Relationship Between Drugs and Functional Activity of Various Mammalian P-Glycoproteins (ABCB1)

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Abstract: P-glycoprotein (Pgp, ABCB1) is an efflux transporter for a variety of amphipathic agents that can affect the pharmacokinetics of drugs. In order to extrapolate transport and pharmacokinetic data of the drug candidates obtained from *in vitro* and animal models to those in humans, it is important to understand the functional differences of Pgps from various mammalian species including human, monkey, dog, rat, and mouse. Here, we review differences/similarities in the properties of Pgp from numerous mammalian species commonly used in preclinical studies and discuss their relevance to the pharmacokinetics of potential drug molecules.

Key Words: ABC transporter, ATP hydrolysis, chemoprevention, multidrug resistance, pharmacokinetics, species differences, structure-activity relationship.

INTRODUCTION

P-glycoprotein (Pgp, ABCB1)[1], which belongs to the ATP-binding cassette (ABC) transporter superfamily, is a mammalian plasma membrane phospho-glycoprotein encoded by the multidrug resistance 1 (*MDR*1) gene. Pgp plays a role in the resistance of tumors to cytotoxic drugs and is known to efflux structurally unrelated diverse amphipathic compounds from cells [1-3]. Pgp is expressed both in multidrug-resistant cancer cells and also in a number of normal tissues, such as those of the liver, kidney, small intestine, colon, and brain, suggesting that the physiological role of Pgp is a protective mechanism against xenobiotics and endogenous metabolites [4-7]. After exposure to a single cytotoxic drug such as one of the Vinca alkaloids, anthracyclines, taxoids, or actinomycin D, cells can over-express Pgp and exhibit the MDR phenotype. Pgp over-expression is induced not only by chemical compounds, but also by physical stress caused by X-rays [8] and ultraviolet light irradiation [9], or heat shock [10].

Approximately 50% of currently marketed drugs have been identified to be Pgp substrates and/or inhibitors [11]. Pgp can influence the pharmacokinetics of drugs by contributing to the processes that govern absorption, distribution, metabolism, elimination, and/or toxicity (ADMET). Additionally, Pgp has been implicated in drug-drug interactions involving co-administered Pgp substrates and modulators. For instance, intestinal Pgp mediates substantial direct transepithelial excretion of drugs including paclitaxel [12, 13], while liver Pgp mediates considerable hepatobiliary excretion of other drugs [14, 15]. The oral bioavailability of paclitaxel is improved by the co-administration of Pgp inhibitors such as PSC833 and cyclosporine A [16-18]. Therefore, pharmaceutical industries have begun to screen drug–Pgp interactions when evaluating profiles of new chemical entities [11].

The availability of transgenic *mdr* knock-out mice or mice genetically *mdr* deficient and numerous *in vitro* systems have provided a means to evaluate the role of Pgp in drug ADMET. When applying *in vitro* and *in vivo* screening models to study Pgp function, it is important to understand the differences between the functional activity of this human transporter and that of other species. In the pharmaceutical industry, preclinical animal models are utilized for determining the relevant ADMET properties and safety assessment of investigational molecules. The most commonly used preclinical species include monkeys, dogs, and rodents. There are a large number of studies dealing with the structure-activity relationships (SAR) between drugs and Pgps. Recently, several studies regarding specific differences of Pgps were reported. However, they do not consider the correlation between SAR and species differences of Pgps, which is an important factor *in vitro* screening system. In this mini-review, therefore, we evaluate data from the current literature regarding the differences and similarities in Pgps of various mammalian species and examine SAR between drugs and Pgps of those species.

COMPARISON OF GENE AND AMINO ACID SEQUENCE OF PGPS

Pgps are encoded by the multigene MDR gene family. The number of Pgps varies between species. Humans, monkeys and rabbits have two genes, *MDR*1 (*ABCB*1) and *MDR*2 (*ABCB*4), while rodents and cows have three, *Abcb*1a (mdr3), *Abcb*1b and *Abcb*2 [19-21], and dogs and pigs are predicted to have four and five, respectively [22]. The reason why some animals have more Pgp genes is not known.

ABC transporter subfamilies have been classified according to the guidelines of the HUGO gene Nomenclature Committee (www.gene.ucl.ac.uk/nomenclature/genefamily/

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Table 1. Multidrug Resistance Gene Family of Selected Mammalian Species

Species	Scientific Name	Gene[a]	Gene ID	Number of Amino Acids	Sequence Identity (%)[b]
Human	*Homo sapiens*	*ABCB*1	5243	1280	100
Cynomolgus Monkey	*Macaca fascicularis*	*ABCB*1	AF537134[c]	1283	96.3
Rhesus Monkey	*Macaca mulatta*	*ABCB*1	574235	1283	96.2
Dog	*Canis familiaris*	*Abcb*1	403879	1281	90.9
Rat	*Rattus norvegicus*	*Abcb*1a *Abcb*1b	170913 24646	1272 1275	86.6 80.4
Mouse	*Mus musculus*	*Abcb*1a *Abcb*1b	18671 18669	1276 1276	87.0 80.6

[a]Only genes involved in development of MDR are included in this analysis.

[b]Compared to human *ABCB*1.

[c]The accession number for mRNA nucleotide.

abc.html). The gene and the amino acid sequences of Pgps from five species are summarized in Table **1** and the phylogenetic tree of *MDR*1 (ABCB1) genes from these species is shown in Fig. **1**. At the gene and amino acid levels, monkey Pgp has the highest level of sequence identity with human Pgp. The Abcb genes can be divided into three classes, *ABCB*1 (*Abcb*1), *ABCB*4 (*Abcb*4), and *ABCB*11 (*Abcb*11). The *ABCB*1 codes for a MDR phenotype, *ABCB*4 functions primarily as a lipid translocase involved in phosphatidylcholine transport [23-26], and *ABCB*11 functions primarily as a bile salt transporter [27, 28]. However, it has been reported that human *ABCB*4 can contribute to the development of MDR under certain conditions [29]. Although no significant research supports the involvement of *Abcb*11 in the MDR phenotype, one study reported that cells transfected with *Abcb*11 demonstrated low level resistance to paclitaxel [30] and a separate study showed *Abcb*11 mediated the transport of vinblastine *in vitro* [31].

COMPARISON OF TISSUE DISTRIBUTION OF PGPS

Human Pgp, 1280 amino acids, is normally expressed on the apical (or luminal) surface of epithelial cells in kidney proximal tubules, liver bile canaliculi, in the large and small intestine, the brain-blood barrier, the cortex, the hippocampus, the cerebellum, the spinal cord, the testes, the breast ductal epithelium, and the endometrium [32-36]. The mouse Pgps (*Abcb*1a and *Abcb*1b) are both 1276 amino acids in length but have different apparent molecular weights (160-kDa and 180-kDa Pgp for *Abcb*1a and *Abcb*1b, respectively), due to non-equivalent glycosylation. Despite their high level of sequence identity (84%), the different pattern of tissue expression suggests that the two rodent isoforms may perform distinct functions [37]. Mouse *Abcb*1a is expressed in the intestine, liver and testis at a high level and also in the lung and brain (BBB) [4]. However, *Abcb*1b is expressed in tissues associated with steroid biosynthesis and distribution, such as the adrenal gland and ovary and is found at higher levels in the uterus during pregnancy [38]. The heart, lung, thymus, and spleen also contain significant and similar levels of both *Abcb*1a and *Abcb*1b [37].

Rat MDR gene expression is similar to that of the mouse. Rat *Abcb*1a is expressed in the intestine, at the blood-brain and at the blood-testes barrier, whereas *Abcb*1b is highly expressed in the adrenal grand, pregnant uterus, and ovaries

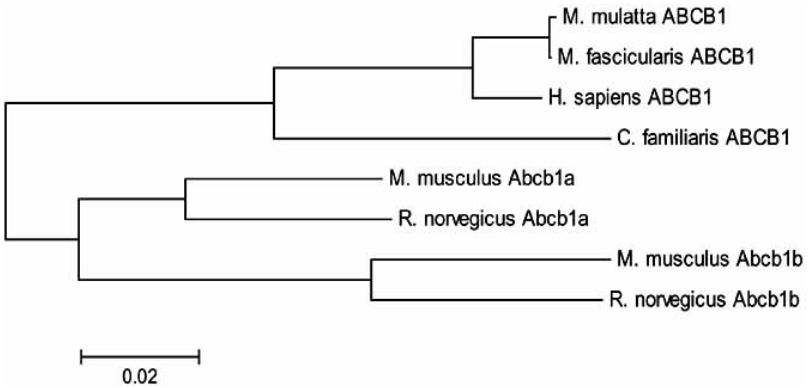

Fig. (1). Phylogenetic analysis of the *MDR*1 (ABCB1) gene from various species. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 3.1 [100]. *M. musculus, Mus musculis; R. norvegicus, Rattus norvegicus; C. familiaris, Canis familiaris; M. fascricularis, Macaca fascricularis; M. mulatta, Macaca mulatta.* Scale bar indicates an evolutionary distance of 0.02 nucleotide substitution per position in the sequence.

[39, 40]. However, the one difference between rats and humans is in the adrenal medulla, where Pgp expression is observed in humans but not rats [32]. In this organ, Pgp activity may involve glucocorticoid transport [41]. Beagle dog *Abcb*1 is highly expressed in the brain, kidney, testis and liver, and expressed at lower levels in the jejunum, colon and lung. It is expressed at very low levels in the ileum and duodenum [42]. *Abcb*1 mRNA levels and protein is comparable in the liver, kidney, duodenum and lung. However, mRNA expression in the brain is lower than expected, whereas mRNA expression in the jejunum and colon is higher than expected, based on protein determined by Western blots. Pgp expression patterns are quite similar in dogs and humans except in the liver and brain, where dog tissues have relatively higher expression than those of humans. The relative amount and overall bio-distribution of Pgp in monkeys is not known. However, one study showed that the liver and kidney of the rhesus monkey are known to contain Pgp [43].

ANTIBODIES AGAINST PGPS FROM VARIOUS SPECIES

Several monoclonal and polyclonal antibodies have been used for monitoring expression levels of Pgp from various sources, which are summarized in Table **2**. C219 was one of the first widely available anti-Pgp monoclonal antibodies [44] and can react with dog, monkey and human as well as mouse and rat Pgps. C219 recognizes two epitopes, 568-VQVALD-573 and 1213-VQEALD-1218 and binds more strongly to the peptide sequence VQAALD in the N-terminal half of mouse and rat Pgps than the sequence VQVALD in the N-terminal half of the human Pgp [45]. This indicates that the difference of a single amino acid in the epitope can change its affinity to an antibody. C494, another antibody raised in the same screen that produced C219, recognizes the sequence 1028-PNTLEGN-1034 in the Pgp molecule. It is reported that dog Pgp was detected by C494 [45], which has the same epitope. Therefore, monkey Pgp, which also has this sequence, should also be detected by C494, because it has the same epitope. However, there is no experimental evidence in the literature demonstrating this. On the other hand, the monoclonal antibody Ab-1 (265/F4) can distinguish between the murine isoforms, as it is reactive with Abcb1b, but not Abcb1a [46]. The monoclonal antibody JSB-1 can detect monkey and human Pgps but not rodent Pgps. Although the JSB-1 antibody has been shown to recognize the intracellular region of Pgp, the exact location of its epitope is unclear [47]. Bruggemann *et al*. developed

Table 2. Summary of Selected Antibodies for Detection by Immonoblotting of P-Glycoproteins from Human, Monkey, Dog, Rat and Mouse

Antibody	Source	Epitope	Human	Monkey	Dog	Rat	Mouse	References
C219	Mouse monoclonal	568-574, 1213-1219	+[a]	+	+	+++	+++	[44, 55, 92]
C494	Mouse monoclonal	1028- 1034	+	N.D.	+	N.D.	--	[42, 45]
Ab-1 (265/F4)	hamster monoclonal	N.D.	-	N.D.	N.D.	N.D.	±	[46, 93]
Ab-2 (Clone F4)	Mouse monoclonal	extracellular	+	+	+	+	--	[94, 95]
JSB-1	Hamster monoclonal	intracellular	+	+	N.D.	N.D.	--	[46, 47, 96]
C19 (sc1617)	Goat Polyclonal	C-terminal	+	N.D.	N.D.	+	+	[97, 98]
Anti-P7	Rabbit polyclonal	28-35	+	N.D.	N.D.	N.D.	--	[51, 52]
PEPG13	Rabbit polyclonal	592-636	+++	N.D.	N.D.	--	--	[48, 49]
4007	Rabbit polyclonal	919-1280	+++	N.D.	+++	N.D.	+++	[52]
4077	Rabbit polyclonal	140-228	+++	N.D.	+	N.D.	+	[52]
H-241 (sc-8313)	Rabbit polyclonal	1040-1280	+	+	+	+	+	[64, 99]

[a] +++, highly detected (100% signal); +, weakly detected (<15% signal); --, not detected; N.D., Not determined; ±, only *Abcb*1b was detected.

polyclonal antisera against specific regions of Pgp [48]. Antisera PEPG13 does not cross-react with human MDR2 (ABCB4) or mouse Pgps [49, 50]. Antiserum 4007 and 4077 recognizes mouse, hamster, dog and human Pgps with approximately equal efficiency. However, the anti-P7 antiserum detects human Pgp with low efficiency and does not detect mouse Pgp [51, 52]. Differences in the amino acid sequences in the epitopes can affect the affinity of an antibody. In order to compare the expression level of Pgps from different species, it would be necessary to develop an antibody with an epitope that is identical across different species.

SPECIES-SPECIFIC DIFFERENCES IN THE FUNCTION OF PGP

ATP binding and hydrolysis are essential for the function of Pgp as a drug transporter. The correct sentence is: ATP binding and hydrolysis are essential for the function of Pgp as a drug transporter. Several groups have demonstrated that Pgp substrates and modulators stimulate ATPase activity in mammalian cells expressing Pgp [1, 53]. Pgp ATPase assays have been utilized to identify the Pgp ligands in a high throughput mode; however, these assays can give false negative results since not all ligands stimulate ATPase activity of Pgp [54]. Limited evaluation of key biochemical characteristics of Pgps among different species has been performed. Xia *et al*. [55] evaluated kinetics of the ATPase activity and its activation profiles with 21 structurally diverse compounds in membranes of insect cells infected with human, rhesus monkey, or beagle dog *MDR*1 (*Mdr*1) baculovirus (Table **3**). These data showed differences in binding affinities and changes in activation levels among these species with several of the compounds tested. For example, erythromycin stimulated Pgp-mediated ATPase activity in only vesicles with human Pgp (6.3-fold) and chloroquine stimulated Pgp ATPase activity of only rhesus monkey Pgp (5.7-fold). Several compounds (cyclosporine A, etoposide, amiodarone, tamoxifen, and thioridazine) stimulated ATPase activity of both human and rhesus monkey Pgp but not that of dog Pgp. Dexamethasone was the only compound shown to stimulate Pgp-mediated ATPase activity in human Pgp (11.4-fold) and dog Pgp vesicles (2.4-fold), but not rhesus monkey Pgp vesicles. Interestingly from the compounds tested, no compound was identified as a 'dog Pgp only' stimulant. These data suggest that the ATPase activation profile depends on the species, as well as the structure of the compound. Thus, monkey Pgp appears to be closer to human Pgp in ATPase activity compared to dog Pgp, which is consistent with the level of similarity among the protein identities (Table **1** and Fig. **1**). This result is also consistent with the fact that the rate and extent of drug absorption are similar in monkeys and humans, but the hepatic enzyme activity of monkeys is quite different from that of humans [56].

The generation of *Mdr*1a/1b knockout mice and the discovery of mice genetically deficient in *Mdr*1a have provided tremendous insight into the role Pgp plays in the various ADMET processes. Schinkel *et al.* [6] reported that *Mdr*1a/1b knockout mice have highly increased levels of drugs such as ivermectin, vinblastine, digoxin and cyclosporine A in the brain. The important role of Pgp in bio-distribution and elimination has also been observed in a subpopulation of Collie dogs that have a 4-bp deletion of the *Mdr*1 gene resulting in synthesis of nonfunctional Pgp [57, 58]. In dogs with this phenotype, toxicity after administration of various drugs including ivermectin, vincristine, vinblastine, and doxorubicin was observed presumably due to impaired Pgp-mediated absorption and clearance [59].

The influence of Pgp under normal physiological conditions has also been observed. Walker *et al*. [60] reported that Pgp was involved in the nonlinear systemic exposure of UK-427,857 in humans presumably by saturation of Pgp-mediated oral absorption at increased doses. Interestingly, the oral dose-exposure relationship observed in humans was not observed in either rat or dog. This aspect needs to be explored further. Other *in vivo* models have also explored the involvement of Pgp using chemical inhibitors. Cutler *et al*. [61] explored the possibility of generating "chemical" knockouts in mice, rats, and guinea pigs using the inhibitor GF120918. These workers were able to use this approach to investigate the effects of Pgp modulation on the brain penetration of SB-487946 and also noted a difference in the optimal blood concentrations of GF120918 required to achieve "chemical" knockout of guinea pigs versus mice or rats.

In vitro systems expressing Pgps of different species have been established to resolve the problems with species differences of Pgp properties and to study differences in transport and metabolism in *in vivo* systems. Yamazaki *et al*. [62] demonstrated that the Pgp substrate susceptibility is different between human and mouse for certain compounds using *in vitro* studies with LLC-PK1 pig kidney epithelial cells transfected with human *Abcb*1 and mouse *Abcb*1a. In addition, Booth-Genthe *et al*. [63] compared LLC-PK1 cells transfected with human *ABCB*1, mouse *Abcb*1a and rat *Abcb*1a by cellular accumulation and trans-cellular transport assays. They found that ~20% of the 179 compounds evaluated were predicted to be substrates in one species but not in other species. Recently, the Katoh and Takeuchi groups [64, 65] developed LLC-PK1 cell lines expressing Pgps from seven mammalian species and compared the transport properties of human, monkey, canine, rat (*Abcb*1a and *Abcb*1b), and mouse (*Abcb*1a and *Abcb*1b) Pgps using the transwell transport assay. These results indicated that there was a good correlation in the efflux ratio between human and monkey Pgp, which is consistent with the level of similarity among their protein identities (Table **1** and Fig. **1**), but a poor correlation in the efflux rate of human Pgp and mouse Pgp, and human Pgp1 and canine Pgp. These studies also demonstrated that the differences in Pgp activity between species depend on the nature of the transport-substrate tested. The efflux ratios of compounds tested in these cell lines are summarized in Table **3**.

STRUCTURE-ACTIVITY RELATIONSHIPS BETWEEN COMPOUNDS AND VARIOUS PGPS

A number of studies establishing Structure-Activity Relationships (SAR) with Pgp have been published. Zamora *et al*. [66] concluded that hydrophobicity, cationic charge, and molar refractory were important properties for modulators of MDR. Pearce *et al*. [67] reported that the relative disposition of aromatic rings and basic nitrogen atom was important for modulators of Pgp-associated MDR using eight analogs of

Table 3. Relationship of Drugs with Pgps Obtained from Different Mammalian Species

Drug or Compound	ATPase Activity[a]	Corrected Efflux Ratio[b]						
		Human *ABCB*1	Monkey *ABCB*1	Dog *Abcb*1	Rat *Abcb*1a	Rat *Abcb*1b	Mouse *Abcb*1a	Mouse *Abcb*1b
Ritonavir	H, D, P	5.4	1.6	4.4	8.0	6.3	3.1	3.0
Saquinavir	H, D, P	2.8	1.5	4.5	2.5	2.7	1.7	2.4
Vinblastine	H, D, P	3.8	4.1	8.9	5.6	4.8	10.2	4.2
Quercetin	H, D, P	N.D.[c]						
Verapamil	H, D, P	6.4	8.4	8.4	4.8	7.2	3.2	3.4
Digoxin	H, D, P	10.2	7.4	18.2	13.9	12.9	14.5	15.4
Progesterone	H, D, P	N.D.						
Rhodamine 123	H, D, P	N.D.						
Propanolol	H, D, P	0.8	1.3	1.2	1.1	1.1	1.1	1.2
Cyclosporine A	H, P	7.0	9.2	1.7	7.5	3.8	4.6	5.9
Etoposide	H, P	4.1	3.3	2.0	1.6	4.0	3.0	4.0
Amiodarone	H, P	N.D.						
Tamoxifen	H, P	N.D.						
Thioridozine	H, P	N.D.						
Physostigmine	H	N.D.						
Paclitaxel	H	5.2	4.6	3.9	2.5	2.9	3.1	3.1
Erythromycin	H	1.6	1.2	1.0	1.7	2.0	1.8	2.8
Chloroquine	P	N.D.						
Daunomycin	--	7.7	4.4	6.3	3.5	4.1	5.2	4.7
Hoechst33342	--	N.D.						
Miconazole	N.D.	N.D.						
Terfenadine	N.D.	N.D.						
Diltiazem	N.D.	2.5	3.0	2.4	2.8	1.9	2.5	1.6
Dexamethasone	H, P	5.0	6.7	6.7	6.1	11.2	5.4	7.1

[a]Adapted from [55]. The drug-stimulated ATPase activity of human (H), monkey (P), and dog (D) Pgps; --, did not stimulate the ATPase activity of Pgp from any species.

[b]Drug efflux data compiled from [64, 65].

[c]N.D. Not determined.

reserpine. Bain and LeBlanc [68] identified the interaction between accumulation of substrate of Pgp and molecular weight, lipophilicity and hydrogen bond potential of pesticides. Litman *et al*. [54] studied how the surface area of some drugs correlated with Pgp-mediated ATPase activity. Many groups [69-72] have compared the correlation between the lipophilicity of drug molecules (octanol/water partition coefficient) and Pgp activity, such as ATPase activity or inhibition of efflux.

However, the mechanism by which a wide variety of structurally unrelated drugs are able to interact with Pgp is unknown. The consensus of these studies is that Pgp substrates are amphipathic with a molecular mass of 300-1,500 Da. Using a combination of experimental and theoretical approaches, Seelig *et al*. [73] have endeavored to establish the physico-chemical determinants of Pgp substrates and modulators. They have distinguished the presence of specific recognition patterns consisting of hydrogen bond acceptor (or electron donor) groups (e.g., carbonyl, ether, hydroxyl, or halide group) with precise spatial separation (Fig. **2**). Type I units consist of two hydrogen bond acceptors with a spatial separation of 2.5 ± 0.3 Å. Type II units comprise two hydro-

gen bond acceptors with a spatial separation of 4.6 ± 0.6 Å or three electron donor groups with a spatial separation of the outer two groups of 4.6 ± 0.6 Å. All molecules that contain at least one Type I or one Type II unit are predicted to be the substrates of Pgp. One molecule of a Pgp substrate has up to eight hydrogen bond acceptor units.

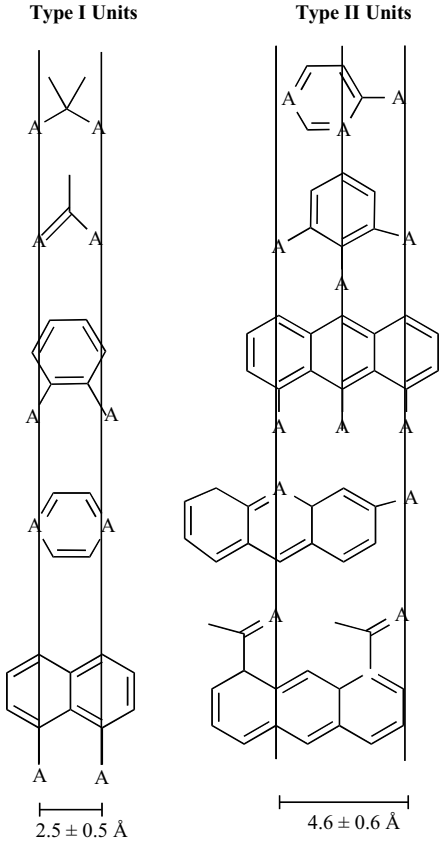

Fig. (2). H-bond acceptor patterns observed in P-glycoprotein substrates. Type I units: patterns formed by electron donor (hydrogen bond acceptor) pairs with a spatial separation of 2.5 ± 0.3 Å. Type II units: patterns form either by three electron donor groups with a spatial separation of the outer two electron donor groups of 4.6 ± 0.6 Å or by two electron donor groups with a spatial separation of 4.6 ± 0.6 Å. "A" denotes a hydrogen bonding acceptor group (electron donor group) and the numbers indicate the first and the nth atom with a free electron pair. Adapted from [73].

Later, Didziapetris *et al*. predicted Pgp substrate specificity using computational analysis [74]. In general, they estimated that compounds with (N + O) ≥ 8, MW > 400, and acid pKa > 4 are likely to be Pgp substrates, whereas compounds with (N + O) ≤ 4, MW < 400, and base pKa < 8 are likely to be non-substrates.

The functional unit of Pgp is composed of two homologous halves, each containing six transmembrane helices and a nucleotide-binding domain separated by a flexible linker region. Loo and Clarke have used a cys-less mutant of Pgp to introduce cysteines at specific locations in the transmembrane domains. Using analogs of Pgp substrates which are bifunctional chemical crosslinking agents, they suggested that transmembrane helices 4, 5, 6 and 10, 11 and 12 contribute to drug binding [75-77]. Pgp is known to have multiple overlapping binding sites and at least two drugs can be bound simultaneously in the drug-binding pocket (reviewed in [53]). Shapiro and Ling have proposed the presence of two functional drug-binding sites within Pgp [78]. The H-site binds Hoechst substrate, quercetin and colchicine, while the R-site binds Rhodamine123, daunorubicin, doxorubicin and other anthracyclines. Pajeva *et al*. identified the pharmacophore points of drugs involved in the verapamil binding site of Pgp, which are two hydrophobic, three hydrogen bond acceptors, and one hydrogen bond donor point [79]. The binding site for the H-site (Hoechst 33342) and R-site (rhodamines) [79] were distinguished by 3D pharmacophore model study. Compared with the nucleotide-binding domains, the amino acid sequences are much more variable in the transmembrane regions of the different species of Pgps. Thus, the different amino acids in various Pgps in the drug-substrate-binding pocket can change drug-binding affinities. However, there have been a few reports on molecular interactions of substrates with the Pgps of different species.

Tang-Wai *et al*. reported different efficiencies for modulators of Pgp, according to species [80]. It was shown that human and mouse Pgps conferred different degrees of resistance to structurally distinct drugs and had different sensitivities to the reversal effect of structurally distinct modulators. Differences in potency can be attributed to changes within the amino acids forming the drug-binding pocket, altering the nature of this pocket by introducing small changes such as hydrogen bond donors or acceptors. Therefore, even minor alterations in the structure of a modulator can change its binding affinity. Iodoarylazidoprazosin (IAAP), a prazosin photoaffinity analog, binds at different sites in hamster and human Pgps, which supports the conclusions of Tang-Wai and colleagues. The binding site was reported to be the amino acid sequences 248-312, 758-800 and 1160-1218 in hamster Pgp [81] but sequences 1135-1169 in human Pgp [3].

Several studies suggest stereoselectivity for Pgp, but this idea is controversial [82-89]. Different stereo selectivity has been reported in the Pgps of different species. In immortalized rat brain capillary endothelial GPNT cells, the (+)-stereoisomer of mefloquine was up to 8-fold more effective than its antipode in increasing cellular accumulation of [^{3}H]vinblastine, while in Caco-2 cells, both enantiomers were equally effective [90]. A high resolution structure in the presence of transport-substrates of Pgp from various species similar to the recently described high resolution structure of a bacterial multidrug transporter, Sav 1866 [91] will be very useful to map out the drug-substrate-binding site(s) on Pgps.

CONCLUSIONS

Pgp is associated with the phenomenon of MDR in cancer cells due to decreased cellular accumulation of drug resulting from Pgp-mediated active efflux. Pgp extrudes a variety of amphipathic compounds and can affect the pharmacokinetics and tissue distribution of many drugs. Therefore, a drug's interaction with Pgp should be considered in the early stage of drug development in order to identify unexpected changes in pharmacokinetics of the drugs as well as undesir-

able clinical outcomes. *In vitro* and animal models are useful tools for screening the interaction between Pgp and drug candidates during drug development. Thus, it is important to investigate the differences in the Pgps of various species and to understand the SAR between drugs and those Pgps. The extrapolation of the results from one assay to another is often disputable (Table **3**). To avoid this problem, both *in vivo* and *in vitro* functional assays have been employed for screening.

One may consider which preclinical species is the most useful for preclinical research. Monkey Pgp-mediated transport activity may more often have the best correlation with human, presumably due to greater similarity between their protein identities relative to other preclinical species. However, it is difficult to ascertain simply on protein identities as to which preclinical Pgp model would be most appropriate. Another consideration is that routine use of *in vivo* monkey models are limited and cost restricted. Thus, the rodent models may be more useful for *in vitro*/*in vivo* correlation between an individual species (i.e. LLC-PK1 cell expressing mouse Pgp *vs*. mdr1a/b(-/-) mice). Having the appropriate screening tool is realized when one considers that an entire chemical class may be a substrate of one species but not the other. We believe that the species tool should be most reflective of the *in vivo* comparator. For example, if the pharmacology model or toxicology model is the guinea pig, then the most appropriate *in vitro* Pgp-predictor would be a guinea pig-based model (e.g. LLC-PK1 cells expressing guinea pig Pgp).

There is limited information available concerning interactions between drug-substrates and modulators with Pgp at a molecular level because no information is available on the high-resolution structure of apo- and ligand-bound Pgp. Additionally, it is not easy to identify exactly which amino acid residues are involved in substrate interactions in the Pgps of various species. However, the identity of the protein segments and amino acid residues responsible for the differences in substrate specificity can be studied in chimeric or mutant proteins. Detailed knowledge of the substrate specificity of the Pgps of different mammalian species used in preclinical studies may provide a molecular approach for the design of drugs with increased efficiency. It may also prevent incorrect extrapolation of preclinical data and facilitate the development of drugs with various cellular targets.

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ABBREVIATIONS

ABC = ATP-binding cassette

MDR = multidrug resistance

Pgp = P-glycoprotein

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