Heterologous Expression of Various P-Glycoproteins in Polarized Epithelial Cells Induces Directional Transport of Small (Type 1) and Bulky (Type 2) Cationic Drugs

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ABSTRACT

We recently showed that absence of mdr1-type P-glycoprotein (P-gp) in mice resulted in profoundly reduced hepatic and intestinal clearance of type 1 and type 2 cationic drugs compared with that in wild-type mice. These data strongly support the concept that mdr1-type P-gps are involved in the disposition of cationic amphiphilic drugs from the body. We tested the hypothesis that mdr1-type P-gps are involved in the transmembrane transport of organic cations in epithelial cells expressing various drug-transporting P-gps. Therefore, transepithelial transport of the P-gp substrate vinblastine, the steroidal (type 2) cation vecuronium, the relatively small (type 1) cationic compound azidoprocainamide methiodide and the aliphatic cation tri-n-butylmethylammonium were measured. Apical expression of the mdr1a, mdr1b or MDR1 gene in confluent grown polarized transformed LLC-PK1 cells resulted in highly enhanced apical directed secretion of all the drugs tested compared with controls. The vectorial transport of tri-n-butylmethylammonium in the apical direction in the P-gp (over)expressing cells could be inhibited by vinblastine. The present observations show that apical secretion of type 1 as well as of type 2 organic cations is enhanced significantly in the presence of apical expressed mdr1-type P-gp. These findings provide evidence for the involvement of drug-transporting P-gp in transmembrane transport of various organic cations, including relatively small molecular weight aromatic and aliphatic compounds.

The disposition of endogenous as well as exogenous cationic compounds from the body occurs through the epithelial secretory cells in the liver, kidney and/or small intestine (Meijer et al., 1997; Pritchard and Miller, 1997; Hunter and Hirst, 1997). The transport steps involved in the secretion of such compounds require both basolateral as well as apical localized transmembrane transport proteins that can mediate efficient uptake and export, respectively. Several organic cation uptake mechanisms have been identified at the level of the basolateral or sinusoidal hepatocyte membrane. In fact, separate uptake mechanisms seem to play a role in the uptake for the small type 1 organic cations and the more bulky type 2 organic cations (for recent review see Meijer et al., 1997). Thus, at least two separate hepatic organic cation uptake mechanisms could be classified: a type 1 organic cation hepatic uptake system, which may be similar to the OCT1 (Gründemann et al., 1994), and the type 2 organic cation uptake system. The latter transport system may be similar to the OATP (Bosuyt et al., 1996). The apical membrane of epithelial cells is highly specialized to export compounds to the exterior environment. Hence it contains various primary or secondary active transport systems to fulfill this export task. Several of these systems have been characterized functionally in the liver as well as in the kidney (Moseley et al., 1996; Inui et al., 1985), whereas some other systems have been characterized recently at the molecular biological level (Paulusma et al., 1996; Müller et al., 1994). At the level of the hepatocyte canalicular membrane, both ATP-independent (Moseley et al., 1996) as well as ATP-dependent (Kamimoto et al., 1989; Müller et al., 1994) transport processes for cationic drugs have been identified. ATP-dependent transport in membrane vesicles was observed for cationic agents such as daunomycin (Kamimoto et al., 1989), and this transport was suggested to be mediated by P-gp. Müller et al. (1994) more definitively established the involvement of P-gp in cationic drug transport, such as APDA and pen-tylquinidine in plasma membrane vesicles of insect cells transfected with the rat mdr1b gene. Similar to this, ATP-dependent transport systems for cationic agents also have been detected in the brush-border membrane of kidney proximal tubule cells. The latter transport system may be similar to the OATP (Bosuyt et al., 1996).

ABBREVIATIONS: mdr/MDR, multidrug resistance; P-gp, P-glycoprotein; APM, azidoprocainamide methiodide; TBuMA, tri-n-butylmethylammonium; APDA, azo-pentyl-deoxyjmalinilium; OCT, organic cation transporter; OATP, organic anion transporting polypeptide.

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imal tubule cells (Dudley and Brown, 1996; Lieberman et al., 1989).

Recently, an important contribution was made toward the understanding of the role of P-gp in the disposition of various drugs from the body by a study on mdr1a gene “knockout” mice (Schinkel et al., 1994). These studies clearly indicated the importance of P-gp in drug elimination and body distribution processes, because transmembrane transport in liver and intestine was reduced profoundly (Smit et al., 1998; Schinkel et al., 1995). However, in mdr1a (−/−) mice the mdr1b P-gp still is expressed in liver and kidney (Schinkel et al., 1994). Therefore a mouse model with simultaneously disrupted mdr1a and mdr1b genes [mdr1a/mdr1b (−/−) mice] was used to further investigate the role of drug-transporting P-gp in the disposition of intravenously injected (cationic) drugs (Schinkel et al., 1997; Smit et al., in press). Absence of both mdr1a and mdr1b P-gp reduced hepatic and intestinal clearance of intravenously injected cationic drugs even further compared with mdr1a (−/−) mice. Collectively these data indicate that under normal conditions in vivo P-gp is involved in the secretion of amphiphilic organic cations. Of particular interest was the finding that absence of drug transporting P-gp resulted in a significant reduction in the excretion rates of relatively small aliphatic organic cations such as TBuMA. TBuMA is an elegant model drug for transmembrane transport studies, because it is not metabolized and has minimal plasma protein binding. The molecular features of TBuMA seem to lack the structural characteristics that are common for P-gp substrates (see also Meijer et al., 1997). We therefore decided to investigate if such small organic cationic agents are indeed substrates for mdr1-type P-gps. The porcine proximal tubule cell line (LLC-PK1) provides an elegant in vitro epithelial cell system to study P-gp-mediated transport (Ueda et al., 1992). LLC-PK1 derived cells transfected with the murine mdr or the human MDR1 genes express the appropriate P-gp that is confined to the apical membrane domain of these epithelial cells (Ueda et al., 1992; Van Helvoort et al., 1996).

Materials and Methods

Chemicals. Vecuronium was a gift from Organon Teknika (Turnhout, Belgium) and [3H]vecuronium was provided by Organon International (Oss, The Netherlands). TBuMA and [3H]TBuMA were synthesized in our laboratory, according to the procedures described by Neel et al. (1984). APM and [3H]APM were synthesized according to Mol et al. (1992). Inulin-[14C]carboxylic acid was from Amersham International (Little Chalfont, UK). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO).

Cell lines, tissue culture and transport assays. The pig kidney epithelial cell line LLC-PK1 was obtained from the American Type Culture Collection (Rockville, MD) and cultured as described (Schinkel et al., 1995). The generation of the human MDR1a, murine mdr1a-transfected LLC-PK1 subclones LLC-MDR1, LLC-mdr1a was described previously (Van Helvoort et al., 1996; Schinkel et al., 1995). LLC-mdr1b cells were generated essentially as described for the subclones described above, except that the murine mdr1b cDNA (a kind gift from Dr. P. Gros, Montreal) was cloned in the eukaryotic expression vector pJ311 (Morgenstern and Land, 1990). Transport assays were carried out as described (Schinkel et al., 1995). Complete medium including t-glutamine, penicillin, streptomycin and fetal calf serum was used throughout. Cells were seeded on microporous polycarbonate membrane filters (3.0-μm pore size, 24.5-mm diameter, Transwell 3414, Costar Corp., Cambridge, MD) at a density of 2 × 10^6 cells per well for parent cell line and subclones. The cells were grown for 3 days in complete medium with one medium replacement. One to two hours before the start of the experiment, medium at the apical and basal side of the monolayer was replaced with complete medium. The experiment was started (t = 0) by replacing the medium at either apical or basal side of the monolayer with complete medium containing radiolabeled drug (3.7–7.4 kBq/ml) and [3H]inulin (0.93 kBq/ml, 4 μM). The cells were incubated at 37°C in 5% CO2, and 50-μl aliquots were taken at 30 min, 1, 2 and 4 h. The appearance of radioactivity in the opposite compartment was measured and presented as the fraction of total radioactivity added at the beginning of the experiment. Directional transport was measured in triplicate in at least three independent experiments, and values are depicted as mean (±S.E.).

The passive paracellular flux was monitored by the appearance of inulin[14C]carboxylic acid in the opposite compartment and was always less than 1% of total radioactivity per hour.

Results

The present study tested whether type 1 and/or type 2 amphiphilic organic cations are substrates for mdr1-type P-gps. For this purpose we used polarized pig kidney cells (LLC-PK1) transfected with either human MDR1, or murine mdr1a (Schinkel et al., 1995) or mdr1b cDNA. Parent and transfected epithelial cells were grown on microporous supports to obtain confluent and highly polarized epithelial cells. This enabled the study of directional transport of organic cations in cells that express the human MDR1, murine mdr1a or mdr1b P-gp. Transfected cells had roughly similar levels of P-gp expression, and P-gp was confined to the apical domain of these epithelial cells (Van Helvoort et al., 1996; Schinkel et al., 1995).

Figure 1 shows directional transport of the known P-gp substrate vinblastine in cells expressing MDR1, mdr1a and mdr1b P-gp. Vinblastine was secreted efficiently into the apical medium in cells expressing the MDR1, mdr1a and mdr1b P-gp (fig. 1, B–D). The parent LLC-PK1 cells showed a lower apical directed flux of vinblastine. This moderate directional flux observed in parent cells may be mediated by endogenous P-gp present in these epithelial cells (Dudley and Brown, 1996; Childs and Ling, 1996). Yet as a result of murine mdr1 or human MDR1 gene expression in the transfected cells, transport in the apical direction was increased ∼3-fold (P < .05). In contrast, the apical-to-basolateral directed flux of vinblastine was 3- to 11-fold lower in P-gp overexpressing cells as compared with the parent cells (P < .05). TBuMA, a small type 1 organic cation, was transported almost equally efficiently in both the apical and basolateral direction in parent LLC-PK1 cells (fig. 2A). A markedly enhanced, apical directed transport was observed for TBuMA if MDR1, mdr1a or mdr1b P-gp were expressed (fig. 2, B–D). Both vinblastine and TBuMA seemed to be transported equally efficiently in cells expressing the MDR1 P-gp (see figs. 1B and 2B). In mdr1a or mdr1b P-gp-expressing cells the TBuMA apical secretion was less efficiently compared with apical transport observed in MDR1-expressing cells.

Parent LLC-PK1 cells also favored apical secretion over basolateral transport of APM, a second type 1 organic cation (P < .05) (fig. 3A). This suggests that endogenous transport proteins are present which can catalyze net transport of APM in the apical direction in these cells (fig. 3A).

A clear enhancement of APM apical secretion was observed.
Fig. 1. Transcellular transport of $[^3]$Hvinblastine (2 µM) in confluent grown polarized epithelial cells. Drug transport is expressed as nanomoles per well measured in the opposite compartment. Open symbols indicate apical-to-basolateral transport, and closed symbols indicate basolateral-to-apical transport of vinblastine. (A) LLC-PK1: control ($n = 6$); (B) LLC-MDR1: MDR1 P-gp-expressing cells ($n = 6$); (C) LLC-mdr1a: mdr1a P-gp-expressing cells ($n = 3$); (D) LLC-mdr1b: mdr1b P-gp-expressing cells ($n = 3$). Values are expressed as the mean ± S.E. *P < .05 (unpaired two-sided Student’s t test).

Fig. 2. Transcellular transport of $[^3]$HTBuMA (10 µM) in confluent grown polarized epithelial cells. Drug transport is expressed as nanomoles per well measured in the opposite compartment. Open symbols indicate apical-to-basolateral transport and closed symbols indicate basolateral-to-apical transport of TBuMA. (A) LLC-PK1: control ($n = 9$); (B) LLC-MDR1: MDR1 P-gp-expressing cells ($n = 9$); (C) LLC-mdr1a: mdr1a P-gp-expressing cells ($n = 6$); (D) LLC-mdr1b: mdr1b P-gp-expressing cells ($n = 9$). Values are expressed as the mean ± S.E. *P < .05 (unpaired two-sided Student’s t test).
in cells expressing mdr1-type P-gps (fig. 3, B–D), and this apical secretion was significantly higher than the apical secretion that was observed in the parent cells.

Note that for both tested small (type 1) organic cations the apical-to-basolateral directed flux was equally efficient in the presence or absence of mdr1-type P-gp.

The contribution of mdr1-type P-gps to the net apical secretion of TBuMA that was found in P-gp-expressing cells was studied further. On addition of the P-gp substrate vinblastine the TBuMA apical secretion was partly inhibited (fig. 4). When TBuMA was added to the apical medium, the apical-to-basolateral directed flux of TBuMA was increased significantly on addition of vinblastine, and this stimulating effect appeared equal in all the cell lines tested (fig. 4B).

The steroidal muscle relaxant vecuronium, a bulky (type 2) organic cation, showed apical directed transport in the parent cell line that was significantly higher than the apical-to-basal directed vecuronium flux (fig. 5A). In epithelial cells expressing MDR1, mdr1a or mdr1b P-gp the apical directed flux of vecuronium was significantly higher than the flux in the parental cells (fig. 5, B–D). All the mdr1-type P-gps investigated seemed to transport vecuronium equally efficiently.

In cells expressing mdr1-type P-gp the apical directed flux of vecuronium found in this study was 4- to 8-fold lower than the fluxes obtained with the small type 1 cationic agents TBuMA and APM.

**Fig. 3.** Transcellular transport of [3H]APM (10 μM) in confluently grown polarized epithelial cells. Drug transport is expressed as nanomoles per well measured in the opposite compartment. Open symbols indicate apical-to-basolateral transport and closed symbols indicate basolateral-to-apical transport of APM. (A) LLC-PK₁: control (n = 9); (B) LLC-MDR1: MDR1 P-gp-expressing cells (n = 9); (C) LLC-mdr1a: mdr1a P-gp-expressing cells (n = 6); (D) LLC-mdr1b: mdr1b P-gp-expressing cells (n = 9). Values are expressed as the mean ± S.E. *P < .05 (unpaired two-sided Student’s t test).

**Fig. 4.** Interaction of TBuMA transcellular transport and vinblastine (2 μM). (A) Basolateral-to-apical transported amounts after 4 h incubation. (B) Apical-to-basolateral levels after 4 h incubation. The amounts measured are expressed as the mean (±S.E.) percentage secreted into the compartment opposite the compartment where TBuMA was administered (n = 6). I, LLC-PK₁; II, LLC-MDR1; III, LLC-mdr1a; IV, LLC-mdr1b. Black and gray columns indicate the levels found in the absence and the presence of vinblastine, respectively. *P < .05 (unpaired two-sided Student’s t test).
LLC-PK₁ cells have been used extensively as a model to characterize cationic drug transport in kidney epithelial cells (Dudley and Brown, 1996; Fauth et al., 1988; Fouda et al., 1990). When grown on a microporous support LLC-PK₁ cells form a polarized monolayer with epithelial characteristics such as tight junctions and apical microvilli (Faller et al., 1990; Gstraunthaler et al., 1990). The transcellular transport of organic cationic compounds or drugs, such as triethanolamide (Fauth et al., 1988), cimetidine (Dudley and Brown, 1996) or procainamide (Takano et al., 1992) have been studied in this system. Uptake of the small cationic compounds into LLC-PK₁ cells is a carrier-mediated process (Takano et al., 1992; Fauth et al., 1988; Fouda et al., 1990). The putative pig homolog of OCT1 (Gründemann et al., 1994) is a potential candidate for such uptake process. After uptake into the epithelial cells subsequent secretion takes place across the apical membrane. Several endogenous transport proteins may contribute to the latter process such as a cation/proton antiporter or P-gp (Maegawa et al., 1988; Childs and Ling, 1996). Thus secretory transport results in a net apical directed output of organic cations resembling renal tubular secretion of organic cationic compounds.

In the present study we investigated P-gp-mediated organic cation transport in LLC-PK₁ cells and in LLC-PK₁ cells transfected with various cDNAs encoding the human MDR1, the murine mdr1a or mdr1b P-gp.

Vinblastine apical directed secretion was highly enhanced in cells that express P-gps (fig. 1, B–D). This was shown previously for the MDR1 and mdr1a P-gp-expressing LLC-PK₁ cells (Van Helvoort et al., 1996; Schinkel et al., 1995). We extended these observations showing that the mdr1b P-gp-expressing cells also display an increased apical directed vinblastine transport (fig. 1D); less than 40% [³H]vinblastine remained in the basolateral medium after addition to this compartment. This effect on the apical directed vinblastine transport rate seemed similar for the various P-gp isoforms. Furthermore, in the present study it was found that vinblastine fluxes that exceeded 1.9 nmol/well would result in an apical vinblastine concentration exceeding that in the basolateral compartment. This tells that vinblastine movement into the apical medium can occur against its own chemical gradient.

The rapid passive net vinblastine influx is a key feature that allows the investigation of enhanced apical directed secretion of vinblastine; if basolateral uptake of vinblastine were rate limiting in the transcellular transport, the presence of heterologously expressed P-gps at the apical domain of transfected LLC-PK₁ cells would not enhance the apical directed transport of vinblastine. We observed that vinblastine was transported equally well into the apical medium in the MDR1 and in the murine mdr1a transfected cells which express similar levels of P-gp (Schinkel et al., 1995). Mdr1b transfected cells also seem to transport vinblastine equally as well as MDR1 and mdr1a P-gp-expressing cells. Another study found at least some differentiation in the transport capacities of the various P-gps toward the anticancer drug vinblastine (Tang-Wai et al., 1995).

In contrast, the apical-to-basolateral directed transport of vinblastine was significantly lower when mdr1-type P-gps were expressed as compared with normal LLC-PK₁ cells.
About 80 to 90% of the administered amount of \([\text{H}]\text{vinblastine}\) remained in the apical medium after administration to cells that express mdr1-type P-gp. This implies that P-gp not only increases the net transepithelial transport after basolateral addition, but also can reduce the net apical-to-basolateral passive movement of vinblastine after apical addition. The 4- to 8-fold decreased basolateral flux of vinblastine in the mdr1-type P-gp-expressing cells, together with the high amount of residual vinblastine in apical medium after 4 h suggests that the hydrophobic drug vinblastine is kept out of the cells. Presently substantial evidence exists that mdr1-type P-gps can function as hydrophobic "vacuum cleaner"-type transporters that can expel hydrophobic drugs like vinblastine from the lipid phase (Higgins and Gottesman, 1992).

Such a drug transport mechanism was elegantly shown for a lactococcal MDR homolog, LmrA. This prokaryote P-gp was shown to catalyze transport of the organic cation 1-[4-(trimethylamino)phenyl]-6-phenylhexa-1,3,5-triene from the inner leaflet to the outer leaflet of the lipid bilayer (Bolhuis et al., 1996). Such a hydrophobic vacuum cleaner mechanism may explain the largely decreased vinblastine transepithelial transport into the basolateral medium after apical addition in mdr1-type P-gp-expressing cells. Such a decrease in net apical-to-basolateral transport was not observed for TBuMA, APM and vecuronium. These less lipophilic agents possibly can not be expelled from the plasma membrane but may only be transported from the cytoplasmic compartment after carrier-mediated uptake. Also the build-up of cytoplasmic drug concentrations may be slow; hence, the driving force for P-gp-mediated export may be too low to observe significant changes.

Next we investigated P-gp-mediated transport of the type 1 organic cations. Because these type 1 cationic compounds are relatively hydrophilic, basolateral uptake of these compounds most likely involves transporter proteins that are embedded in the basolateral membrane domain, such as OCT1 (Busch et al., 1996). Hence, basolateral organic cation uptake into the LLC-PK1 cells and its transfectants via such a transporter is a very fast process (Busch et al., 1996). We observed that the basolateral uptake during the first 2 to 3 h is a faster process than the apical secretion process in the same period (data not shown).

In normal LLC-PK1 cells, approximately 5% (~1.8 nmol/well) of the basolateral administered TBuMA was transported into the apical medium (see fig. 2A), which indicates that a relatively slow transcellular process takes place compared with vinblastine transport in LLC-PK1 cells. The (over)-expression of the human MDR1 P-gp highly enhanced the apical directed transport of the type 1 cationic compound compared with controls, which resulted in a net secretion of about 60% TBuMA into the apical medium (fig. 2B). The expression of murine drug-transporting P-gps similarly significantly enhanced TBuMA transport in the apical direction (fig. 2C and D). Despite the similar expression levels of MDR1, mdr1a (Van Helvoort et al., 1996; Schinkel et al., 1995) and probably also mdr1b P-gp in the transfected epithelial cells, the mdr1a and mdr1b P-gp stimulated the apical secretion of the small (type 1) cationic compound TBuMA less than MDR1 P-gp. Finally, it was found that TBuMA fluxes that exceeded 9.5 nmol/well would result in an apical TBuMA concentration exceeding that in the basolateral compartment. This implies that TBuMA movement into the apical medium can occur against its own chemical gradient, similar to what was observed with vinblastine.

For comparison, we also studied the directional transport of a second type 1 cationic agent, APM. The apical secretion of both type organic cations in murine P-gp-expressing cells was similar in size, which suggests that mdr1a and mdr1b P-gp accommodate both type 1 organic cations. In contrast to TBuMA, MDR1 and mdr1a and mdr1b P-gp enhanced the APM secretion into the apical medium to a similar extent (fig. 3, B–D). Differences in affinity of these compounds for the particular P-gps might be involved.

Next, the transepithelial transport of a more bulky steroidal cation, vecuronium, was investigated (fig. 5). The net apical secretion of vecuronium was relatively slow compared with the apical secretion of small type 1 organic cations. Yet, the apical secretion of vecuronium was enhanced significantly in epithelial cells expressing P-gp compared with the basolateral directed flux and with the basolateral-to-apical secretion in parent LLC-PK1 cells.

The relatively slow apical secretion of vecuronium in this cell system may be in accordance with the much slower renal secretion of type 2 organic cations than type 1 organic cations, as observed by us in vivo (Smit et al., 1998, in press). Such slow renal secretion of vecuronium in vivo could be caused by rate limitation in the uptake into renal tubular cells along with an efficient (competing) uptake process in hepatocytes.

Until now there has been no evidence in the literature that indicates the presence of basolateral transport proteins in kidney epithelial cells which could be involved in the epithelial uptake of type 2 (bulky) organic cations. Clearly, this situation differs from the liver. Recently, an OATP was identified in the liver (Bossuyt et al., 1996). This OATP is expressed in the basolateral domain of the hepatocytes, and it can transport the type 2 cationic compound APDA (Bossuyt et al., 1996); hence, this protein also may be involved in the hepatic uptake of bulky cations such as APDA or vecuronium in vivo. Transporters such as OATP have not been detected yet at the basolateral domain of the renal proximal tubular cells and this may explain the less efficient renal uptake, a process that may become rate limiting. Immunological evidence recently has been presented showing that OATP may be present in the S3 segment of the proximal tubular cells at the apical domain (Bergwerk et al., 1996); however, its role at this pole of the cell remains to be clarified.

The potential involvement of mdr1-type P-gp in TBuMA transport was substantiated further by investigating the effect of vinblastine on the apical secretion of this type 1 cationic compound. Indeed, the net apical directed secretion of TBuMA in P-gp-expressing cells was inhibited partially upon the addition of the P-gp substrate vinblastine (2 μM) (fig. 4A). Concomitantly, cellular TBuMA content increased in line with an inhibition of apical secretion of TBuMA (not shown). The partial inhibition of TBuMA apical secretion by vinblastine could be caused by incomplete inhibition of P-gp TBuMA transport. In addition, such residual apical TBuMA secretion also could be ascribed to endogenous cation-transporting proteins such as the cation/proton exchanger (Pietruck and Ulrich, 1995; Imi et al., 1985; Takano et al., 1992) and/or the recently identified OCT2 (Gründemann et al., 1997).

The net apical-to-basolateral directed transport of TBuMA...
was increased significantly upon addition of vinblastine in all the epithelial cells tested (fig. 4B).

Mdr1-type P-gp activity likely counteracts the net apical reabsorption process, and the inhibition of P-gp activity by vinblastine therefore may lead to an increased net apical influx.

The present findings support our earlier observations in mdr1a and mdr1a/b gene “knockout” mice, which indicates that the murine mdr1-type P-gps mediate TBuMA and APM elimination (Smit et al., 1998, in press). Considering the molecular features of APM, P-gp-mediated transport of this agent can be envisioned considering that cimetidine, another cationic drug of about equal molecular weight, is also a P-gp substrate (Dudley and Brown, 1996; Pan et al., 1994). More surprising is that the relatively small nonmetabolized aminocyclic cation TBuMA behaves as a P-gp substrate. However, the presence of three butyl groups at the positively charged onium center in TBuMA may provide a sufficiently bulky character to be accommodated by P-gp.

In conclusion, we have shown that the apical secretion of organic cations in transfected epithelial cells is mediated by P-gp localized in the apical membrane. This observation is in line with our recent findings in mice devoid of mdr1a or both mdr1a and mdr1b P-gps, which shows a markedly reduced biliary and intestinal secretion of several organic cations. The data in the present study support the idea that P-gp also mediates the secretion of relatively small cationic compounds (Smit et al., in press). Consequently, mdr1-type P-gps may be involved in the elimination of a much broader spectrum of cationic compounds from the body than assumed previously.

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