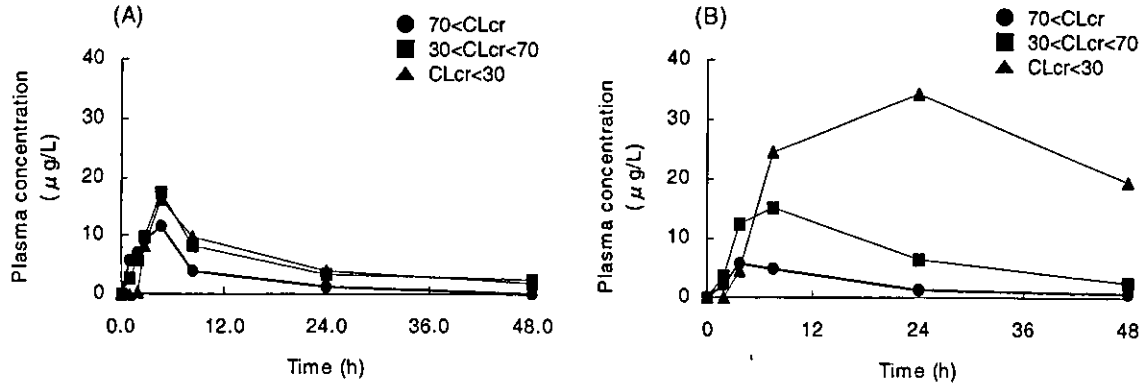


Fig. 4. Plasma concentrations of temocaprilat (A) following a single oral dose of temocapril 1 mg and enalaprilat (B) following a single oral dose of enalapril 5 mg in patients with different degrees of renal function. CLcr = creatinine clearance in ml/min. (From Ref. 85)

desirable for drugs with toxic CNS effects. Some quinolone antibacterial agents or anti-cancer agents exhibit low brain distribution, in spite of having a high lipophilicity, because they are prevented from entering the brain by P-gp, probably explaining their relative lack of CNS side-effects.[41] P-gp-deficient mice display a significant increase in the brain distribution of these drugs.[40,41] A key tool for investigating the role of transporters in drug disposition has been transporter gene knockout mice.[62-65] Use of efflux transporters is expected to help prevent such CNS toxicity. During drug development, a lead compound has to be selected that may, or may not, interact with brain efflux transporters, depending on whether this interaction is desirable.

However, toxicity may occasionally be caused by concentrative tissue distribution due to active transport. The organic anion transporter-1 (OAT1/*SLC22A6*), mainly expressed in the kidney, plays a key role in the renal tubular secretion of negatively charged molecules (**Fig. 1**).[66,67] Antiviral nucleotide analogs, such as adefovir, β-lactam antibiotics, and ochratoxin A, are efficiently transported by OAT1 which, presumably, mediates their accumulation in renal proximal tubules.[68-71] In some cases, compounds interacting with OAT1 cause nephrotoxicity so that a type of drug not transported by these transporters or co-administration of inhibitors of these transporters should be effective.[72] A fluorescence assay to screen for novel human OAT1 inhibitors has been developed and it has been suggested that nonsteroidal anti-inflammatory drugs (NSAIDs) may reduce adefovir nephrotoxicity since NSAIDs efficiently inhibit the human OAT1-specific transport of adefovir at clinically relevant concentrations.[73-75]

Although irinotecan (CPT-11) is an effective anti-cancer drug, its use is associated with severe gastrointestinal toxicity (diarrhea) in clinical situations.[76] The action of its active metabolite, SN-38, on gastrointestinal cells is believed to be responsible for this toxicity.[77] The biliary excretion of SN-38 and SN-38 glucuronide and subsequent uptake by GI epithelial cells may be associated with this diarrhea.[78] Since MRP2 is involved in the biliary excretion of SN-38 and SN-38 glucuronide, MRP2 blockers help reduce their biliary excretion and subsequent side-effects on gastrointestinal epithelial cells.[79-81]

Control of Elimination using Drug Transporters

As far as the liver is concerned, a wide variety of transporter families are known to be present at the sinusoidal and canalicular membranes and to play a significant role in hepatobiliary excretion (**Fig. 1**).[15,16,22,35,82,83] In the kidney, transporters at the basolateral and luminal membranes are also involved in the active renal secretion of drugs (**Fig. 1**).[14,66,84] If drugs for use in patients with kidney diseases are predominantly excreted into urine, the plasma concentrations will exhibit significant inter-patient variability, depending on the renal function. A multiple elimination pathway via both the liver and kidney will result in a relatively stable pharmacokinetic profile compared with only a single elimination pathway. Temocaprilat, an ACE- inhibitor (ACEI), is a typical case of multiple elimination pathways via both liver and kidney, thereby avoiding a large degree of interindividual variability in its pharmacokinetic profile compared with other ACEIs which are mainly eliminated via urine.[85] In patients with renal failure, the AUCs of other ACEIs like enalapril are markedly increased because these are eliminated primarily via renal excretion. On the other hand, the AUC of temocaprilat in these patients is scarcely affected because temocaprilat is excreted via both bile and urine (**Fig. 4**).[85] Although the biliary excretion of temocaprilat is governed by MRP2 at the canalicular membrane, it has been suggested that other ACEIs are not good substrates of MRP2.[86,87] Thus, temocaprilat may have a different elimination pathway compared with other ACEIs. The route of elimination may be controlled by using transporters that are expressed selective-

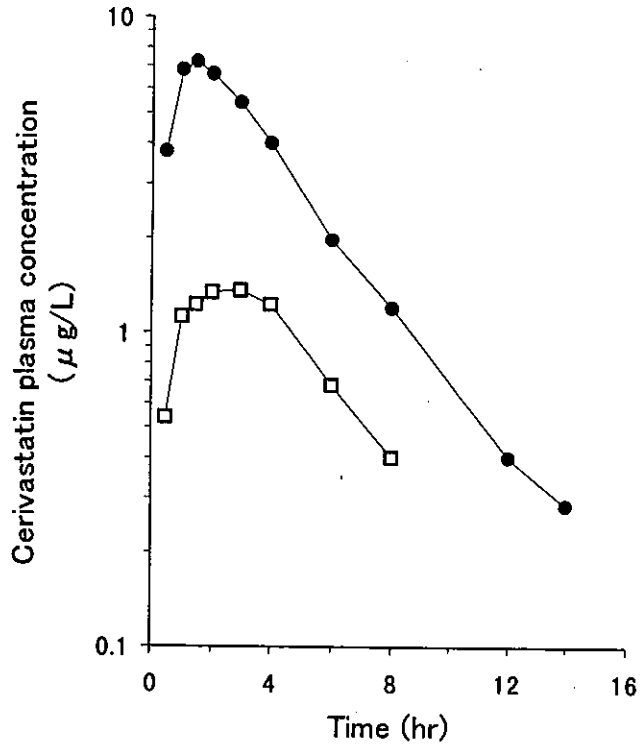


Fig. 5. Change in disposition of cerivastin following cyclosporin treatment. Cerivastin plasma concentration-time profiles after oral administration of 0.2 mg cerivastatin in kidney transplant recipients receiving stable individual cyclosporin treatment (●) compared with healthy subjects receiving no cyclosporin (□) are shown. (From Ref. 95)

ly in either the liver or kidney. For instance, OATP-C/*SLC21A6* (also referred to as OATP2 and LST-1) and OATP8/*SLC21A8* have been shown to be expressed exclusively in liver, and appear to play a key role in the hepatic uptake of various drugs (**Fig. 1**).[20,21,36,88-90] Human OCT1/*SLC22A1* is expressed primarily in the liver whereas OAT1 is expressed predominantly in the kidney (**Fig. 1**).[67,91]

Drug-Drug Interactions Involving Drug Transporters

Due to the broad substrate specificity of drug transporters, drug-drug interactions involving these transporters are very likely. Recently, both inhibition and induction of transporters have been implicated as one mechanism responsible for certain drug-drug interactions.[12]

Drug-Drug Interactions Involving Elimination: P-gp inhibitors, such as quinidine, verapamil and valspodar, are known to increase plasma concentrations of digoxin because they block biliary and/or urinary excretion of digoxin via P-gp.[92-94] Since the therapeutic range of digoxin is small, changes in its plasma concentration are potentially very serious. Co-administration of an HMG-CoA inhibitor, cerivastatin, and cyclosporin to kidney transplant recipients results in a significant increase in cerivastatin plasma concentrations (**Fig. 5**).[95] Since CYP3A4 is responsible for the metabolism of both cerivastatin and cyclosporin, metabolism possibly plays a role in this interaction. However, the effect of erythromycin, a suicide substrate of CYP3A4, on the AUC of cerivastatin is minimal, suggesting that this hypothesis may not be valid.[12] The hepatobiliary transport of pravastatin, an HMG-CoA reductase inhibitor, has been shown to be carrier-mediated (OATP-C and MRP2).[21,36,96] This transporter is also expected to be involved in distribution to the liver, the main site of cerivastatin distribution, resulting a drug-drug interaction between cerivastatin and cyclosporin during hepatic uptake. Recently, cerivastatin was withdrawn from the market because of severe rhabdomyolysis associated with cerivastatin-gemfibrozil combination therapy.[97,98] It is possible that the transporter in the sinusoidal membrane of the liver is involved in this drug-drug interaction.

Drug-Drug Interactions Involving Absorption: **Table 2** shows examples suggesting that P-gp may be involved in human drug-drug interactions associated with absorption. For example, the plasma concentration of talinolol is increased by co-administration of erythromycin.[99] It is suggested that erythromycin inhibits talinolol secretion from enterocytes into the lumen via P-gp, resulting in increased net absorption because of the lack of any significant metabolism of talinolol. The metabolism by CYP3A in the human small intestine is a major factor limiting oral bioavailability, accompanied by P-gp-mediated efflux. Digoxin or talinolol are good substrates of P-gp, but not of CYP3A. Thus, the role of P-gp can be directly demonstrated by these examples. Since the substrate specificities of CYP3A and P-gp overlap, many drugs may be substrates of both.[100,101] In such cases, it is difficult to distinguish between the contribution of CYP3A and that of P-gp to the increased oral bioavailability.[100,101] Other reports have shown that grapefruit juice, orange juice and apple juice reduce the oral bioavailability of fexofenadine, an OATP substrate, in healthy volunteers.[102,103] Inhibition of OATP-mediated drug uptake at the intestinal wall is believed to play an important role in this phenomenon.

Induction of intestinal P-gp can be seen as a new type of drug-drug interaction. Rifampin and St John's wort induce intestinal P-gp, resulting in reduced oral bioavailability of digoxin (**Table 2**).[24,104,105] Since rifampin also induces intestinal MRP2, co-administration of rifampin is expected to increase secretion into the lumen of MRP2 substrates, such as glutathione or glucuronide conjugates.[106] Recently, it has been reported that steroid xenobiotic receptor (SXR; also known as PXR), a member of the nuclear hormone receptor superfamily,

Table 2. Examples of the possible involvement of intestinal P-gp with clinical drug-drug interactions

	inhibitor or inducer	substrate	effects	Ref.
P-gp inhibition	atorvastatin	digoxin	C_{max} + 20%, AUC + 15%	135
	cyclosporine	docetaxel	BA 11-fold increase[a]	136
	cyclosporine	paclitaxel	BA 8-fold increase[a]	137
	erythromycin	atorvastatin	C_{max} + 38%, AUC + 32%, $T_{1/2}$ no change[a]	138
	erythromycin	cyclosporin	C_{max} + 113%, AUC + 97%, $T_{1/2}$ no change[a]	139
	erythromycin	cyclosporin	C_{max} + 180%, AUC + 115%, $T_{1/2}$ no change[a]	140
	erythromycin	cyclosporin	AUC + 115%[a]	141
	erythromycin	fexofenadine	C_{max} + 82%, AUC + 109%	142
	erythromycin	talinolol	AUC + 34%, $T_{1/2}$ no change	99
	erythromycin	saquinavir	C_{max} + 106%, AUC + 99%[a]	143
	GF120918	paclitaxel	BA 7-fold increase[a]	50
	ketoconazole	fexofenadine	C_{max} + 135%, AUC + 164%	142
	ketoconazole	saquinavir	C_{max} + 171%, AUC + 190%[a]	143
	ketoconazole	tacrolimus	BA 2-fold increase[a]	144
	ritonavir	saquinavir	AUC × 58, C_{max} × 33[a]	60
	talinolol	digoxin	C_{max} + 45%, AUC + 23%, $T_{1/2}$ no change	145
	valspodar	digoxin	AUC + 76%	92
	valspodar	paclitaxel	AUC + 100 ~ + 200%[a]	34
	verapamil	talinolol	secretion rate − 29 ~ − 56%	146
P-gp induction	rifampin	digoxin	C_{max} − 58%, AUC − 37%, $T_{1/2}$ no change, intestinal P-gp 3.5-fold increase	24
	rifampin	fexofenadine	C_{max} − 41%, AUC − 56%, $T_{1/2}$ no change	147
	rifampin	saquinavir	C_{max} − 65%, AUC − 70%[a]	143
	rifampin	tacrolimus	BA 14% → 7%, CL_{tot} + 47%[a]	148
	rifampin	talinolol	AUC − 35%, intestinal P-gp 4.2-hold increase	149
	St John's wort	digoxin	intestinal P-gp 1.4-hold increase	105
	St John's wort	digoxin	C_{max} − 26%, AUC − 25%	104

[a] CYP3A is also supposed to be involved in drug-drug interactions.

involved in xenobiotic induction of CYP3A, can also regulate the expression of the gene MDR1 encoding the protein P-gp.[107,108] SXR is activated by paclitaxel or rifampin and is responsible for inducing expression of CYP3A and MDR1. It is plausible that CYP3A and P-gp act synergistically, affecting intestinal availability. SXR-binding assays will be a useful tool for screening compounds which will induce not only CYP3A but also MDR1 and be a potential cause of drug interactions.[109] Prediction of *In vivo* Drug-Drug Interactions from *In vitro* Data: Ueda *et al.* have developed a rational strategy for using *in vitro* data to predict drug-drug interactions between probenecid and methotrexate involving biliary excretion.[12,110] By using isolated hepatocytes and canalicular membrane vesicles for the analysis of hepatic uptake and excretion, the degree of reduction in methotrexate biliary excretion by probenecid can be quantitatively predicted based on the inhibition constant found *in vitro* as well as the concentration of the inhibitor drug in circulating plasma and liver, respectively.[12,110] Establishing such strategies is important for predicting the degree of drug-drug interactions or side-effects in clinical situations.

Genetic Polymorphism of Drug Transporters

The latest trend is to develop drug prescription or treatment protocols based on the genetic constitution of individual patients. It is also important to develop drugs which are relatively unaffected by polymorphisms and exhibit little interindividual variability. Polymorphisms in drug transporters, such as MDR1, MRP1/ABCC1, MRP2, OATP-C, novel organic cation transporter 2 (OCTN2/*SLC22A5*), have been demonstrated.[111-114] It has been reported that a single nucleotide polymorphism (SNP) in exon 26 (C3435T) of MDR1 results in the reduced intestinal expression of P-gp, along with increased oral bioavailability of digoxin.[115] The frequency of this mutation is relatively high and significantly affected by ethnic origin.[116,117] Furthermore, Kim *et al.* have demonstrated that two synonymous SNPs (C1236T in exon 12 and C3435T in exon 26) and a non-synonymous SNP (G2677T, Ala893Ser in exon 21) are linked.[118] It is suggested that P-gp activity is enhanced in subjects with this allele, resulting in a reduction in absorption of the P-gp substrate, fexofenadine (**Table 3**). This finding contradicts previous results on the effect of exon 26 C3435T SNP on digoxin disposition.[115] The reason for this discrepancy and failure to detect exon 21 SNP in a previous study is currently unclear. A polymorphism of MDR1 may change the brain penetration of P-gp substrates more dramatically than that into the small intestine. It is possible that MDR1 polymorphisms

Table 3. MDR1 alleles and fexofenadine disposition in Caucasians (From Ref. 118)

Genotype	Frequency (%)	$AUC_{(0-4)}$ ($ng \cdot mL^{-1} \cdot h$)	C_{max} ($ng \cdot mL^{-1}$)	T_{max} (h)	$T_{1/2}$ (h)
*1/*1	16	1316±543	508±205	2.7±0.8	2.8±0.4
*1/*2	11	1171±967	400±282	3.3±1	3.1±0.7
*2/*2	14	837±311	317±185	2.4±1.7	3.5±0.9

180 mg fexofenadine was administered orally. The first published MDR1 sequence is shown as the MDR1*1 allele. MDR1*2 allele contain 3 SNPs simultaneously; C1236T in exon 12, G2677T in exon 21, and C3435T in exon 26.

cause inter-individual differences in the degree of CNS toxicity of P-gp substrates. In addtion, SNPs of OATP-C associated with a reduction in transport activity have been found.[119] In particular, OATP-C polymorphisms may influence the interindividual variability in the pharmacological/toxicological effects of those drugs which have the liver as their pharmacological target and/or a plasma clearance governed by OATP-C mediated hepatic uptake.

How can we use Drug Transporters to Optimize Drug Discovery and Development?

Optimizing the pharmacokinetic properties during the early stages of drug development is now widely accepted as being essential. In this context, attention is now being focused on optimizing pharmacokinetic profiles by using transporter function. The expression system of transporters is an efficient tool for screening transport activities. Recent studies show that *in vivo* P-gp function can be quantitatively predicted by using MDR1-transfected cell monolayers (**Fig. 6**).[120] The "$K_{p,brain}$ ratio" ($K_{p,brain(mdr1a/1b(-/-))}/K_{p,brain(mdr1a/1b(+/+))}$ is the most suitable parameter for describing P-gp function on the blood-brain-barrier (BBB) (**Fig. 7**). By normalizing the brain-to-plasma concentration ratio ($K_{p,brain}$) in mdr1a/1b knockout mice with reference to that in normal mice, the P-gp function parameter can be simply estimated as shown in **Fig. 7**. Furthermore, *in vivo*, this parameter corresponds to the "corrected flux ratio" across the MDR1-transfected cell monolayers (**Fig. 7**). The "corrected flux ratio" represents the normalizing ratio of B→A flux to A→B flux in parental cell monolayers with respect to that in MDR1-transfected cell monolayers. Indeed, a clear correlation between both parameters *in vitro* and *in vivo* has been obtained experimentally (**Fig. 6**). Another report also showed an equally good correlation.[121] Although one can calculate the net flux by subtracting A→B flux from B→A flux in MDR1-transfected cell monolayers, no theoretical background can be given to relate these to *in vivo* parameters.[122] In the case of CNS-active drugs, the concentration of "free" drug in the brain is most important for predicting pharmacological effects, not the concentration of "total" drug, since the term "total" drug includes the fraction bound nonspecifically to brain components. We should also note that the value of the "$K_{p,brain}$ ratio" indicates the brain-to-plasma concentration ratio of "free" drug ($K_{p,brain,free}$), and this important parameter, which can reflect the pharmacological effect of drugs on the central nervous system, can be estimated by using the expression system (See the equations in **Fig. 7**). Recently, double-transfected cell monolayers expressing OATP-C or OATP8 in the basolateral membrane and MRP2 in the apical membrane have been constructed.[96,123] The double-transfected cells may be used to analyze the hepatic vectorial transport of drugs and to screen the transport profiles of new drug candidates.

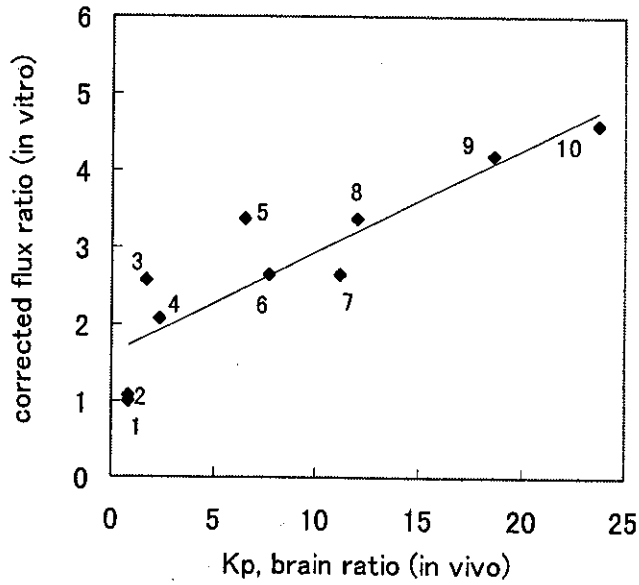
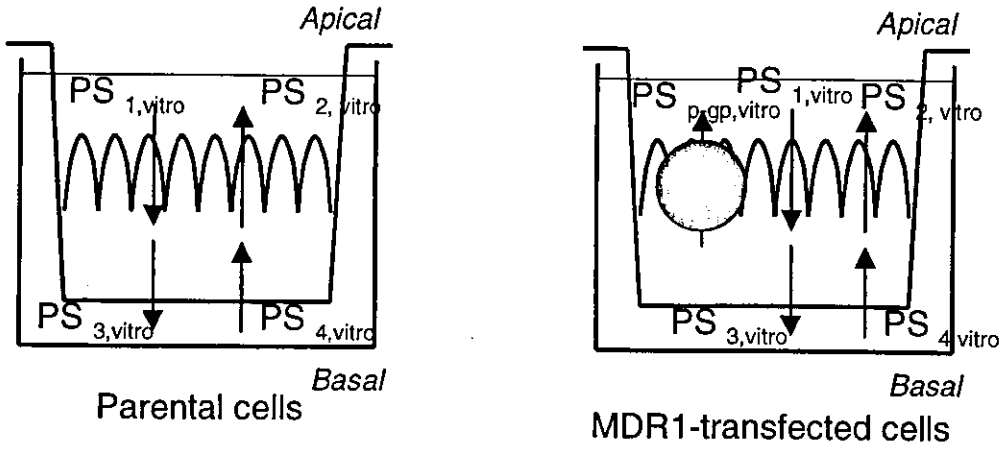


Fig. 6. Correlation of P-gp function determined in *in vitro* transcellular transport studies using MDR1-transfected cells/control cells and *in vivo* brain penetration studies using mdr1a/1b knockout mice/wild type mice. 1, diazepam; 2, progesterone; 3, daunomycin; 4, dexamethasone; 5, loperamide; 6, verapamil; 7, vinblastine; 8, cyclosporin A; 9, digoxin; 10, quinidine. (From Ref. 120)

In addition, a high-throughput assay to indirectly detect compounds interacting with P-gp has been described. Such methods are based on inhibition of the efflux of radiolabeled or fluorescent P-gp substrates.[124-126] In the case where radiolabeled ligands are used, the scintillation proximity assay is a useful tool for the sequential detection of radioactivity in the 96-well plate format.[127] Also shown are assays measuring drug-stimulated ATPase activity in baculovirus-infected insect cells expressing human P-gp.[128] We need to choose suitable assays, depending on the properties of drug candidates or the purpose of the evaluation. Furthermore, the next step towards improved high-throughput screening should involve computational (*in silico*) ap-

A. In vitro (cultured cell monolayers)

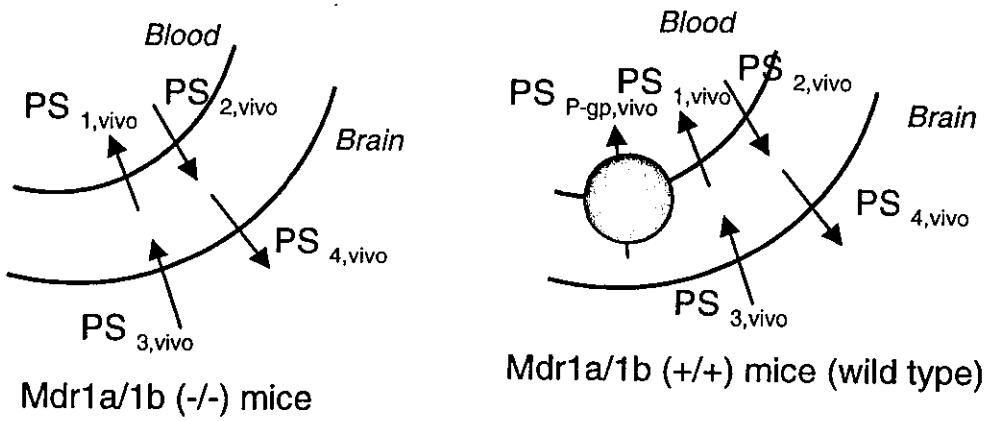


Corrected flux ratio

$$= \frac{PS_{B\rightarrow A\ (parent\ cells)}}{PS_{A\rightarrow B\ (parent\ cells)}} \Big/ \frac{PS_{B\rightarrow A\ (MDR1\ transfected\ cells)}}{PS_{A\rightarrow B\ (MDR1\ transfected\ cells)}} = 1 + \frac{PS_{P\text{-}gp,vitro}}{PS_{2,vitro}}$$

PS : the permeability-surface area product

B. In vivo (BBB)



$$K_{p,brain}\ ratio = \frac{K_{p,brain\ (mdr1a/1b\ (-/-))}}{K_{p,brain\ (mdr1a/1b\ (+/+))}} = 1 + \frac{PS_{P\text{-}gp,vivo}}{PS_{2,vivo}}$$

$K_{p,brain}$: the brain-to-plasma concentration ratio

Fig. 7. Schematic diagram illustrating the permeability-surface area products (PS) for the penetration of ligands across the plasma membrane. A and B represent the PS products across the cultured cell monolayers and those across the cerebral endothelial cells. $PS_{1,vitro}$ and $PS_{2,vitro}$ represent the PS products for the influx and efflux across the apical membrane of the cultured cell monolayers, respectively. $PS_{3,vitro}$ and $PS_{4,vitro}$ represent the PS products for the influx and efflux across the basal membrane, respectively. $PS_{P\text{-}gp,vitro}$ represents the PS products for P-gp mediated efflux across the apical membrane. $PS_{1,vivo}$ and $PS_{2,vivo}$ represent the PS products for the influx and efflux across the luminal membrane of cerebral endothelial cells, respectively. $PS_{3,vivo}$ and $PS_{4,vivo}$ represent the PS products for the influx and efflux across the antiluminal membrane of cerebral endothelial cells, respectively. $PS_{P\text{-}gp,vivo}$ represents the PS products for P-gp mediated efflux across the luminal membrane. $PS_{A\text{-}B}$ and $PS_{B\text{-}A}$ represent the PS products across the monolayer in the apical-to-basal direction and the basal-to-apical, respectively. (From Ref. 120)