pravastatin. As hepatic clearance of pravastatin is rate-limited by uptake (Hsiang et al., 1999), low transport activity of OATP-C may lead to a reduction of hepatocellular uptake of pravastatin, resulting in lower total clearance. OATP-C polymorphisms may also influence the interindividual variability in the pharmacological effects of pravastatin that have the liver as their pharmacological target.

In addition, it has been reported that the genotypic frequencies of MDR1 and OATP-C are dependent on race (Table 14) (Ameyaw et al., 2001; Tirona et al., 2001). Polymorphisms in drug transporters may be involved in not only the interindividual variability but also the ethnic differences in drug disposition, like the polymorphisms of cytochrome P450. In any case, it is important to know whether each polymorphism has any clinical significance. In particular, since a change in the functional properties of transporters frequently does not alter the plasma concentration, unlike metabolizing enzymes, it is difficult to detect a change in these functional properties in vivo. To determine the in vivo function of transporters, positron emission tomography may be a useful tool and a correlation between genotype and phenotype will need to be established in the future. Studies of the polymorphisms in human drug transporters have been recently initiated and, in the future, the information obtained could be used for establishing the most appropriate drug treatment for individual patients.

TABLE 14
Summary of nonsynonymous polymorphisms in OATP-C (Tirona et al., 2001)

Exon	Region	Nucleic Acid Substitution	Amino Acid Substitution	Allelic Frequency				[^{3}H]Estrone Sulfate Transport Activity by OATP-C Variants		
				European-American (n = 49)	African-American (n = 44)	Japanese[a] (n = 267)	Japanese[b] (n = 120)	K_m (μM)	V_{max} (pmol/min/mg)	V_{max}/K_m (μl/min/mg)
			Wild type					0.54 ± 0.21	19.8 ± 3.4	41 ± 9.7
2	TM	T217C	Phe73Leu	0.02	0.00	NE	0.00	5.9 ± 1.5*	20 ± 2.8	4.3 ± 1.1*
3	TM	T245C	Val82Ala	0.02	0.00	NE	0.00			
4		A388G	Asn130Asp	0.30	0.74††	0.54	0.63	0.33 ± 0.12	18 ± 2.2	90 ± 25
4		A452G	Asn151Ser	0.00	0.00	NE	0.04			
4		C463A	Pro155Thr	0.16	0.02†	NE	0.00	0.72 ± 0.28	17 ± 3.7	30 ± 5.4
4		A467G	Glu156Gly	0.02	0.00	NE	0.00			
5	TM	T521C	Val174Ala	0.14	0.02†	0.11	0.16	0.34 ± 0.089	5.7 ± 0.75**	20 ± 3.2
8	TM	C1007G	Pro336Arg	0.00	0.00	NE	0.01			
8	TM	T1058C	Ile353Thr	0.02	0.00	NE	0.00	2.4 ± 0.9*	20 ± 5.5	9.4 ± 1.4*
9	Ex loop5	A1294G	Asn432Asp	0.01	0.00	NE	0.00	0.37 ± 0.18	12 ± 0.94**	51 ± 16
10	Ex loop5	A1385G	Asp462Gly	0.01	0.00	NE	0.00	0.14 ± 0.073	8.3 ± 1.7*	116 ± 38
10	Ex loop5	G1454T	Cys485Phe	0.00	0.00	NE	0.01			
10	Ex loop5	G1463C	Gly488Ala	0.00	0.09†	NE	0.00	4.3 ± 2.2	13 ± 4.9	5.9 ± 2.1*
14		A1964G	Asp655Gly	0.02	0.00	NE	0.00	2.7 ± 0.56*	19 ± 3.3	8.8 ± 2.2*
14		A2000G	Glu667Gly	0.02	0.34††	NE	0.00	0.63 ± 0.21	16 ± 3.2	33 ± 3.3

NE, not examined.
* $P < 0.05$; ** $P < 0.01$ relative to wild type (GenBank accession numbers AB026657, AJ132573).
† $P < 0.05$; †† $P < 0.01$ relative to European-American using Fisher's exact test.
[a] Adapted from Nozawa et al., 2002.
[b] Adapted from Nishizato et al., 2003.

IX. Methods for Assessing Drug Transporter Activities in Drug Discovery

The pharmaceutical industry is now at a turning point and strategies for drug discovery and development are changing rapidly. A significant number of drug candidates entering clinical development are dropped at some stage due to unacceptable pharmacokinetic properties. Thus, optimizing the pharmacokinetic properties during the early stages of drug development is now widely accepted as being essential (White, 2000; Roberts, 2001). Drug discovery based on the transport mechanisms and substrate specificities of drug transporters will become increasingly important. Identification of compounds that are substrates for transporters can aid the optimization and selection of new drug candidates. High-throughput assays for transporters are needed during the early stages of drug discovery and 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 using MDR1-transfectd cell monolayers (Adachi et al., 2001) (Fig. 8). 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 in vivo on the BBB (Fig. 9). By normalizing the brain-to-plasma concentration ratio ($K_{p,brain}$) in mdr1a/1b knock-

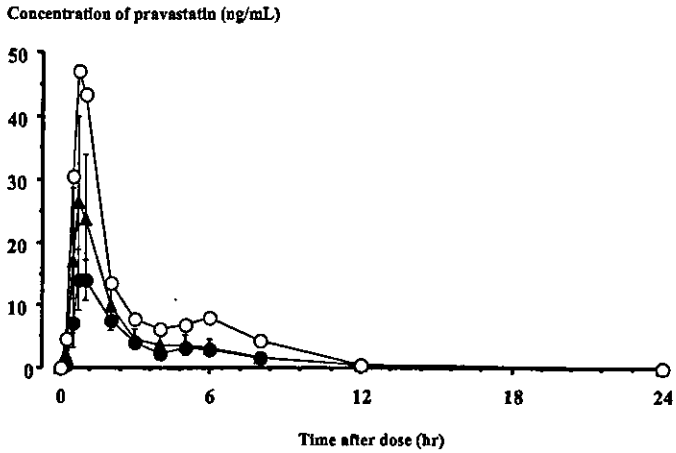


FIG. 7. Mean serum concentration over time after a single oral pravastatin dose of 10 mg in 3 OATP-C genotypic groups. ●, OATP-C*1b/*1b (n = 4); triangles, *1b/*15 subjects (n = 9); ○, *15/*15 subject (n = 1). The OATP-C*1b allele possesses mutations of Asn130Asp, and the OATP*15 allele possesses two SNPs, Asn130Asp and Val174Ala, simultaneously (Nishizato et al., 2003).

out mice with reference to that in normal mice, the P-gp function parameter can be simply estimated as shown in eq. 2 of Fig. 9. Furthermore, in vitro, this parameter corresponds to the "corrected flux ratio" across MDR1-transfected cell monolayers (Fig. 9). The corrected flux ratio represents the normalizing ratio of basal-to-apical (B→A) permeability versus apical-to-basal (A→B) permeability in parent cell monolayers with respect to that in MDR1-transfected cell monolayers (eq. 1 of Fig. 9). Indeed, a clear correlation between both parameters in vitro and in vivo has been obtained experimentally (Fig. 8). Another report also described a similar result (Yamazaki et al., 2001). Although one can calculate the net flux by subtracting the A→B flux from the B→A flux in MDR1-transfected cell monolayers, it is difficult to know to which in vivo parameters these correspond. In the case of CNS-active drugs, the concentration of "free" drug in the brain is very important for predicting pharmacological effects, not the concentration of "total" drug, since the term "total" drug includes the fraction bound nonspecifically to brain macromolecules such as proteins and lipids. We should also note that the value of the $K_{p,brain}$ ratio can be an index of the brain-to-plasma concentration ratio of "free" drug ($K_{p,brain,free}$), the most important parameter as far as the index of pharmacological activity is concerned. Supposing that the activities of passive diffusion and the function of transporters other than P-gp are not altered between mdr1a/1b knockout mice and wild-type mice, the values of PS_1, PS_2, PS_3, and PS_4 of eq. 3 in Fig. 9 are unaffected by P-gp function. Thus, as shown in eq. 3 in Fig. 9, the "$K_{p,brain,free}$" is inversely proportional to the $K_{p,brain}$ ratio. Moreover, this important parameter, which can reflect the pharmacological effect of drugs on the CNS, can be estimated by using a suitable expression system (see the equations in Fig. 9).

Recently, double-transfected cell monolayers have been established that express uptake transporters (OATP-C or OATP8) and MRP2 on the basolateral and apical membranes, respectively (Cui et al., 2001a; Sasaki et al., 2002). Most substrates of MRP2 are negatively charged under physiological conditions and thus cannot penetrate the plasma membrane without an uptake transporter. Therefore, it remains difficult to study MRP2 function in whole cells and MRP2 has been mostly studied using inside-out membrane vesicles prepared from MRP2-expressing cells. The use of double-transfected cell monolayers makes it possible to assess MRP2 activity more easily with intact cells. The basal-to-apical transport of pravastatin, which is a substrate of OATP-C and MRP2, was 2.5 times higher than that in the opposite direction in double-transfected cells (Fig. 10D), whereas a symmetrical flux of pravastatin was observed across the MRP2-expressing cell monolayer (Fig. 10 C) (Sasaki et al., 2002). Because of the easier handling of double-transfected cells grown on Transwell membrane inserts compared with the preparation and handling of membrane vesicles, it may be possible to develop throughput screening systems using double-transfected cells. These in vitro models, reproducing the polarity of transporters and the direction of transport, may be useful for predicting the in vivo hepatic vectorial transport of drugs from blood to bile. Moreover, the combination of an uptake and efflux transporter may be modified for certain purposes. For example, a combina-

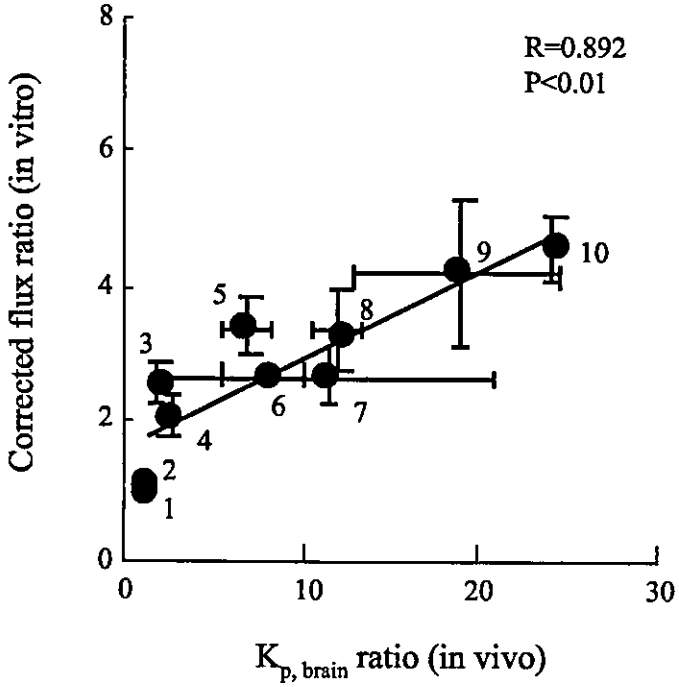
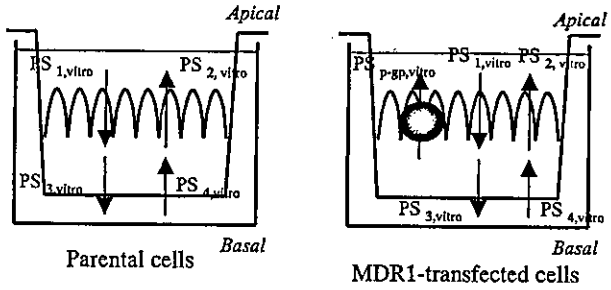


FIG. 8. Correlation of P-gp function determined in in vitro transcellular transport studies using MDR1 transfected cells/control cells and in vivo brain penetration studies using mdr1a/1b knockout mice/wild-type mice. 1, diazepam; 2, progesterone; 3, daunomycin; 4, dexamethasone; 5, loperamide; 6, verapamil; 7, vinblastine; 8, cyclosporin A; 9, digoxin; 10, quinidine (Adachi et al., 2001).

(A)In vitro (cultured cell monolayers)

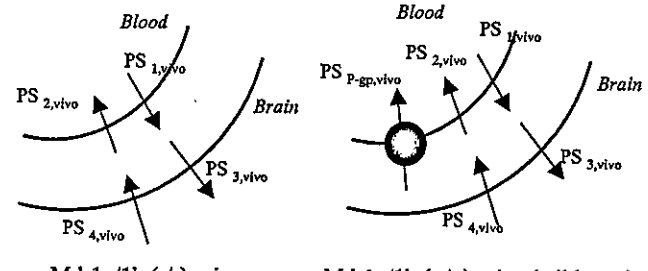


Corrected flux ratio

$$= \frac{PS_{B\rightarrow A\ (\text{parent cells})}}{PS_{A\rightarrow B\ (\text{parent cells})}} \Big/ \frac{PS_{B\rightarrow A\ (\text{MDR1 transfected cells})}}{PS_{A\rightarrow B\ (\text{MDR1 transfected cells})}}$$

$$= 1 + \frac{PS_{P\text{-}gp,vitro}}{PS_{2,vitro}} \quad \text{(Eq. 1)}$$

(B)In vivo (BBB)



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

$$K_{p,brain,\ free} = \frac{\text{Concentration of free drugs in brain of wild type mice}}{\text{Concentration of free drugs in plasma of wild type mice}}$$

$$= \frac{PS_1 \times PS_3}{PS_4 (PS_2 + PS_{P\text{-}gp})}$$

$$= \frac{1}{K_{p,brain}\ \text{ratio}} \times \frac{PS_1 \times PS_3}{PS_2 \times PS_4} \quad \text{(Eq. 3)}$$

FIG. 9. 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 non-P-gp-mediated efflux across the apical membrane of the cultured cell monolayers, respectively. $PS_{3,vitro}$ and $PS_{4,vitro}$ represent the PS products for the efflux and influx 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 non-P-gp-mediated efflux across the luminal membrane of cerebral endothelial cells, respectively. $PS_{3,vivo}$ and $PS_{4,vivo}$ represent the PS products for the efflux and influx across the abluminal 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 (Adachi et al., 2001).

tion of OCT1 with P-gp may serve to study the biliary excretion of organic cations. Although the identification of many transporters localized on the apical membrane in the kidney is awaited, a combination of transporters in human kidney tubule cells may be a suitable system for studying the urinary excretion of drugs. Sample analysis is a major limitation of the throughput in a monolayer transport assay. Liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS), due to its superior sensitivity, selectivity, and rapidity along with significantly reduced method development time, is an ideal analytical tool for high-throughput analysis of transport samples. In addition, the exceptional capability of LC/MS/MS for the simultaneous determination of multiple drug mixtures has allowed sample pooling (i.e., multiple samples to be pooled before analysis), which forms the throughput of the monolayer transport assay (Bu et al., 2000).

In addition, higher throughput assays to detect indirectly compounds interacting with P-gp have also been described. Such methods are based on inhibition of the efflux of radiolabeled or fluorescent P-gp substrates (Doppenschmitt et al., 1998; Wang et al., 2000; Eneroth et al., 2001). When radiolabeled ligands are used, the scintillation proximity assay is a useful tool for the sequential detection of radioactivity in the 96-well plate format (Fernandes, 1998). Also shown are assays measuring drug-stimulated ATPase activity in human P-gp-expressing cells (Polli et al., 2001), whereas the monolayer transport assay is regarded as the standard for identifying P-gp substrates because it measures efflux in the most direct manner. However, monolayer transport assays are relatively labor-intensive due to the cell culture and analytical requirements, which limit assay throughput. Indirect assays offer higher throughput, a generic readout (release of inorganic phosphate or increase in fluorescence or radioactivity), and are readily automated. However, these assays are not designed to distinguish P-gp substrates from inhibitors and do not directly measure transport. Polli et al. have compared assays used to determine whether compounds are P-gp substrates (Polli et al., 2001). Sixty-six compounds were tested in a transcellular transport assay using an MDR1-transfected cell monolayer and an inhibition assay for calcein-AM uptake. Although more than half of the compounds exhibited concordance across the assays, there were compounds that exhibited interassay differences that related to their apparent permeability (P_{app}).

TABLE 15
Efflux transport of estradiol-17β-glucuronide from the brain across the blood-brain barrier in rats (Sugiyama et al., 2001)

	Effects of Each Inhibitor (K_i) on the uptake of [^{3}H]$E_2$17βG into Gene-Transfected LLC-PK1 Cells				Maximum Inhibitory Effects of Each Inhibitor on the Efflux of [^{3}H]$E_2$17βG from the Brain in Rats[a]
	Oatp1	Oatp2	Oat1	Oat3	
[^{3}H]$E_2$17βG	K_m = 2.58	K_m = 17.0	Not Transported	K_m = 8.43	
Probenecid	74.4	72.9	31.0[b]	20.0	100%
TCA	10.8	39.4	2770[b]	790	100%
PAH	>5000	>5000	K_m = 85.1[b]	301	20%
Digoxin	>300	0.037	>330[b]	>330	40%

[a] Effects at the maximum inhibitor concentration on the efflux of [^{3}H]E_2-17βG from the brain after microinjection into the rat cerebrum.
[b] Effects on the uptake of [^{3}H]PAH into Oat1-transfected LLC-PK1 cells.

E_2-17βG from the brain were investigated. Probenecid and TCA inhibited the elimination of E_2-17βG via the BBB completely, whereas PAH and digoxin reduced the total efflux to about 80% and 60% of the control value, respectively. The selectivity of these inhibitors was confirmed by examining their inhibitory effects on the transport via each type of organic anion transporter gene-transfected cell. Digoxin specifically inhibited the transport via Oatp2, TCA inhibited Oatp1 and Oatp2, PAH inhibited Oat1 and Oat3, and probenecid inhibited all these transporters. Taking the selectivity of these inhibitors into consideration, the maximum contribution made by the Oatp2 and Oat family to the total efflux of E_2-17βG from the brain appears to be about 40 and 20%, respectively. A similar analysis has been applied to the renal uptake mechanism of PAH and pravastatin (Hasegawa et al., 2002). Furthermore, the contribution of rat Oat1 and Oat3 to the total renal uptake of anionic compounds and nucleoside derivatives has been examined (Hasegawa et al., 2003). The uptake of test compounds was investigated using kidney slices from male rats and rOat1- and rOat3-expressed LLC-PK1 cells. The uptake clearance of test compounds by kidney slices was compared with the value predicted from the transport activity by cDNA transfectants using PAH and pravastatin as reference compounds. The renal uptake of PAH and pravastatin was predominantly accounted for by rat Oat1 and Oat3, respectively (Hasegawa et al., 2002), and these drugs can be used as reference compounds for rat Oat1 and Oat3. The Oatp family is responsible for the hepatic uptake of pravastatin. Thus, pravastatin is taken up by the liver and kidney via different transporters. Furthermore, it is suggested that, using specific inhibitors, rat Oat3 is mainly responsible for the uptake of benzylpenicillin and PAH by the choroid plexus and the efficient removal of its substrates from the cerebrospinal fluid (Nagata et al., 2002).

Recently, drug-drug interactions involving drug transporters and genetic polymorphisms of drug transporters have been described. 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. Due to the rapid progress in the analysis of SNPs, it is likely that functionally relevant SNPs will be found for many transporters in the near future. Furthermore, the SNP mutations in the promoter/enhancer region of transporters and/or those in the DNA binding proteins (such as PXR) may be taken into consideration in accounting for interindividual differences in the expression level of transporters (Forman, 2001; Hustert et al., 2001; Zhang et al., 2001). Since the substrate specificity of drug transporters is generally broad and multispecific, if the strategy of targeting specific transporters is adopted then one should develop drug candidates that take into consideration the possibility of drug-drug interactions involving transporters. If the genetic polymorphisms and drug-drug interactions involving transporters mentioned above are likely, it will be crucial to predict quantitatively the degree of any changes in pharmacokinetics caused by these factors. Pharmaceutical companies need to identify which drug candidates are substrates for which transporters, and should investigate the contribution of each transporter to total transport by integrating the data from transporter gene-transfected cells. Providing this information to clinicians should lead to the safer use of drugs.

Current information regarding the molecular and cellular aspects of drug transporters has grown steadily and encouraged studies of the mechanisms of drug disposition. Clarification of the role of each transporter in drug disposition in vivo is of potential importance. The information on substrate selectivity and tissue distribution of the drug transporters will aid in the prediction of the in vivo kinetic profile of drugs from in vitro data. Research on drug transporters will lead to the more efficient development of new safer and more effective drugs.

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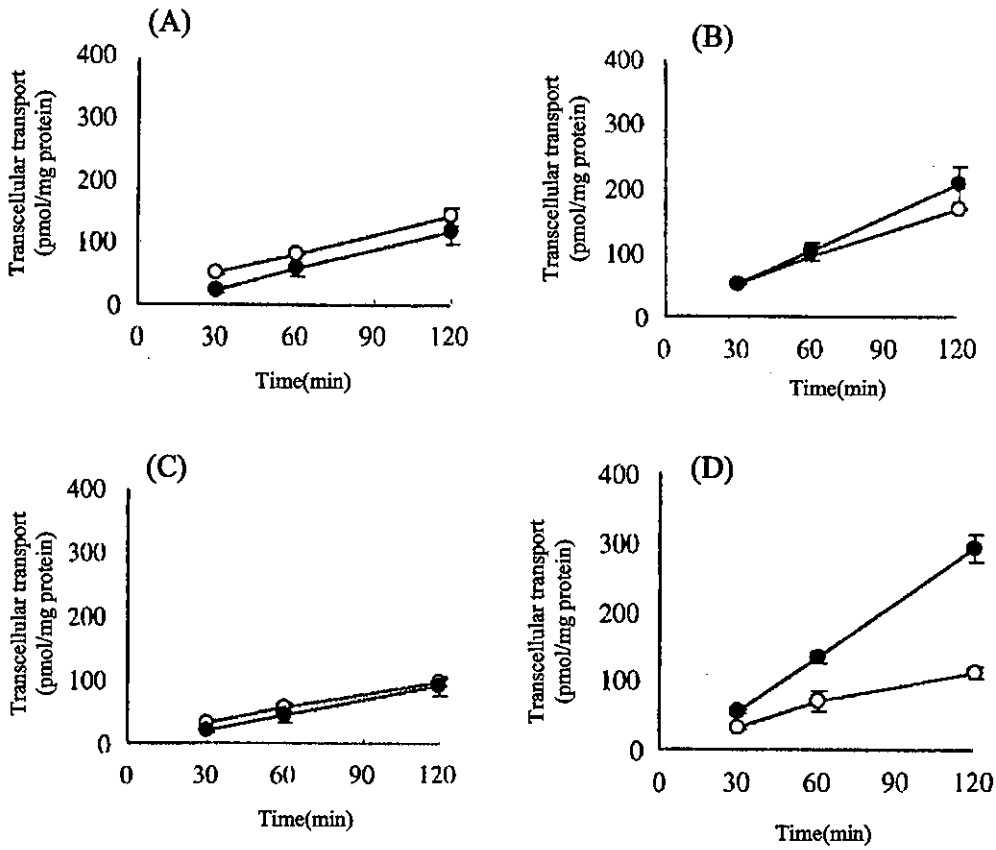

FIG. 10. Time profiles for the transcellular transport of [^{3}H]pravastatin across MDCK II monolayers. Transcellular transport of [^{3}H]pravastatin (1 μM) across MDCK II monolayers expressing OATP-C (B), MRP2 (C), and both OATP-C and MRP2 (double transfectant, D) was compared with that across the control MDCK II monolayer (A). Open and closed circles represent the transcellular transport in the apical-to-basal and basal-to-apical directions, respectively (Sasaki et al., 2002).

All assays detected substrates across a broad range of P_{app} values but the monolayer efflux assay was more prone to fail with high-P_{app} compounds, whereas the calcein-AM assay was more prone to fail with low-to-moderate P_{app} compounds. In the calcein-AM assay, tested compounds cannot inhibit calcein transport via P-gp unless they enter the cells and, thus, it may be difficult to observe P-gp activity with low-P_{app} compounds. As shown in eq. 1 in Fig. 8, P-gp activity is estimated as the ratio of P-gp clearance to passive permeability clearance in the monolayer efflux assay. Therefore, highly permeable compounds may be difficult to detect due to the masking of transport via P-gp. We need to choose suitable assays, depending on the properties of the drug candidates or the purpose of the evaluation. The monolayer efflux assay is more reliable at low-to-moderate P_{app} values and is the method of choice for evaluating drug candidates, despite the relatively low throughput and reliance on LC/MS/MS. In addition, computational (in silico) studies of transporter activity are being studied intensively. An attempt has been made to predict the transport activity of P-gp, MRP2, PEPT1, or ASBT from the structure or physicochemical parameters of compounds (Ekins et al., 2000; Seelig and Landwojtowicz, 2000; Han et al., 2001; Ekins et al., 2002a,b; Stouch and Gudmundsson, 2002). The in silico approach allows the design and optimization of the structures of drug candidates before their synthesis, resulting in an extremely efficient drug discovery process.

The prediction of pharmacokinetics in humans from an understanding of transport mechanisms should allow therapeutic agents to be used more safely. When there are species differences in transporters, the prediction of in vivo transport activity from in vitro data are important. Thus, methods allowing the rational prediction and extrapolation of in vivo drug disposition from in vitro data are also essential (Kusuhara and Sugiyama, 2001a). Since there are drugs that are recognized by several transporters localized on the same membrane, multiple transporters are expected to be involved in membrane transport of one drug. Therefore, the contribution of each transporter to the net membrane transport has to be taken into consideration when observations made in gene expression systems are extrapolated to in vivo situations. For example, Sugiyama et al. have estimated the contribution of each transporter to the efflux of E_2-17βG via the BBB using cDNA-transfected cells and specific inhibitors of each transporter (Table 15) (Sugiyama et al., 2001). Using the Brain Efflux Index method, the inhibitory effects of probenecid, taurocholate (TCA), PAH, and digoxin on the total efflux of

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特集

上皮組織ベクトル輸送の分子基盤と機能制御

序論

倉智嘉久・杉山雄一

1. 生命活動は上皮の賜物

特定の構造・機能をもった細胞どうしが，目的に応じて集合し，機能上・構造上の合目的性をもった1つの有機体が“組織”であり，さらにさまざまな性質の組織が組み合わさったものが“器官”である．“器官”の集合体が“生命体・個体”となるので，生命現象を考えるとき，その最小単位である個々の細胞・組織の機能を十分に理解することはきわめて重要である．組織は上皮・支持・筋・神経の4つの組織に大別される．本特集は上皮組織に関するものであるが，そもそも“上皮”組織という概念は，19世紀にチューリッヒ，ハイデルベルク，ゲッチンゲンの大学で教授として活躍した組織学者Jacob Henle(1809～1885)により確立されたもので，その系統的な組織学の開拓書“Allgemeine Anatomie”に“Epithel, epithelium(上皮)”の記載が見られる．ちなみに腎臓における「ヘンレの係蹄」の名は彼の名前に由来する．

上皮細胞は体のあらゆる腔所と体表に層をなして並んでいるが，われわれはまさにこの上皮細胞の恩恵を受けて生命活動を維持しているといっても過言ではない．たとえば，「食物や水を摂取し消化する」という過程を考えてみても，まず，嗅覚“上皮”細胞を介して臭いを認識し，味覚“上皮”細胞の働きで味わい，食物は“上皮”細胞を含む食道により嚥下される．胃の“上皮”細胞である壁細胞から分泌される酸により消化は助けられ，腸においてはもちろん種々の機能をもった腸管“上皮”細胞により，水分・栄養素が選択的に吸収され，必要に応じて各臓器部分で“上皮”を介して分配される．水については，われわれは1日約1～2 l を摂取し，尿，他の体液，不感蒸泄で約2 l が体外に排泄されているが，上皮細胞の集合体ともいうべき腎臓においては，1日約180 l もの体液が濾過されている．しかし，その99%は尿細管を経過している間に“上皮”細胞により再吸収されており，最終的に尿として排泄されるのは1日1～1.5 l にすぎない．われわれが病気をした際に飲む薬物も，多くは小腸“上皮”細胞により体内に取り込まれ，全身へと循環してゆき，肝臓“上皮”細胞に取り込まれたのちに代謝/胆汁中排泄を受け，また腎尿細管“上皮”細胞で尿中へと排泄される．まさに，われわれは上皮細胞・上皮組織と切っても切れない関係である．

2. 上皮細胞の多様性と機能

読者の皆様はもうお気づきかと思うが，上皮細胞には実にさまざまな種類が存在し，多彩な機能や選択性が，臓器別あるいは同じ臓器内でも機能区域に応じて備わっている．上皮細胞・上皮組織は複雑な臓器機能を維持するため，障壁として働き，化学組成の異なる液体を分けなければならないと同時に，選択的に必要な分子のみを移動させなければならない．たとえば腎尿細管では，上皮細胞が尿の通過する管腔部分と間質液が満たされた対側組織を分離しており，尿と間質液が混じらないようにしている一方で，水や特定のイオン・蛋白質などの必要な小分子の分泌・吸収を司っており，いわばフィルターのような役割を担っている．そのために，①上皮細胞は密着結合(tight junction)や接着結合(adherens junction)とよばれる特殊な構造体を用いて互いを接着し結合することでバリアをつくっている．また，上皮細胞は，②密着結合で細胞を頂上膜(apical membrane，管腔)側と基底外側膜(basolateral membrane)側に分けており，この場所の区分化に従って，イオンや水，小分子を選択的に運搬するチャネル，担体，能動輸送体が特定の場所に局在し，互いに協調することで機能制御されているという仕組みになっている．その結果，イオン，水，アミノ酸などの，“極性”をもった選択的な一方向性の輸送(“ベクトル輸送”)が成立している．さらに，このシステムは種々のホルモン・細胞内シグナルにより時間的・空間的制御を受け，ダイナミックに調節されている．体液環境の恒常性維持には，この上皮細胞の輸送システムが中心的役割を果たしており，ベクトル輸送は個体の生命保持のために不可欠な要素となっている(図1)．

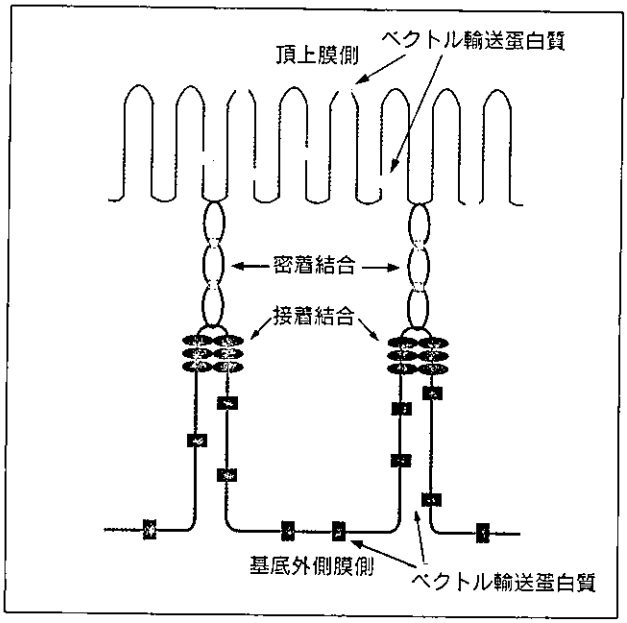

図1　上皮細胞の構成要素

3. 上皮細胞間接着構成分子・上皮ベクトル輸送分子の研究の現状

近年の分子生物学・生理学の進歩により，上皮細胞間接着やベクトル輸送を司る機能蛋白質が多く単離・同定されてきている．特定のイオンを選択的に通すイオンチャネル［→今月のKey Words（p.104）］については，1980年代より種々のクローニングの手法を用いてCa^{2+}，Na^{+}，K^{+}，Cl^{-}チャネルをはじめ，多くのチャネルの遺伝子やアミノ酸配列が明らかになった．ここ10年あまりの研究で，細胞間の密着結合はオクルディン，クローディン，ZOなどの膜貫通蛋白質，アンカー蛋白質で構成されている一方で，接着結合は細胞内蛋白質，カテニン，アクチニンなどにより接着分子カドヘリンが裏打ちされる構造となっていることが明らかにされた．また，薬物取り込み輸送体を含めた種々の担体やトランスポーターの同定により，薬物の作用点や循環動態が分子レベルで解明されるようになった．最近では，さらなる技術の進歩により，蛋白質-蛋白質結合を解析する酵母two-hybrid法やGST pull down法，免疫沈降法などが開発・改良され，ベクトル輸送蛋白質を特異的な場所へと局在させる分子機構が，一部は細胞内裏打ち蛋白質やアンカー蛋白質に結合することで成立することも明らかになってきた．また，ベクトル輸送蛋白質を直接GFPなどの蛍光蛋白質で標識し，タイムラプス機能をもつ顕微鏡で観察することにより，リアルタイムの分子の動きを追うことが可能となり，新たに合成された特定の蛋白質が，どのような道筋を通り，どこに向かって運ばれ，どのくらい決まった部分に維持されるかといった興味深い実験結果も報告されている．

一方で，ジーンターゲティングや遺伝子解析の技術進歩も，ここ10年で目覚ましいものがあり，次々とベクトル輸送蛋白質の新しい側面を見ることができるようになってきた．特定の分子を欠失したノックアウト動物の解析で，上皮ベクトル輸送分子，上皮細胞間接着構成分子が予想もつかなかった生理機能をもつことが報告されている．本特集では詳しく触れないが，たとえば，哺乳類の皮膚にはバリアとして機能的な密着結合は存在しないというのが通説であったが，クローディンのノックアウトマウスは皮膚からの水分蒸発過多により致死となることから，その通説は覆された．さらに，ベクトル輸送蛋白質の遺伝子異常が，長い間謎であったいくつかの遺伝子病の原因であることも見いだされている．後述する嚢胞線維症，Bartter症候群，Liddle症候群，Dubin-Johnson症候群［→今月のKey Words（p.104）］，進行性家族性肝内胆汁うっ滞は，いずれも上皮組織においてベクトル輸送を司るイオンチャネルやトランスポーターの異常によりひき起こされる遺伝性疾患である．このように，ベクトル輸送蛋白質が実体として捉えられ，その生理機能と疾患とのかかわりが議論されてきた，というのがここ数年間の現状である．

4. 今後の展望

上述してきたように，上皮細胞接着構成システム・上皮バリアシステム・ベクトル輸送システムは，各々の分子が同定され，それらが個々の機能を果たしつつ協調することが明らかとなってきた．演劇や映画に例えるならば，役者がそろい，台詞が入ったといったところであろうか．しかしながら，それを実際にまとまったパフォーマンスとして見る視点にはいまだ十分には至っていない．つまり，今後は新たな役者の発掘もむろん必要であるが，得られた結果や知識を統合し，器官機能を総合的に理解していくことが求められる．そのことが，より詳細な病態把握や，より効果的な治療法の開発につながっていくと考えられる．とくに薬物の開発においては，肝，腎，小腸，脳などの各組織のベクトル輸送のメカニズムをより明らかにし，それらを全体的に捉えながら理解し

ていくことで，異物からの生体防御の仕組みの解明のみならず，最適の動態特性をもち薬効の優れた副作用の少ない化合物の開発につながるものと期待される．

さらに，基礎研究を実際の臨床治療の場へと積極的に還元し，現実化していくためには，①適切な疾患モデルの確立と検討が重要な位置を占めてくる．そのためには，最近脚光を浴びつつある，組織特異的・時間特異的なノックアウト動物や，ノックイン動物，トランスジェニック動物が作製され，解析されなければならない．また同時に，②素過程から全体を再構成し定量的にベクトル輸送を理解する方法の確立が必要である．そのためには，コンピュータシミュレーションを用いて，ベクトル輸送蛋白質・上皮細胞間接着構成蛋白質の素子から，器官全体の機能を再構成し理解することが不可欠になってくる．

5. 本特集の概略とメッセージ

そこで本特集では，上皮細胞における細胞間接着構成や種々のベクトル輸送に焦点をあて，その各臓器における多様性をもたらすメカニズム，機能制御，分子基盤，さらにベクトル輸送蛋白質の局在化の分子機構について，最新の知見を紹介したい．高井らは，どのような分子やメカニズムにより細胞どうしが結合・接着し，上皮組織機能を成立させているかを，新しく同定した細胞間接着機構を中心に概説する．イオン・水や小分子の上皮組織における輸送のメカニズムや，疾患・薬物との関連については，倉智ら・杉山らが説明する．堅田らは，ベクトル輸送蛋白質の局在に重要である細胞内小胞輸送の分子機構について述べる．畑らは，ベクトル輸送の基盤である細胞極性の形成・維持における裏打ち蛋白質の機能について紹介する．各編とも，今後の展望についての興味深い記述を含んでいる．本特集を，個体生命維持の基本原理を考える糸口に少しでもしていただけたら幸いである．

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杉山雄一

略歴：1971年東大薬学部卒業，1973年同修士課程修了．1974年同博士課程在学中，製剤学の助手になる，1979-1981年米国UCLA医学部(肝臓生理学)に留学，1989年東大薬学部製剤学助教授，1991年より東京大学大学院薬学系研究科製剤設計学教授．研究テーマ：(1)*in vitro* から *in vivo* への薬物動態の構築，バーチャル薬物動態シミュレーターの開発，(2)肝臓，腎臓，脳における薬物動態の速度論的・分子生物学的解析．関心事・抱負：薬物速度論と生化学・分子生物学を統合することにより，薬物体内動態(とくに肝臓，腎臓，脳)の支配機構を明らかにする"molecular pharmacokinetics"の研究領域を構築中．世界をリードする研究領域にしたい．狂のつくジャイアンツファン．

今月のKey Words

ネクチン [nectin]

ネクチンは免疫グロブリンスーパーファミリーに属する接着分子で，細胞外ドメインに3つの免疫グロブリン様ループを有するI型膜糖蛋白質である．細胞内領域のC末端にはPDZドメインと結合するコンセンサス配列を有し，この部位を介してアクチンフィラメント結合蛋白質アファディンと結合することにより，アクチン細胞骨格に連結している．ネクチンは，ネクチン-1，-2，-3，-4の4種のメンバーからなるファミリーを構成しており，それぞれのネクチンのメンバーはホモフィリックまたはヘテロフィリックに結合する．ネクチン-アファディン系は，上皮細胞ではアドヘレンスジャンクションに局在し，カドヘリン-カテニン系と連結しながら，アドヘレンスジャンクションとタイトジャンクションの形成に重要な役割を果たしている．また，ネクチン-アファディン系は，神経シナプスの形成やセルトリ細胞-精子細胞間の接着にも関与している．さらに，ネクチン-1と-2はアルファヘルペスウイルスの受容体として機能することが知られている．

[→pp.105-112] (高井義美)

イオンチャネル [ion channel]

イオンチャネルは生体細胞膜を貫通する蛋白質であり，蛋白質内にイオンの通り道となる親水性の小孔(ポア)を形成している．細胞膜を介する無機イオンの透過性を制御し，細胞の電気的興奮を司る．イオンチャネルにはイオン選択性(selectivity)があり，特定のイオンしか通過できない構造(イオン選択性フィルター)を有している．各々のイオンは，細胞膜を介する電気化学ポテンシャル(細胞内外のイオン濃度差と膜電位によって決まる)に従って，チャネルを通過する．多くのイオンチャネルは，生体内で多量体をつくって機能している．たとえば，上皮型 Na^+ チャネルは4量体，Cl^- チャネルである ClC は2量体を構成している．さらに，ATP 感受性 K^+ チャネルにおけるスルホニル尿素受容体や，Ca^{2+} チャネルにおける β，$\alpha_2\delta$，γ サブユニット，アンカー蛋白質など，異なった種類の蛋白質と結合することによって，本来の機能的チャネルが形成されている場合も多い．また，イオンチャネルは，G蛋白質やセカンドメッセンジャーなど，細胞内シグナル系により活性制御を受ける．

[→pp.101-103，113-121]（倉智嘉久）

Dubin-Johnson 症候群 [Dubin-Johnson syndrome]

MRP2 の機能欠損に起因する劣性型の遺伝病で，グルクロン酸抱合ビリルビンの胆汁排泄低下により，致死性ではないものの慢性持続性黄疸を示す．通常，肝機能検査薬ブロモスルホフタレイン(BSP)投与後の血中消失プロファイルに再上昇があれば患者と判定される．正常人では BSP やビリルビンなどの異物，老廃物は血中→肝臓→胆汁という一方向性のベクトル輸送で消失するが，患者群では胆管側の排泄過程が低下することでベクトル輸送が障害される．BSP 血中濃度の再上昇はいったん肝細胞に取り込まれたのち，細胞内で生じた BSP グルタチオン抱合体が MRP2 欠損のため胆汁側へ排泄されず，血管側に発現誘導された MRP3 によって血中へ汲み出されるためと説明される．これまでに Dubin-Johnson 症候群で同定されているいくつかのアミノ酸置換においては，MRP2 蛋白質が胆管側膜へ正常に移行せず，細胞質で分解される，いわゆるソーティング障害が原因であることが示されている．

[→pp.101-103，122-132]（伊藤晃成）

Rab [Rab small GTPase]

Rab はエンドサイトーシス，エキソサイトーシス，トランスサイトーシスといった細胞内小胞輸送の制御で重要な役割を担う低分子量G蛋白質ファミリーである．現在までに40種類以上のメンバーが同定されている Rab には，極性細胞や非極性細胞にかかわらず発現し普遍的な機能を担う Rab と，極性細胞にのみ発現し特有の機能を担っている Rab とがあるが，それぞれの Rab は特定の細胞内小器官に局在し，供与膜からの出芽，標的膜への輸送，および融合過程を制御している．また，Rab は活性型の GTP 型と不活性型の GDP 型という2つのコンホメーションをとっており，GEF(GDP/GTP 交換因子)や GAP(GTPase 活性促進蛋白質)をはじめとしてさまざまな因子の働きを受容体刺激などの上流からのシグナルが支配することで，Rab の活性および局在が調節されている．

[→pp.133-139]（梶保博昭）

MAGUK [membrane-associated guanylate kinase]

MAGUK は字義のとおり細胞膜を裏打ちする分子のうち，酵母のグアニル酸キナーゼに類似した配列を共通してもつ分子群の総称である．赤血球の p55，上皮細胞のタイトジャンクションの ZO-1，神経シナプスの PSD-95 などが代表例．いずれもグアニル酸キナーゼ領域のほかに，PDZ 領域，SH3 領域をもつ．そのほかにカルモジュリンキナーゼ領域をもつ CASK や，SH3 領域の代わりに WW 領域をもつ S-SCAM，BAP1/MAGI-1 も MAGUK に属する．これらの領域はいずれも蛋白質相互作用を仲立ちするモジュールとして機能し，グアニル酸キナーゼ領域にも酵素活性がないことが確認されている．したがって，MAGUK の一義的な役割は，受容体・接着分子などの膜貫通蛋白質や，細胞骨格あるいはシグナル伝達にかかわる分子と相互作用して，特定の組合せからなる分子の集合体を形成することにあると理解されている．しかし，ZO-1 や CASK については，細胞核に移行して遺伝子転写制御にかかわる可能性も示唆されている．

[→pp.140-148]（畑 裕）

◆表紙解説◆

腸管上皮における K^+ チャネル(緑)と
βカテニン(赤)の分布

腸管上皮細胞では，βカテニンは細胞外側膜に分布し接着結合を構成しているが，一方で K^+ チャネルは頂上膜側に強く発現しており，腸管腔への K^+ 輸送にかかわっていると考えられる．このように，上皮細胞では各々の分子が機能的な局在を示している．（日比野 浩）

特集

上皮組織ベクトル輸送の分子基盤と機能制御

薬物トランスポーターの局在とベクトル輸送

伊藤晃成・鈴木洋史・堀江利治・杉山雄一

薬物輸送体の多くは極性細胞の頭頂(apical)側，基底膜(basolateral)側の一方に局在し，基質の方向性輸送(ベクトル輸送)に関与する．薬物輸送体は本来，生体に必要な物質の吸収，毒性物質の排泄解毒などに関与し，その機能を最大限発揮するべく配置されている．肝細胞は医薬品の解毒排泄を担う最も主要な臓器を形成する極性細胞であり，薬物体内動態を考慮するうえで重要であるとともに，いくつかの輸送体の局在化障害が遺伝病として表在化することでも知られる．本稿では，とくに肝臓における薬物輸送体の局在化機構に関して，一般的な局在化機構との比較を行ないながら紹介する．

Key words 極性細胞　ソーティング　内在化　一次性能動輸送担体

はじめに

薬物が標的部位に達し，薬効を発揮し体外へ排泄されるまでには，さまざまな細胞を透過する必要がある．たとえば経口投与後の抗精神薬の生体内運命を考えてみる．小腸上皮細胞を経由して門脈へと吸収された薬物の一部は，門脈から肝実質細胞に取り込まれ，胆汁排泄により消化管に排泄される．この消失を免れた薬物は，循環血を介して全身に廻る．腎臓は肝臓とならび主要な薬物消失臓器であり，薬物分子の一部は，腎上皮細胞を経由して尿細管管腔に分泌される．薬効部位である脳実質細胞に到達するには，さらに強固な関門を透過する必要がある．脳の血管内皮細胞は小腸，肝臓，腎臓に比べ細胞間の結合がタイトないわゆる関門(血液脳関門)を形成し，異物薬物の侵入を防いでいる．また本稿では触れないが，胎盤や精巣には，循環血からの薬物の侵入を制限するために，やはり血管内皮細胞が血液胎盤関門，血液精巣関門を形成している．このように，薬物の吸収・分布・排泄過程で極性細胞を介した透過性が問題となり，それは輸送体の種類，発現量はもちろん，血管側と管腔側のどちらの膜に発現しているかによって左右される．

これら薬物輸送体群は，1990年代後半になって遺伝子クローニングがほぼ終了し，薬物代謝酵素などと同様，基質特異性，臓器における発現量情報が，医薬品開発，とくに薬物動態にかかわる研究者のおもな興味対象であった．一方で，これら薬物輸送体のsingle nucleotide polymorphism(SNP)情報も蓄積しつつあり，基質特異性，発現量の個人差に加え，輸送体によっては膜移行効率の個人差も示唆されてきている．また，いくつかの輸送体は，生体機能維持に必要な内因性物質輸送にも関与し，その遺伝性機能障害は疾患に繋がることもしばしばである．その要因として輸送体分子中の1アミノ酸変異による膜への移行，あるいは局在が障害されるタイプのものも報告されている．

本稿では，医学・薬学の両観点から注目を集めつつあるこれら輸送体に関して，とくにその膜移行，局在化機構に関するこれまでの知見をまとめてみたい．

I. 薬物輸送体の臓器特異的な極性分布の意義

複数のトランスポーターが協奏的に作用して化合物の体内動態に影響を与える例としては，腸肝循環があげられる．生体内因性物質として胆汁酸を，薬物としてプラバスタチンを例として，その腸肝循環を図1に示す．胆汁酸は，肝臓で合成され胆汁中に排泄されるが，腸管で

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Hiroshi Suzuki, Yuichi Sugiyama, 東京大学大学院薬学系研究科
Polarized expression of drug transporters and its physiological significance

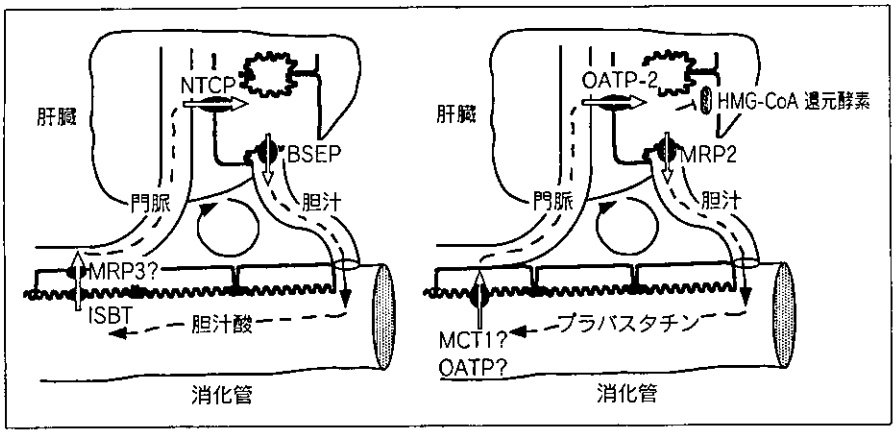


図1　胆汁酸とプラバスタチンの効率的な腸肝循環
胆汁酸はNTCP，BSEP，ISBTによって効率的な腸肝循環を受ける．経口投与されたプラバスタチンはOATP2，MRP2によって効率的な腸肝循環を受け，標的臓器である肝臓以外の臓器への移行は抑えられ，薬効が最大限に発揮される．MRP3は消化管上皮細胞血管側，モノカルボン酸輸送体(MCT1)，OATPファミリーは管腔側に発現がみられるものの，腸肝循環への寄与は不明である．

再吸収され再び肝臓に戻る．1日に6～10回循環するが，1回循環するごとに，わずか1～2%が糞中に失われるにすぎない，非常に効率のよい循環である．タウロコール酸などの一価胆汁酸に対しては，少なくとも図に示す4つの輸送体が腸肝循環に寄与する．それらは消化管管腔側(apical)に発現するNa^+依存性の胆汁酸取り込み輸送体ISBT(ileal Na^+-dependent bile salt transporter)と血管側(basolateral)に発現し門脈血側への排出に関与する未同定のトランスポーター，肝細胞の血管側(basolaeral)に発現し，Na^+依存性の取り込みに関与するNTCP(Na^+-dependent taurocholate transport protein)，肝細胞の胆管側(apical)に発現しATP加水分解によって胆汁中に排泄するBSEP(bile salt export protein)が関与する．なお一次性能動輸送担体MRP3(multidrug resistance-associated protein 3)は消化管血管側に発現し，胆汁酸を基質にすることが示されているが，胆汁酸の腸肝循環への寄与率は明らかとされていない．

略　号

BSEP：bile salt export protein
ISBT：ileal Na^+-dependent bile salt transporter
MDR：multidrug resistance protein
MRP：multidrug resistance-associated protein
NTCP：Na^+-dependent taurocholate transport protein
OAT：organic anion transporter
OATP：organic anion transporting polypeptide
OCT：organic cation transoporter

また高脂血症治療薬であるプラバスタチンは，肝細胞のコレステロール合成律速酵素であるHMG-CoA還元酵素を標的とする薬剤であるが，この薬物に対しても腸肝循環が観察される．本薬物は経口投与後，ヒト肝臓ではOATP2 (organic anion transporting polypeptide 2)により取り込まれ，MRP2により排出を受けることにより，効率よい腸肝循環を受ける(図1)．プラバスタチンは水溶性が高く，トランスポーターにより肝選択的に取り込まれることもあり，副作用臓器への薬物の分布が抑えられ，肝での薬効が最大限持続する．

これら有機化合物の取り込み，および排泄輸送体の臓器分布，細胞内局在パターンによって，基質となる薬の体内動態特性が左右される．逆に，これら輸送体に認識されやすい，あるいはされにくい化合物設計をすることにより，臓器特異的な薬のデリバリーも理論上可能と考えられる．

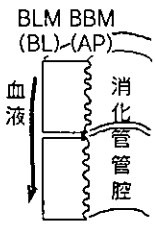
II. 各臓器における薬物輸送体の細胞内局在分類

薬物輸送体の分布を各臓器ごとにまとめたのが表1である．簡便のため以降はすべての臓器でapicalとbasolateralという表記に統一する．すなわち小腸上皮細胞，腎臓尿細管上皮細胞，血液脳脊髄液関門を形成する脈絡叢上皮細胞では，血液側がbasolateral，管腔側あるいは脳脊髄液側がapicalである．血液脳関門を形成する脳毛細血管内皮細胞では血液(luminal)側がapical，脳(antiluminal)側がbasolateralに相当する(表1参照)．

小腸上皮ではペプチド，糖類，リン酸など生体に必要な成分の取り込み輸送体がapicalに局在する．先述した胆汁酸の再吸収に関与するISBTもapicalに局在する．一方で，異物排出蛋白質である多くの一次性能動輸送担体もapical側に発現し，外界からの異物・薬物を管腔側に押し戻す役目を担い，吸収低下の原因となる．薬物吸収性を改善する試みのひとつとしてこれらの取り込み，排泄輸送体への親和性を考慮した薬剤形態が考慮

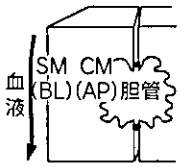
表 1　各臓器における薬物輸送体局在

小腸



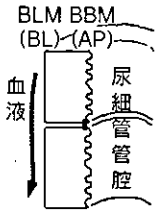
	ヒト 名称	ヒト 局在	ラット 名称	ラット 局在
ペプチド輸送体				
	PEPT1	BBM	PepT1	BBM
有機アニオン輸送体				
	OATP-B	ND	Oatp3	BBM
	OATP-D	ND		
	OATP-E	ND		
	ISBT	BBM	Isbt	BBM
有機カチオン輸送体				
			Oct1	BLM
			Oct3	ND
			Octn1	ND
一次性能動輸送担体				
	MDR1	BBM	Mdr1	BBM
	MRP2	BBM	Mrp2	BBM
	MRP3	BLM	Mrp3	BLM
	BCRP	BBM		

肝臓



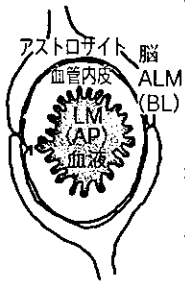
	ヒト 名称	ヒト 局在	ラット 名称	ラット 局在
有機アニオン輸送体				
	NTCP	SM	Ntcp	SM
	OATP-2	SM	Oatp1	SM
	OATP8	SM	Oatp2	SM
	OATP-B	SM	Oatp4	SM
	OAT2		Oat2	SM
			Oat3	SM
有機カチオン輸送体				
	OCT1	ND	Oct1	SM
			Oct1A	ND
	OCT3			
			Octn1	ND
一次性能動輸送担体				
	MRP1	SM		
	MRP3	SM	Mrp3 (EHBR, TR⁻)	SM
	MDR1/2	CM	Mdr1/2	CM
	MRP2	CM	Mrp2	CM
	BSEP	CM	Bsep	CM

腎臓



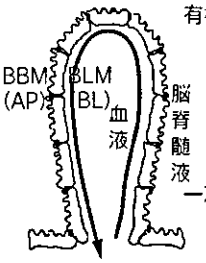
	ヒト 名称	ヒト 局在	ラット 名称	ラット 局在
ペプチド輸送体				
			PepT1	BBM
	PEPT2	ND	PepT2	BBM
有機アニオン輸送体				
	OAT1	BLM	Oat1	BLM
	OAT3	BLM	Oat3	BLM
	OAT4	BBM		
			Oatp1	BBM
			Oatp3	
			Oat-K1	BBM
			Oat-K2	ND
有機カチオン輸送体				
			Oct1	BLM
			Oct1A	ND
	OCT2	BLM	Oct2	BLM
			Oct3	ND
	OCTN1	ND	Octn1	BBM
	OCTN2	ND	Octn2	BBM
一次性能動輸送担体				
	MDR1	BBM	Mdr1	BBM
	MRP2	BBM	Mrp2	BBM

血液脳関門



	ヒト 名称	ヒト 局在	ラット 名称	ラット 局在
有機アニオン輸送体				
			Oatp2	ALM, LM
			Oatp3	ND
			Oat1	ND
			Oat3	ND
有機カチオン輸送体				
			Oct2	ND
一次性能動輸送担体				
	MDR1	LM	Mdr1	LM
			Mrp1	ND
			Mrp5	ND

血液脳脊髄液関門



	ヒト 名称	ヒト 局在	ラット 名称	ラット 局在
有機アニオン輸送体				
	OATP-A	ND	Oatp1	BBM
			Oatp2	BLM
			Oatp3	BBM
			Oat3	BBM
一次性能動輸送担体				
			Mrp1	BLM

ALM : abluminal membrane, AP : apical, BBM : brush border membrane, BCRP : breast cancer resistance protein, BLM : basolateral membrane, BL : basolateral, CM : canalicular membrane, ISBT : ileal Na^+-dependent bile salt transporter, LM : luminal membrane, MDR : multidrug resistance protein, MRP : multidrug resistance-associated protein, ND : not determined, NTCP : Na^+-dependent taurocholate transporter, OAT : organic anion transporter, OATP : organic anion transporting polypeptide, OCT : organic cation transporter, PEPT : peptide transporter, SM : sinusoidal membrane.

されつつある．

腎臓ではbasolateralに取り込み輸送体が，apicalに排泄輸送体が配備され，多くの薬物に対しては，血液(basolateral)→尿管腔(apical)という方向に沿った分泌が生じている．一方，生体に必要なペプチドや糖類，カルニチンなどに対しては，再吸収のための取り込み輸送体が局在している．

血液脳関門では，局在の確認できていない薬物輸送体が多いなか，MDR1(multidrug resistance protein 1)に関してはノックアウトマウスを用いた解析などから，luminal側に発現して基質を血液側にくみ出すことにより，高脂溶性の基質薬物の脳移行性を制限していることが示されている．同様に，血液脳脊髄液関門を形成する脈絡叢上皮細胞では，脳脊髄液側(apical)に取り込み輸送体が，血管側(basolateral)に排泄輸送体MRP1が発現し，脳脊髄液から血液への薬物排出を促進するとともに，血液から脳脊髄液への薬物移行性を制限している．

肝臓は腎臓同様に，薬物に対しては，血液(basolateral)→胆汁(apical)への流れを生じるよう輸送体の配置がなされている．薬物取り込み輸送体としてOAT(organic anion transporter)，OCT(organic cation transporter)，OATP(organic anion transporting polypeptide)ファミリーや，胆汁酸取り込み輸送体NTCPのすべてがbasolateralに局在する．取り込まれた薬物，胆汁酸を含む内因性化合物の多くは，apicalに局在する一次性能動輸送担体の基質となって一方向的に濃縮的に胆汁排泄を受ける．この一方向的な物質の流れが駆動力となり，胆汁流が生成される．生理的条件下では，MRP2やBSEPなどの排出輸送体はapicalに発現し，基質の胆汁排泄に関与するが，胆管閉塞や炎症性のストレス下では，それら輸送体と基質特異性の類似するサブファミリーであるMRP1やMRP3がbasolateralに一時的に発現する．これは，胆汁中に排泄されなくなった基質を血液側に排出することにより，肝細胞を防御するためと考えられる．

III. 肝臓の形態・極性とベクトル輸送再現系

肝臓は小腸，腎臓，脈絡叢などの上皮細胞，あるいは血液脳関門を形成する血管内皮細胞と形態学的に異なっている．これら上皮細胞あるいは内皮細胞は，*in vitro*培養条件下で*in vivo*同様のモノレイヤーを形成し，プレートとの接着面がbasolateral(厳密にはプレートとの接着面はbasal，細胞どうしの接着側面はlateralとよばれるが，膜蛋白質のソーティングを考えるうえではbasolateralと統一して議論する)，培地に面する上面がapicalに相当する(図2a)．トランスウェルと称される容器で培養することにより，経細胞輸送を容易に測定することが可能となり，ヒト結腸由来極性細胞Caco-2，MDR1発現腎上皮細胞LLC-PK1などを用いた医薬品の上皮細胞透過性の評価が可能となっている．しかしながら，同様に薬物の胆汁排泄評価を*in vitro*で評価しようとする場合，以下に示す形態的な問題から輸送透過実験は困難である．すなわち，*in vitro*で初代培養肝細胞あるいは肝癌由来の細胞を培養すると図2bに示すように，隣り合った細胞間に微小胆管とよばれる閉じたapicalスペースが形成される．イヌ遠位尿細管上皮細胞由来MDCK細胞のような極性配置を示さないため，トランスウェルを用いた透過性の評価は不可能である．この問

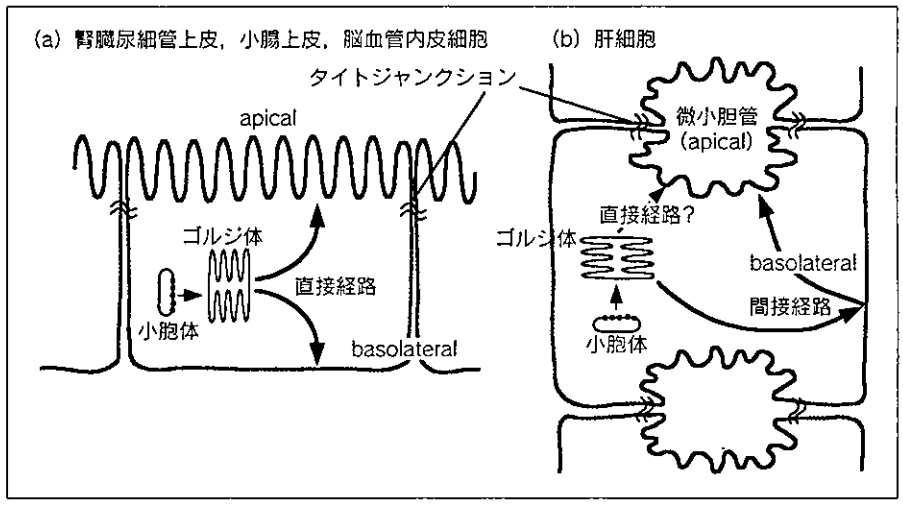


図2 極性細胞の形態と膜蛋白質のソーティング経路
小胞体で合成された膜蛋白質はゴルジ体へ移行し，そこでapicalとbasolateralへ移行する小胞に振り分けられる(直接経路)．肝細胞ではすべての膜蛋白質はいったんbasolateralへ移行し，その後basolateralに留まるものとapicalへ移行する経路(間接経路)のみが存在すると考えられてきたが，apical側の一次性能動輸送担体BSEPやMDR1では直接経路でapical側に移行することが示されている(本文参照)．