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# Hepatobiliary and intestinal clearance of amphiphilic cationic drugs in mice in which both *mdr1a* and *mdr1b* genes have been disrupted

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**1** We have used mice with homozygously disrupted *mdr1a* and *mdr1b* genes (mdr1a/1b (−/−) mice) to study the role of the mdr1-type P-glycoprotein (P-gp) in the elimination of cationic amphiphilic compounds from the body. These mice lack drug-transporting P-gps, but show no physiological abnormalities under laboratory conditions and have normal bile flow.

**2** $^{3}$H-labelled cationic drugs were administered intravenously (i.v.) to mice as a single bolus dose and the disposition of the studied cationic drugs was investigated by focusing on drug secretion into bile, intestinal lumen and urine.

**3** Hepatobiliary secretion of the investigated cationic drugs was profoundly reduced in mice devoid of the mdr1-type P-gps. In fact, the cumulative biliary output, measured during 1 h, of the small type 1 compounds tri-butylmethyl ammonium (TBuMA) and azidoprocainamide methoiodide (APM), as well as of the more bulky type 2 cationic drug vecuronium, was reduced by at least 70% in the mdr1a/1b (−/−) mice compared to wild-type.

**4** The intestinal secretion of TBuMA, APM and vecuronium was also profoundly reduced in mdr1a/1b (−/−) mice compared to wild-type mice. The absence of the mdr1-type P-gp resulted in virtual elimination of intestinal secretion of TBuMA and APM (>90% reduced as compared to wild-type ($P=0.0001$ and 0.0022, respectively)). The intestinal secretion of the type 2 cation drug vecuronium was reduced by 58% ($P=0.0004$) compared to the wild-type mice.

**5** Increased renal clearances of both the type 1 compounds TBuMA and APM and also of the type 2 cationic compound vecuronium in the mdr1a/1b (−/−) mice were observed. Furthermore, the balance between hepatic, intestinal and renal clearances of small type 1 organic cations clearly shifted towards a predominant role for renal clearance. Increased renal clearance may be explained by (over)expresion of additional mechanisms for renal organic cation secretion, alternatively they may also point to an as yet undefined role of P-glycoprotein in kidney physiology and renal secretory function.

**6** We conclude that the elimination from the body of a broad spectrum of cationic amphiphilic drugs via liver and intestine, is largely dictated by the activity of mdr1-type P-glycoproteins.

**Keywords:** P-glycoprotein; cationic drug elimination; hepatobiliary- and intestinal secretion

## Introduction

The mdr1-type (drug transporting), P-glycoproteins (P-gps) are plasma membrane glycoproteins which can expel toxic compounds from the cells cytoplasm, at the expense of adenosine 5′-triphosphate ATP; (Higgins, 1992). As export proteins, the mdr1-type P-gps can keep cytoplasmic drug concentrations below cytotoxic levels resulting in tumour cell survival. The mdr1-type P-gp can mediate transport of a remarkably broad array of substrates, implicating P-gp function in the resistance of tumour cells against many therapeutic anti-cancer drugs (Endicott & Ling, 1989, Gottesman & Pastan, 1993).

Murine drug transporting P-gps are encoded by two genes, the *mdr1a* and *mdr1b* which encode the mdr1a and the mdr1b P-gp respectively, whereas in man drug transporting P-gp is encoded by a single gene: *MDR 1* (Croop *et al*., 1989; Dhir *et al*., 1990, Gros & Buschman, 1993). Tissue distribution studies of the mdr1a and mdr1b P-gps and the MDR1 P-gp have shed light on the expression pattern and the cellular localization of these proteins. P-gp is localized at the apical domain of epithelial cells in many normal tissues, e.g. the small intestine, liver, kidney and blood-brain barrier (Cordon-Cardo *et al*., 1989; Kamimoto *et al*., 1989; Hsing *et al*., 1992; Hori *et al*., 1993; Dutt *et al*., 1994). Such a tissue distribution of P-gp suggests a protective and/or a secretory role of this transmembrane protein.

Several investigators have proposed that the poor oral absorption of certain drugs is not only due to a low intestinal permeation, but also to counteractive drug secretory processes (Turnheim & Lauterbach, 1997; 1980; Neef, 1983). Recent findings with *mdr1a* knockout mice clearly showed that mdr1a P-gp is involved in such drug counterflux phenomena at the level of the intestinal mucosa (Schinkel *et al*., 1994; Mayer *et al*., 1996). Disruption of the *mdr1a* gene largely reduced intestinal transport of the anti-cancer drug paclitaxel and also drastically altered the bio-distribution of paclitaxel in mice (Sparreboom *et al*., 1997). Similar observations were obtained in the mdr1a (−/−) mice with digoxin (Mayer *et al*., 1996) and vinblastine (Van Asperen *et al*., 1996). The findings with the mdr1a (−/−) mice revealed the importance of mdr1-type P-gps in the body with regard to the secretion of amphiphilic drugs (Schinkel *et al*., 1994; Van Asperen *et al*., 1996; Mayer *et al*., 1996). It follows that the abundant presence of mdr1-type P-gp at the intestinal mucosa may largely hinder the absorption rate and oral bio-availability of clinically

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important drugs (Hunter *et al*., 1993), while drug interactions at the intestinal transport level can be expected as well (Van Asperen *et al*., 1997).

In previous studies we have shown that the absence of the mdr1a P-gp considerably decreased the hepatobiliary and intestinal elimination of several amphiphilic drugs (Schinkel *et al*., 1994; 1995; Mayer *et al*., 1996; Smit *et al*., 1996; 1998). The observed residual secretion via the liver was hypothesized to be due to the presence of mdr1b P-gp, that is (over)expressed in the mdr1a (−/−) mice, most likely as a consequence of the disruption of the *mdr1a* gene (Schinkel *et al*., 1994). Therefore, we expected that mice lacking both the mdr1a and mdr1b P-gp would provide an even more powerful model to investigate the role of P-gp in the body elimination of cationic drugs.

In the present study we employed mice with homozygously disrupted *mdr1a* and *mdr1b* genes (mdr1a/1b (−/−) mice) (Schinkel *et al*., 1997), with the aim of investigating the role of mdr1-type P-glycoprotein in the body elimination of cationic model compounds more definitely. The excretion of these cationic model agents into bile, urine and intestinal lumen was simultaneously measured. Although some of the model compounds tested, at first sight, do not share the structural features of well known P-gp substrates, our results show that mdr1-type P-glycoproteins both at the intestinal mucosa, as well as at the bile canalicular level, function as excretory systems for a very broad array of amphiphilic cationic drugs.

Part of this work has been published in preliminary form (Smit *et al*., 1996).

## Methods

### *Pharmacokinetic studies*

Animals were housed and handled according to institutional guidelines complying with Dutch legislation. Male wild-type and mdr1a/1b (−/−) mice (Schinkel *et al*., 1997) of a mixed genetic background (~50% FVB, ~50% 129/Ola) between 12 to 16 weeks of age were used in all experiments. All animals were fed standard chow (RHM, Hope Farms, Woerden, The Netherlands) and given acidified water *ad libitum*, and were kept on a 12/12 h light/dark cycle. In all studies, mice were anaesthetized with a mixture of Hypnorm (fentanyl 0.2 mg $ml^{-1}$, fluanisone 10 mg $ml^{-1}$), diazepam (5 mg $ml^{-1}$) and saline (0.9% (w/v) NaCl) (1 : 1 : 18) injected i.p. (100 $\mu$l 5 $g^{-1}$ body weight (BW)). Pharmacokinetic studies were performed with animals receiving 1–2 $\mu$Ci (37–74 kBq) of the radiolabelled drug per animal. Bolus injections were administered with saline as a vehicle in a dose from 1 to 5.2 mg $kg^{-1}$ BW (see figure legends for the particular conditions). Injection volumes were set to 0.1 ml size in all cases and were administered via the penal vein. Blood samples (~20 to 25 $\mu$l) were drawn at 10, 20, 30, 40, 50 and 60 min from the tail vein, with the total volume withdrawn never exceeding 7 $\mu$l $g^{-1}$ BW. Plasma was obtained after centrifugation and was stored at −20°C until analysis. Biliary excretion was monitored in anaesthetized mice. Briefly, mice were laparotomized by a median incision. After ligation of the common bile duct, the gall bladder was cannulated with polyethylene tubing (Portex Limited, Hythe, U.K.), with an internal diameter of 0.28 mm. The cannula was ligated into the gall bladder to obtain bile samples at a 5 min interval during 1 h. The body temperature was maintained between 37.5 and 37.9°C by use of a standard heating pad and a lamp that was connected to a rectal temperature probe. The tissues at the open surface were kept moist with saline at 37°C. Additional anaesthetic (~250 $\mu$l) was also given directly into the abdominal cavity, if needed. After termination of the experiments the total urine bladder content and small intestinal lumen content were collected and stored at −20°C until analysis. At that time, organs were removed and homogenized in 4 volumes of saline. Homogenates were solubilized by putting them in a 2 : 1 (v/v) mixture of Soluene (Packard, Groningen, The Netherlands) and isopropanol for 2 h at 50°C. The solubilized samples were treated with $H_2O_2$ (30%) to minimize quenching during counting procedures. Likewise plasma samples were treated with $H_2O_2$ before radioactivity was determined.

Quantification of drugs in the biological samples, including the bile samples, was performed by liquid scintillation counting with Hionic Fluor scintiliation fluid (Packard, Groningen, The Netherlands) in a Beckman LS1701 counter.

Previously, the pharmacokinetic behaviour of the cationic model compounds used here, were extensively investigated in one of our laboratories in a rat model (Neef *et al*., 1984a,b). Similar to findings in the rat, no metabolites have been detected for TBuMA and APM in the present study with mice. In rats, it is known that vecuronium is metabolized to 17-OH vecuronium to a minor extent but that this metabolite is excreted by liver in an identical manner to vecuronium (Paanakker *et al*., 1987; Mol *et al*., 1988; Bencini *et al*., 1988).

### *Pharmacokinetic analysis*

MW/Pharm (Proost & Meijer, 1992) was used to calculate the area under the plasma concentration versus time plot (AUC), measured until the last data point. Biliary clearance ($Cl_{BILE}$), renal clearance ($Cl_{RENAL}$) and the intestinal clearance ($Cl_{INT}$) values were calculated by dividing the cumulative amount of drug secreted via the individual secretory organs by the AUC (calculated until the last data point) (Rowland & Tozer, 1989).

ANOVA was used to test the significance of differences between plasma and biliary drug levels observed in wild-type versus mdr1a/1b (−/−) mice and were considered significant if $P<0.05$. Differences in cumulative secretion values, tissue/plasma ratios as well as clearance values obtained in the two animal groups were tested by use of a two-tailed Student's $t$ test and were considered significantly different if $P<0.05$.

### *Chemicals*

Vecuronium was a gift from Organon Teknika (Turnhout, Belgium). [$^3$H]-vecuronium was provided by Organon International (Oss, The Netherlands) and characterized as described earlier by Mol *et al*. (1988). Tri-n-butylmethylammonium (TBuMA) and [$^3$H]-TBuMA was synthesized in our laboratory, according to the procedures described by Neef *et al*. (1984a). Azido-procainamide methoiodide was synthesized according to Mol *et al*. (1992). Azopentyldeoxyajmalinium (APDA) was obtained from Dr G. Kurz (Freiburg, Germany) (Müller *et al*., 1994a). Hypnorm was purchased from Janssen Pharmaceuticals (Tilburg, The Netherlands) and diazepam was from Dumex B.V. (Hilversum, The Netherlands). Deionized water was obtained by use of the Milli-Q Plus System (Millipore, Milford, MA, U.S.A.).

## Results

In the present study the fate of cationic drugs in mice that are completely devoid of mdr1-type P-gp was investigated. These mice have been shown to develop normally under laboratory conditions and show no major pathology (Schinkel *et al.*, 1997). Furthermore, bile constituents and bile flow are similar in the wild-type and the mdr1a/1b (−/−) mice (Schinkel *et al.*, 1997).

Both the biliary and intestinal secretion of the small (type 1) cationic (model) agent TBuMA was profoundly reduced (Figures 1b and 2a) in mdr1a/1b (−/−) mice compared to the wild-type mice. In fact, the cumulative TBuMA secretion in the mdr1a/1b (−/−) mice via liver or the small intestine was decreased to only ~15% and ~7% of the wild-type secretion, respectively ($P<0.05$) (see Figure 2a). In contrast, both the total urinary output in 1 h (Figure 2a) as well as the urinary clearance (Figure 3c) were significantly increased compared to controls.

The plasma concentrations following i.v. injection of the drug were higher in mdr1a/1b (−/−) mice particularly during the initial phase of the experiment (Figure 1a). Accumulation levels of TBuMA in liver, kidney an intestine, the three major secretory organs, in relation to the plasma concentrations of TBuMA at the end of the 60 min period were calculated. Figure 2b shows that the liver to plasma ratio was significantly increased compared to the controls. The kidney to plasma ratio was unchanged whereas the intestinal tissue to plasma

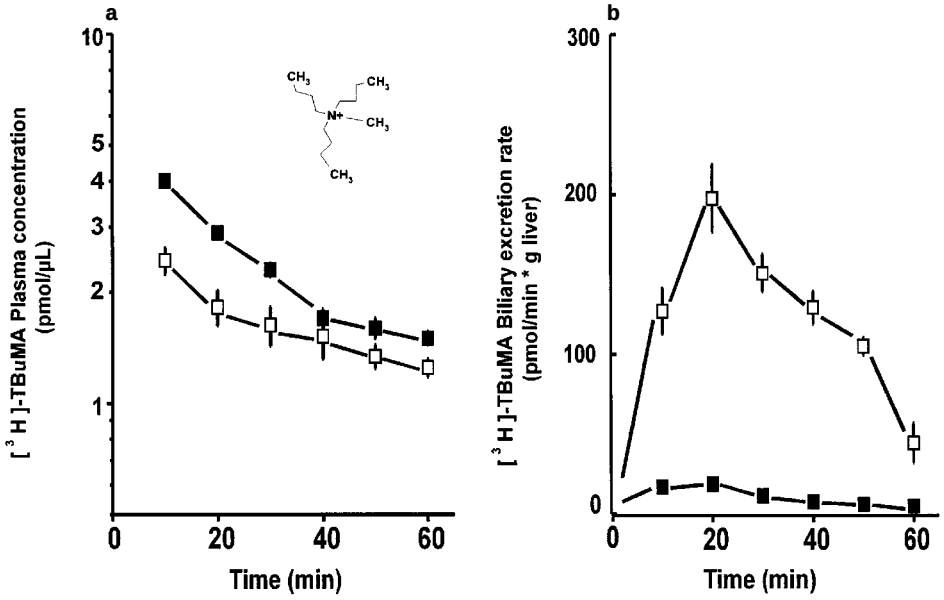


**Figure 1** TBuMA plasma concentration curves (a, pmol TBuMA $\mu l^{-1}$ plasma versus time curve) and biliary excretion rate curves (b, pmol $min^{-1}$ $g^{-1}$ liver versus time curve) in mdr1a/1b (−/−) mice (open symbols) and wild-type (solid symbols) mice, after i.v. injection of 4.1 mg TBuMA $kg^{-1}$ body weight (BW) (13 $\mu$mol $kg^{-1}$). Vertical lines indicate the s.e.mean ($n=3$ to 6). ANOVA statistical analysis confirmed a significant difference ($P<0.05$) between genotypes.

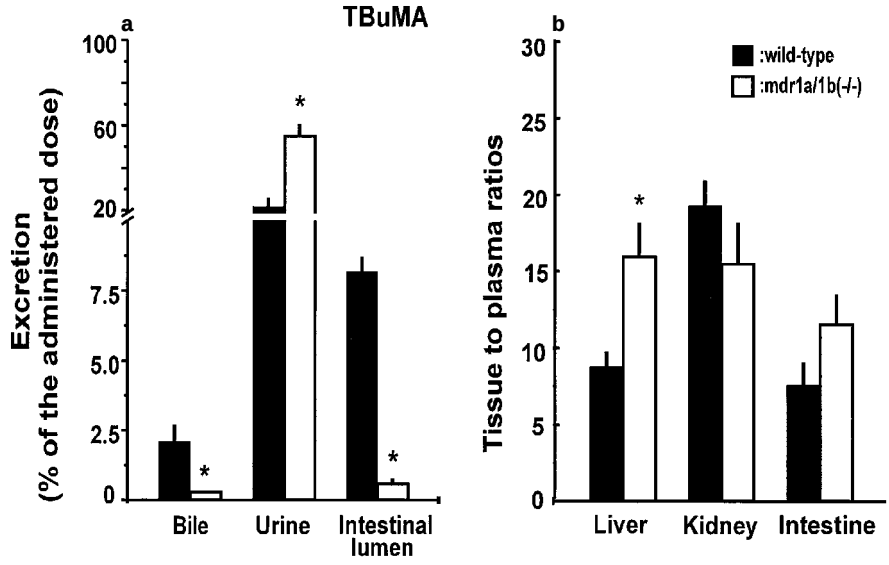


**Figure 2** (a) Cumulative output of [$^3$H]-TBuMA 60 min after i.v. injection of 4.1 mg TBuMA $kg^{-1}$ BW expressed as the percentage of the administered dose. (b) [$^3$H]-TBuMA tissue to plasma ratios 60 min after the i.v. injection of 4.1 mg TBuMA $kg^{-1}$ BW. Values are expressed as the mean ± s.e. ($n=3$ to 6). *$P<0.05$ (two-tailed Student's $t$ test).

ratio was increased ~1.5 fold, but this was not statistically significant ($P=0.06$). The hepatic and small intestinal clearances of TBuMA were significantly decreased (Figure 3a and b, respectively), whereas the renal clearance of TBuMA was increased (Figure 3c) in mdr1a/1b (−/−) mice compared to the wild-type.

APM, a second type 1 cationic model compound that contains an aromatic group, behaved similarly to TBuMA with regard to its pattern of secretion. The biliary output of APM was reduced to about 33% ($P<0.05$) (see Figure 4b and 5a), whereas the intestinal secretion was even reduced to ~9% of the control values in the absence of the mdr1-type P-gp ($P<0.05$) (Figure 5a). The cumulative urinary output (Figure 5a) was increased in the mdr1a/1b (−/−) mice compared to the wild-type. Figure 5b shows tissue to plasma ratios obtained 60 min after i.v. injection of a bolus of APM. The intestinal tissue to plasma concentration-ratio was significantly higher in mdr1a/1b (−/−) mice compared to the wild-type mice, whereas liver to plasma and kidney to plasma ratios where not significantly altered. The APM clearances via liver and intestine were significantly reduced in mdr1a/1b (−/−) mice compared to the wild-type, whereas the renal clearance was increased ($P<0.05$) in the mdr1a/1b (−/−) mice compared to the wild-type. The plasma concentration in the elimination phase of APM was significantly higher in mdr1a/1b (−/−) mice compared to wild-type mice (Figure 4a).

Finally, the pharmacokinetic behaviour of the steroidal muscle relaxant vecuronium (a type 2 bulky cation) was investigated in the mdr1a/1b (−/−) mouse model and compared to the wild-type. Following an i.v. vecuronium bolus injection, plasma vecuronium levels were higher in the mdr1a/1b (−/−) mice compared to the wild-type only during the elimination phase (see Figure 6a). The biliary secretion was strongly reduced by the absence of the mdr1-type P-gps (Figure 6b). Also the intestinal secretion was significantly reduced in mdr1a/1b (−/−) mice compared to the wild-type (Figure 7a). Interestingly, the cumulative renal output was increased in the mdr1a/1b (−/−) mice compared to the wild-type (Figure 7a).

Tissue accumulation of vecuronium correlated with the altered secretion pattern that was observed. Liver to plasma as well as intestinal tissue to plasma ratios were significantly higher in mdr1-type P-gp deficient mice (Figure 7b), whereas the kidney to plasma ratios were unchanged (Figure 7b). The vecuronium clearance values are depicted in Figure 3. From this it can be seen that hepatic as well as intestinal clearances were significantly reduced in the mdr1a/1b (−/−) mice compared to the wild-type. Surprisingly, the vecuronium renal clearance was increased about 5 fold (Figure 3c) in mdr1a/1b (−/−) mice compared to the wild-type. This resembles the findings with regard to renal clearances of the type 1 cationic compounds, although the renal clearance of vecuronium was smaller than both TBuMA and APM renal clearance (see Figure 3c for comparison).

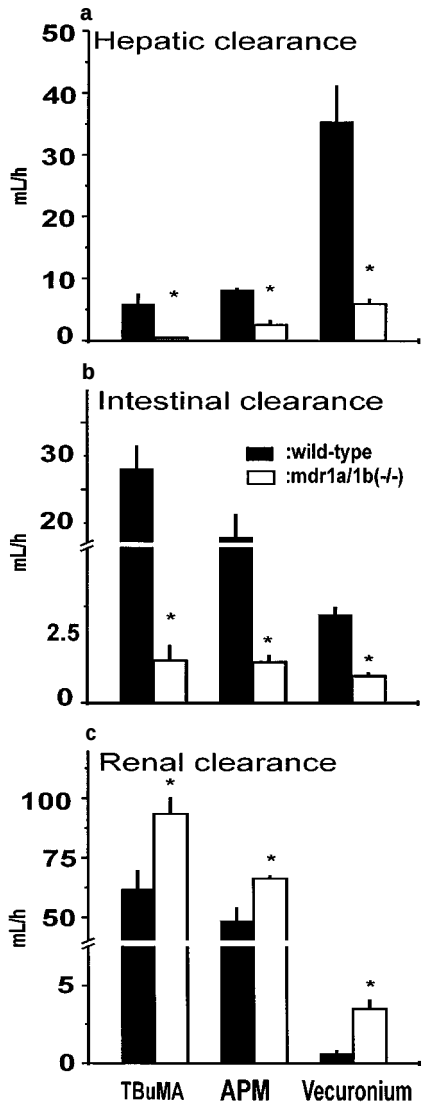


**Figure 3** Hepatic, intestinal and renal drug clearances after the i.v. administration of the cationic drugs tri-n-butylmethylammonium (TBuMA), azido-procainamidemethoiodide (APM) and vecuronium (vec) to the wild-type (solid symbols) and the mdr1a/1b (−/−) (open symbols) mice with a cannulated gall bladder. The parameters were calculated from 3 to 6 independent experiments. Values are expressed as the mean and vertical lines show s.e.mean. *$P<0.05$ (two-tailed Student's $t$ test).

## Discussion

### *Biliary secretion*

Cationic drugs are efficiently removed from the blood by the liver. Multiple transporter proteins have been identified, for small cationic compounds as well as for the more bulky compounds, that are involved in the uptake of these compounds into the hepatocytes (reviewed by Meijer *et al.*, 1997). These proteins mediate facilitated diffusion uptake (Gründemann *et al.*, 1994; Martel *et al.*, 1996; Busch *et al.*, 1996) and operate in conjunction with the secretory proteins at the canalicular domain of the hepatocyte plasma membranes, among which are the drug transporting P-glycoproteins (Thiebaut *et al.*, 1987; Dutt *et al.*, 1994).

The complete absence of mdr1-type Pgps in mice resulted in a dramatically reduced biliary secretion of the cationic agents that were investigated in the present study. Until now, mostly indirect evidence was available to define a possible role of mdr1-type P-gp at the bile canalicular membrane in organic cation secretion into bile; e.g. transport studies in isolated plasma membrane vesicles with hydrophobic anti-cancer drugs (Kamimoto *et al.*, 1989; Watanabe *et al.*, 1995), and less lipophilic cationic agents (Müller *et al.*, 1994a,b).

Using a direct approach, employing mice in which the *mdr1a* and *mdr1b* genes were disrupted, we showed that the

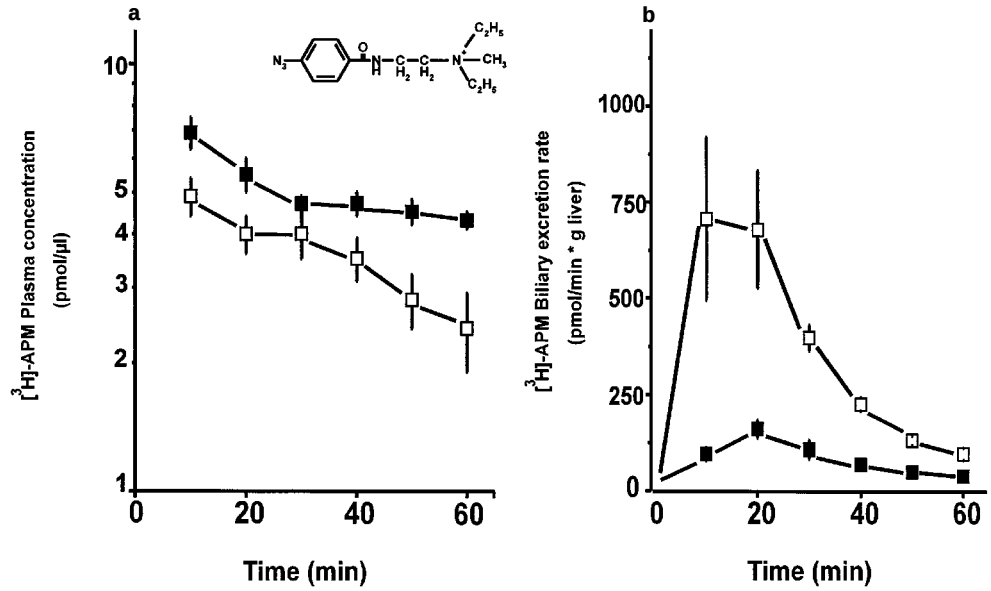


**Figure 4** APM plasma concentration curves (a, pmol APM $\mu l^{-1}$ plasma versus time curve) and biliary excretion rate curves (b, pmol $min^{-1}$ $g^{-1}$ liver versus time curve) in mdr1a/1b (−/−) mice and wild-type mice, after i.v. injection of 5.2 mg APM $kg^{-1}$ BW (13 $\mu$mol $kg^{-1}$). Vertical lines indicate the s.e.mean ($n=3$ to 6). $P<0.05$ between genotypes (ANOVA).

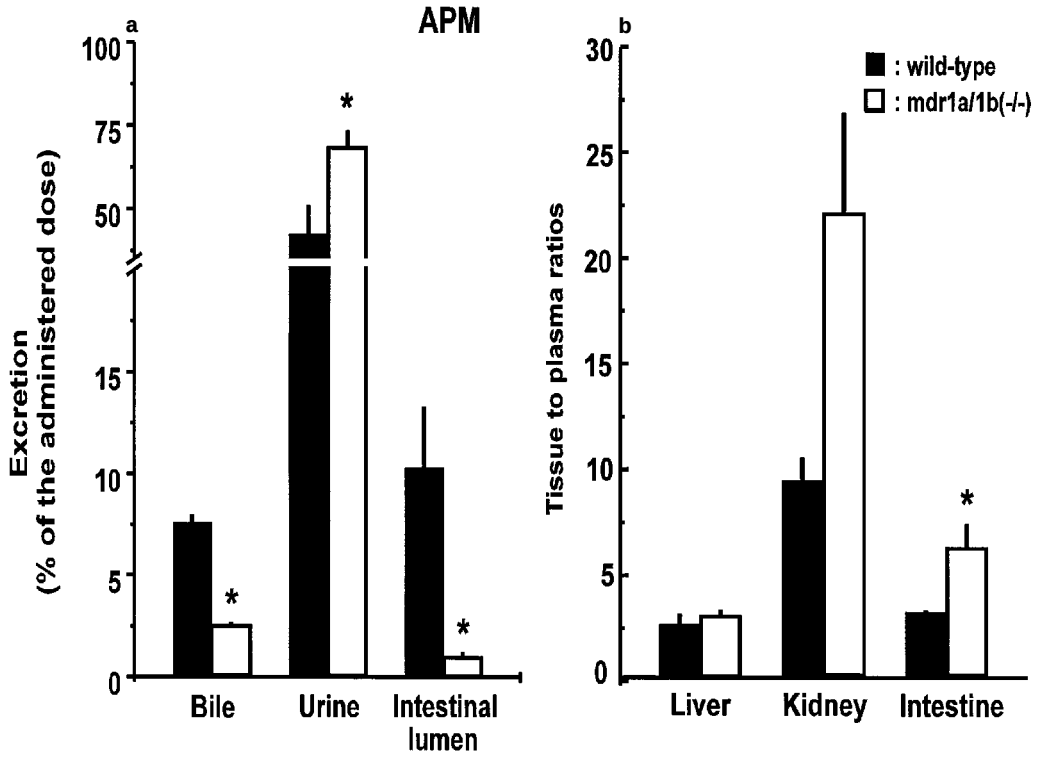


**Figure 5** (a) Cumulative output of [$^3$H]-APM 60 min after i.v. injection of 5.2 mg APM $kg^{-1}$ BW expressed as the percentage of the administered dose. (b) [$^3$H]-APM tissue to plasma ratios 60 min after the i.v. injection of 5.2 mg APM $kg^{-1}$ BW. Values are expressed as the mean ± s.e. ($n=3$ to 6). *$P<0.05$ (two-tailed Student's $t$ test).

secretion of both the small type 1 and more bulky type 2 organic cations into bile is greatly reduced (see Figures 1b, 4b and 6b). The TBuMA cumulative bile secretion was reduced to about 15%, while the APM and vecuronium bile secretion were reduced to about 30% compared to the wild-type cumulative biliary output (see Figure 2a, 5a and 7a, respectively). When the biliary secretion was related to the increased plasma levels it was also evident that the biliary clearance of the investigated compounds was strongly reduced (Figure 3a). These data clearly indicate that drug transporting P-gps mediate the bulk of biliary secretion of cationic compounds, both in the cases of small (type 1) and more bulky (type 2) cationic drugs.

It is important to note we cannot exclude entirely the possibility of secondary changes induced in the apical membrane domains of epithelial cells as a consequence of the absence of both the mdr1a and mdr1b P-gp. However, Schinkel *et al.* (1997) obtained no evidence in their study that

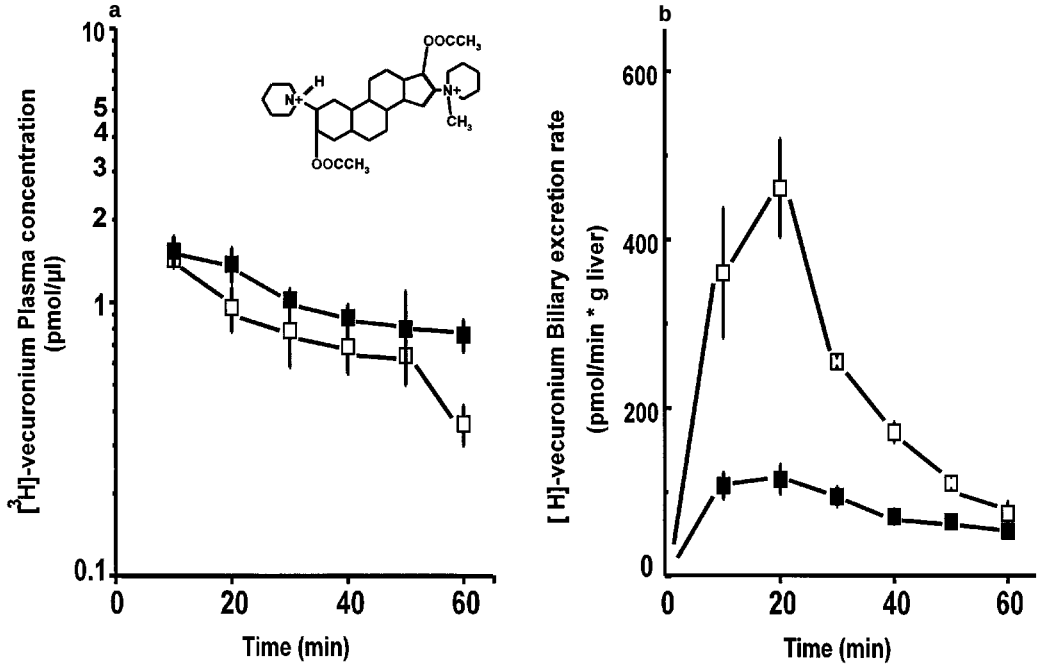


**Figure 6** Vecuronium plasma concentration curves (a, pmol APM $\mu l^{-1}$ plasma versus time curve) and biliary excretion rate curves (b, pmol $min^{-1}$ $g^{-1}$ liver versus time curve) in mdr1a/1b (−/−) mice (open symbols) and wild-type mice (solid symbols), after i.v. injection of 1 mg vecuronium $kg^{-1}$ BW. Vertical lines indicate s.e.mean ($n=3$ to 6). $P<0.05$ between genotypes (ANOVA).

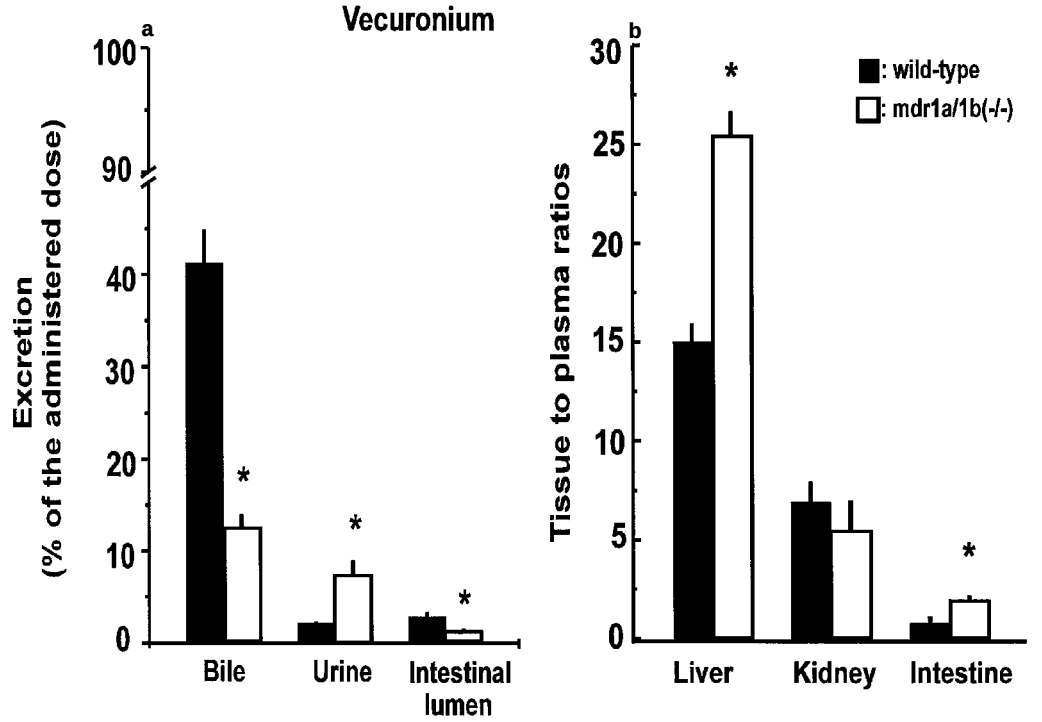


**Figure 7** (a) Cumulative output of [$^3$H]-vecuronium 60 min after i.v. injection of 1 mg vecuronium $kg^{-1}$ BW expressed as a percentage of the administered dose. (b) [$^3$H]-vecuronium tissue to plasma ratios 60 min after the i.v. injection of 1 mg vecuronium $kg^{-1}$ BW. Values are expressed as the mean ± s.e. ($n=3$ to 6). *$P<0.05$ (two-tailed Student's $t$ test).

complete absence of mdr1-type P-gps led to phenotypic alterations in the mdr1a/1b (−/−) mice.

### *Intestinal secretion*

A sharp reduction of the intestinal secretion (see Figure 2a, 5a and Figure 7a) as well as of the intestinal clearance (Figure 3b) of all tested drugs was found in the mdr1a/1b (−/−) mice as compared to wild-type. Interestingly the magnitude of the intestinal organic cation secretion, measured in the present study was significantly larger than that found for mdr1a (−/−) mice (Smit *et al*., 1998) ($P<0.05$, see also below).

Collectively the present data show that the mdr1-type P-gps make an important contribution to type 1 cationic drug

secretion into the gut lumen. Based on these observations it is anticipated that MDR reversal agents like quinidine (Schenck-Gustafsson, 1986; De Lannoy *et al*., 1992; Su & Huang, 1996) and verapamil (Lehnert *et al*., 1991; Bauer *et al*., 1996), may influence the oral absorption and the bio-availability of many cationic drugs, although these drugs have adverse effects like cardiotoxicity. New MDR reversal agents or mdr1-type inhibiting compounds have begun to be developed that have less side effects and have a far higher potency of inhibiting P-gp mediated transport or efflux of chemotherapeutic drugs (Van Asperen *et al*., 1997).

Of note, the intestinal secretion of the type 1 cationic agents across the enterocyte involves both carrier-mediated uptake into the enterocytes, as well as secretion processes out of these cells into the gut lumen. Secretion into the gut lumen most likely occurs at least in part via mdr1-type P-gp, whereas the preceding uptake into the enterocyte probably is mediated by the organic cation transporter OCT1, which has been detected in the intestine by Northern blot and *in situ* hybridization analysis (Gründemann *et al*., 1994). Hence the intestinal clearance process may occur through a concerted action of OCT1 at the serosal uptake level in the enterocyte and subsequent secretion into the gut lumen mediated by P-gp.

### *Urinary secretion*

The absence of mdr1-type P-gps in renal tissue did not result in a reduced organic cation output in the urine. Surprisingly, the cumulative urinary secretion as well as the renal clearance of the drugs investigated here were significantly increased in mdr1a/1b (−/−) mice compared to the renal clearance in wild-type mice. This seems controversial since extensive studies on renal drug secretion revealed that the mdr1-type P-gp both *in vivo* and *in vitro* may be involved in such secretory processes (Okudaria *et al*., 1989; De Lannoy *et al*., 1992; Dutt *et al*., 1994; Pan *et al*., 1994). However, the relative contribution of P-gp can be easily obscured by the abundant presence of other transport systems for organic cations in the proximal tubular cells (Maegawa *et al*., 1988; Kim *et al*., 1991; Katsura *et al*., 1991; Takano *et al*., 1992; Pritchard & Miller, 1993). The present findings may suggest that mdr1-type P-gp is of minor importance for renal cationic drug secretion in the mouse. Yet, observations made by others in other species (David *et al*., 1995; Pietruck & Ullrich, 1995) indicate that renal secretion of cationic drugs, resembling those tested in the present study, may at least partly be mediated by the drug transporting P-gp (Dudley & Brown, 1996). Increases in renal clearance such as observed in the present study may be due to secondary changes imposed by the disruption of both the *mdr1a* and the *mdr1b* gene. Since the plasma protein binding of the tested organic cations is rather low and consequently the glomerular filtration rate of the investigated compounds is likely to be unchanged, these findings imply that the net renal secretion is increased compared to wild-type mice. On the other hand, the apical reabsorption of the organic cations could be reduced at the level of the proximal tubular cells. In principle, such reduced tubular reabsorption and/or an increased luminal secretion process should result in an increased renal clearance and a reduced renal tissue to plasma concentration ratios. However, the absence of mdr1-type P-gp did not significantly reduce the renal tissue to plasma drug ratios in the case of TBuMA and vecuronium. At present investigations are ongoing in our laboratories to reveal potential changes in the expression levels of other tubular reuptake and secretion proteins as a consequence of the *mdr1a* or *mdr1a/1b* gene disruption.

In order to confirm that the organic cations studied here are P-gp substrates in a renal context, we performed a preliminary study on P-gp mediated apical directed transport in confluently grown polarized pig kidney cells (LLC-$PK_1$) transfected with the *mdr1a* or *mdr1b* cDNA. P-gp expression enhanced the apically directed flux of TBuMA, APM and vecuronium (J.W.S., B.W., A.H.S. & D.K.F.M., unpublished data).

The relative importance of the excretory routes of organic cations in the mdr1a/1b (−/−) mice differs for the two classes of cationic agents tested. Although biliary and intestinal secretion was profoundly affected, the total clearance via the liver, kidney and intestine of TBuMA (91 versus 100 ml $h^{-1}$, respectively, in wild-type and mdr1a/1b (−/−)) and of APM (69 versus 70 ml $h^{-1}$, respectively, in wild-type and mdr1a/1b (−/−)) was quite similar in wild-type and mdr1a/1b (−/−) mice. The total elimination rate of these drugs from the blood was therefore basically unaffected in mdr1a/1b (−/−) mice, due to the compensatory increase in the renal secretion. In contrast, in the case of vecuronium, the total clearance value via the three major secretory organs was markedly decreased (35.5 versus 10.2 ml $h^{-1}$, respectively, in wild-type and mdr1a/1b (−/−)), due to a marked reduction in hepatobiliary secretion of vecuronium, which is the meain elimination pathway for this substrate.

### *Organic cation clearance in mdr1a/1b (−/−) versus mdr1a (−/−) mice*

To highlight important pharmacological differences between the mdr1a/1b (−/−) mice and the previously analysed mdr1a (−/−) (Smit *et al*., 1998), we compared the drug clearances in both mouse strains. The rationale to perform such a comparison is that the mdr1a (−/−) mice overexpress the mdr1b P-gp in kidney and liver (Schinkel *et al*., 1994).

*Biliary clearance* Complete absence of the mdr1-type P-gps resulted in a more pronounced reduction in biliary clearance of TBuMA and vecuronium: whereas TBuMA biliary clearance in mdr1a (−/−) mice was decreased to 21% of the wild-type value, in mdr1a/1b (−/−) mice this was down to 9%. For vecuronium the respective biliary clerance values were 42% and 17%. In contrast, APM biliary clearance was comparable in mdr1a (−/−) and mdr1a/1b (−/−) mice (24% versus 22% of the wild-type values). This suggests that the mdr1b P-gp that is localized at the bile canaliculus contributes to the output of at least some organic cationic compounds into bile, although other transport proteins may also be involved. It also appears that in mice, the mdr1b P-gp contributes less to organic cation transport into bile than the mdr1a P-gp.

*Intestinal clearance* The intestinal output of several amphiphilic drugs in mice was shown to be severely reduced in the absence of the mdr1a P-gp (Smit *et al*., 1998) and further disruption of both the *mdr1a* and *mdr1b* genes had at best a modest additional effect. This is in line with earlier observations by Schinkel *et al*. (1997). Of note, in the intestinal tissue of mdr1a (−/−) mice no increased level of mdr1b mRNA was found (Schinkel *et al*., 1994), in contrast to what was observed in liver and kidney. This observation as well as the very low expression of mdr1b P-gp in murine intestine may explain the moderate effect of *mdr1b* disruption on intestinal clearance.

*Renal clearance* Surprisingly, the TBuMA and APM renal clearances in mdr1a/1b (−/−) mice were increased to 151% and 137% of the wild-type values, whereas in the mdr1a (−/−) mice renal clearances were decreased to 65% and 73%, respectively, of the wild-type values. This finding was all the more unexpected since a further reduction in urinary clearance was anticipated based on the decreases found in mdr1a (−/−) mouse model (Smit *et al*., 1998) in which the mdr1b P-gp is still intact and (over)expressed in kidney (Schinkel *et al*., 1994). Similar to the vecuronium renal clearance in mdr1a (−/−) mice, the vecuronium renal clearance in mdr1a/1b (−/−) mice was increased 5 fold compared to the wild-type. It was unclear until now what causes the increased renal clearance of these drugs. Perhaps transporters that differ from mdr1-type P-gps are over-expressed as a consequence of the disruption of the *mdr*1a and *mdr*1b gene in the kidney. Taken together, it appears that liver and intestinal secretion of TBuMA and APM predominantly involves the mdr1a P-gp, whereas mdr1b P-gp plays only a minor quantitative role for these drugs. In the case of vecuronium, mdr1a P-gp mediated secretion in liver and intestine is also the most important route for the disposal of vecuronium from the body, but the relative contribution of mdr1b P-gp is larger than for the two tested type 1 organic cationic compounds. The renal clearance of all these drugs can apparently be mediated by transporters other than the mdr1-type P-gps, which may be upregulated in the mdr1a/1b (−/−) mice.

In conclusion, mdr1-type P-gps in the liver as well as in the intestinal mucosa accommodate secretion of a broad spectrum of cationic amphiphilic drugs from the body. The fact that intestinal secretion as well as biliary output of the organic cations was not completely abolished in mice with disrupted *mdr1a* and *mdr1b* genes indicates that other transport processes may be involved in the overall elimination process, acting in concert with P-gp. Potential candidates are the multidrug resistance related proteins (mrp) of which various isoforms have been identified. As yet, only the *cmoat (mrp2)* gene product has been found to be abundantly expressed at the apical domain of the hepatocyte (Paulusma *et al*., 1996). In addition, a protein closely related to P-gp, the so-called 'sister of P-glycoprotein' (Childs *et al*., 1995) that was identified in porcine liver, is a potential candidate for active organic cation export. The observation that in the mdr1a/1b (−/−) mice the absence of P-gp in the urinary tract results in a markedly increased urinary clearance requires further investigation.

On the basis of this study, we conclude that drug transporting P-glycoprotein enables mammals to remove toxic organic agents from the body through active secretion of such compounds via the liver and the small intestine. These secretory processes provide a protective mechanism through an active contra absorptive flux, limiting the absoprtion and bio-availability of orally ingested organic compounds.

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