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Involvement of Mrp2 (Abcc2) in biliary excretion of moxifloxacin and its metabolites in the isolated perfused rat liver

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Abstract

Moxifloxacin is a novel antibacterial agent that undergoes extensive metabolism in the liver to the glucuronide M1 and the sulfate M2, which are eliminated via the bile. To investigate the role of the multidrug resistance-associated protein (Mrp2) as the hepatic transport system for moxifloxacin and its conjugates, livers of Wistar and Mrp2-deficient TR^- rats were perfused with moxifloxacin (10 μM) in a single-pass system. Values for the hepatic extraction ratio (E) and clearance (Cl) were insignificantly higher in TR^- rats than Wistar rats (0.193 ± 0.050 vs 0.245 ± 0.050 for E; 6.85 ± 1.96 vs 8.73 ± 1.82 mL min^{-1} for Cl), whereas biliary excretion and efflux into perfusate over 60 min were significantly lower in the mutant rat strain. Cumulative biliary excretion of M1, M2 and moxifloxacin was significantly reduced to 0.027%, 19.1%, and 29.6% in the TR^- rats compared with Wistar rats, indicating that the biliary elimination of M1 is mediated exclusively by Mrp2, whereas that of M2 and moxifloxacin seems to depend mostly on Mrp2 and, to a smaller extent, a further unidentified canalicular transporter. Moxifloxacin stimulates bile flow by up to 11% in Wistar rats, but not in TR^- rats, further supporting an efficient transport of this drug and its glucuronidated and sulfated metabolites by Mrp2. Moxifloxacin (10 μM) also reversibly inhibited the Mrp2-mediated biliary elimination of bromsulphthalein in Wistar rats by 34%, indicating competition with the elimination of Mrp2-specific substrates. In conclusion, we found that Mrp2 mediates the biliary elimination of moxifloxacin and its glucuronidated and sulfated metabolites in rats. MRP2 may therefore play a key role in the transport of moxifloxacin and its conjugates into bile in humans.

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Introduction

Moxifloxacin is a novel 8-methoxy-fluoroquinolone antibacterial agent with a broad spectrum of activity against Gram-negative and Gram-positive bacteria as well as anaerobes. Moxifloxacin has broad clinical applications, including the treatment of respiratory tract, gastrointestinal, gynaecological and some skin and soft tissue infections. Furthermore, moxifloxacin is also highly active against *Mycobacterium tuberculosis* (Woodcock et al 1997; Muijsers & Jarvis 2002; Oliphant & Green 2002). Bacterial activity is achieved by inhibition of DNA gyrase and topoisomerase IV, which are required for bacterial DNA relocation, transcription, repair and recombination (Pestova et al 2000).

Following oral or i.v. administration, moxifloxacin is rapidly metabolized to the acyl glucuronide (M1) and the N-sulfate (M2) (Figure 1), both of which are pharmacologically inactive (Stass et al 1998; Stass & Kubitza 1999). However, like the biologically inactive oestrogen sulfate and glucuronide, which are transformed by cellular sulfatases or by β-glucuronidase into the biologically active oestradiol, moxifloxacin conjugates may also serve as inactive pools for moxifloxacin. Formation of M1 is also of clinical importance, as severe adverse reactions associated with moxifloxacin may be related to acyl glucuronide–protein adducts. Phase I biotransformation by cytochrome P450 enzymes was not observed, which means that moxifloxacin is not likely to have pharmacokinetic interactions when co-administered with drugs that either induce or inhibit oxidative enzyme systems (Stass & Kubitza 2001a, b). About 26% of an administered dose is excreted unchanged via faeces and 20% via urine. However, the elimination pathways for the glucuronide and sulfate seem to be organ

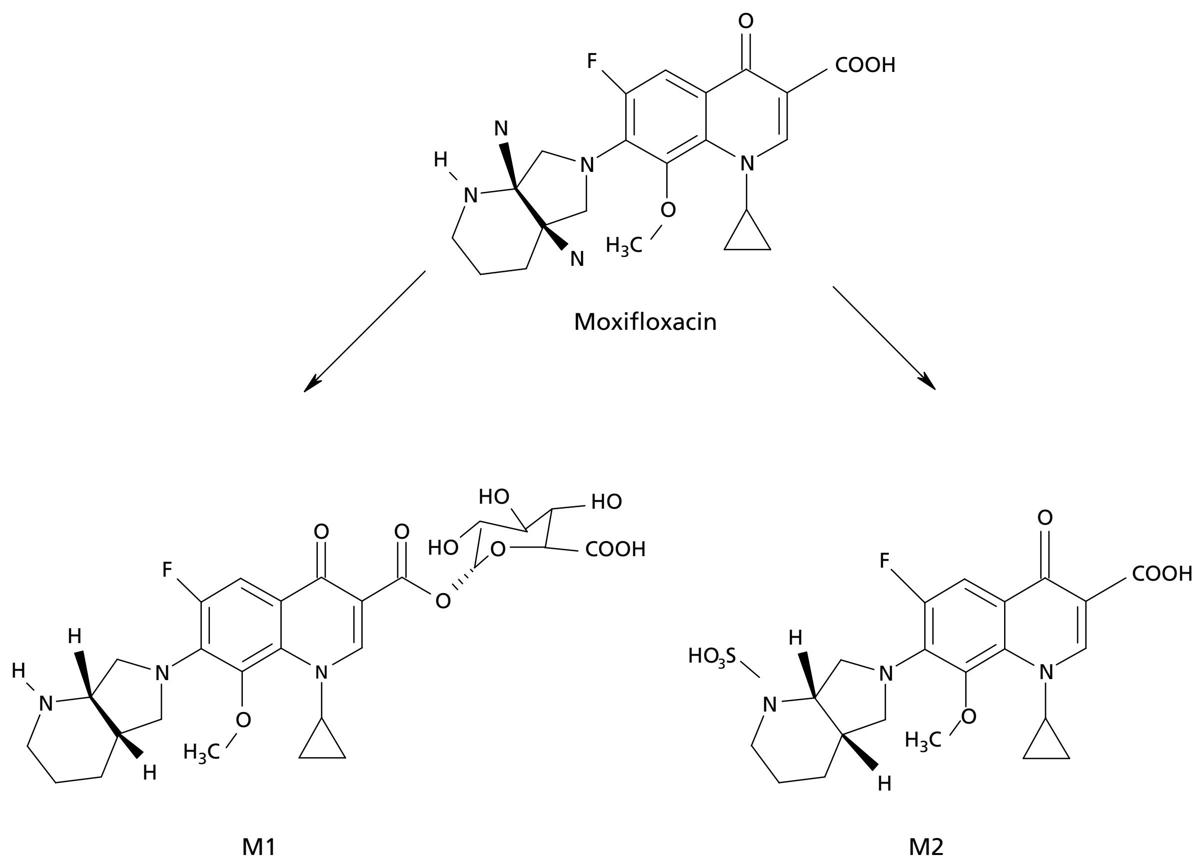

Figure 1 Metabolism of moxifloxacin to moxifloxacin acyl glucuronide (M1) and moxifloxacin N-sulfate (M2).

specific, as the glucuronide was found almost exclusively in the urine (14% of the dose) while the sulfate conjugate could be recovered only in the faeces (35% of the dose) (Stass & Kubitza 1999). Hepatic clearance and metabolism of moxifloxacin are therefore important determinants of drug exposure in patients treated with this compound.

In common with other fluoroquinolones, moxifloxacin is known to accumulate in human cells, most notably in human polymorphonuclear leucocytes and macrophages, where it shows pronounced activity against several types of intracellular bacteria. Recent data in J774 macrophages demonstrated that ciprofloxacin, garenoxicin, levofloxacin and norfloxacin, but not moxifloxacin, are subject to active efflux by the multidrug-resistance-related protein 1 (MRP1) in humans (Michot et al 2005). Since MRP2 shows the same substrate specificity as MRP1, we hypothesize that moxifloxacin and/or its metabolites may also be actively transported across cellular membranes by MRP2. Our hypothesis is supported by recent studies showing that the hepatobiliary transport of the unconjugated and glucuronidated fluoroquinolones – prulifloxacin and grepafloxacin – are also recognized by MRP2 (Sasabe et al 1998; Yagi et al 2003).

MRP2 (ABCC2) is expressed in the canalicular membrane of hepatocytes, the apical membranes of the small intestine and the proximal tubules of the kidney (Chu et al 2004). MRP2 is therefore involved in hepatobiliary, renal and intestinal excretion of clinically important anionic drugs, as well as the intracellularly formed glucuronide and sulfate conjugates of many compounds (Mizuno et al 2003). Homologues of human MRPs have also been found in pathogenic bacteria and are becoming increasingly recognized as a major component of antibiotic drug resistance (Rouveix 2007).

The substrate specificity of MRP2 has been defined in detail using the mutant rat strain TR^-, which lacks functional Mrp2 at the canalicular membrane (Kitamura et al 1992; Elferink & Groen 2002).

Here we have used isolated perfused rat livers of this Mrp2-deficient rat strain to investigate the role of MRP2 in the cellular transport of moxifloxacin and its glucuronidated and sulfated conjugates. Mrp2-competent Wistar rats were used as controls. Compared with in-vivo studies, the isolated perfused rat liver is a better model to study the metabolism and biliary elimination of drugs, since pharmacokinetic variables such as renal clearance and drug distribution are eliminated.

Materials and Methods

Materials

Moxifloxacin hydrochloride was obtained from Bayer Corporation (Leverkusen, Germany). Bromsulphthalein

(BSP), sulfatase type V from limpets (*Patella vulgata*), β-glucuronidase type B-3 from bovine liver, heptane sulfonic acid and phosphoric acid were purchased from Sigma (Munich, Germany). The methanol and water used were of HPLC grade (Mallinckrodt Baker, Deventer, Holland). All other chemicals and solvents were commercially available and of analytical grade.

Liver perfusion

Livers of male Wistar rats (body weight 246 ± 5.77 g; liver weight 12.3 ± 0.760 g) and male TR^- rats (body weight 260 ± 20.0 g; liver weight 14.9 ± 1.07 g) were purchased from the Department of Animal Research and Genetics of the Medical University of Vienna, Austria. Animals were housed in a temperature- and humidity-controlled room with a 12 h light–dark cycle and free access to water and food. The study was approved by the committee for animal welfare at the institution.

Single-pass liver perfusion experiments were carried out using the techniques previously described (Jäger et al 1997). Perfusions were conducted using Krebs–Hensleit bicarbonate buffer, pH 7.4 (KHB), equilibrated with 95% O_2/5% CO_2 and delivered at a flow rate of 3.1 mL (g liver)$^{-1}$ min^{-1} in livers from Wistar rats and 2.8 mL (g liver)$^{-1}$ min^{-1} in livers from TR^- rats (in order to correct for higher liver weights of TR^- rats) using a peristaltic pump (Model PA-SF, IKA-Labortechnik, Staufen i. Br., Germany). Delivery was via the portal vein. The effluent perfusate was collected from the inferior vena cava. The total volume of the perfusion reservoir was 2.5 L for each liver perfusion. The temperature of the perfusion cabinet and perfusion medium was thermostatically controlled at 37°C. Single bile drops falling from the bile duct cannula were weighed (approx 8 mg) and collected. Bile flow was determined from the time interval between drops and the liver weight given in mg g liver^{-1} min^{-1} and was not stimulated by the addition of taurocholate to the perfusion medium. If bile salts are absent in the perfusate, the main forces driving bile secretion are the increased excretion of other osmotically active compounds into bile, which cause an increase in water flow (hydrocholeresis) (Graf 1983). In the rat, so-called ‘bile-salt-independent bile flow’ amounts to more than 60% of total bile secretion (Boyer & Klatskin 1970). In the isolated perfused rat liver, during perfusion with KHB for 30 min, bile flow reaches a constant rate and can be considered as bile salt independent because under these conditions bile acids in bile are nearly absent. After 30 min of perfusion with KHB, a stock solution of moxifloxacin, 10 mM in DMSO (final DMSO concentration 0.05%), was added to give a final concentration of 10 μM. The water-soluble BSP was added directly to the KHB. Perfusion pressure was approximately 6 cm H_2O (15 mmHg) and remained constant during control and moxifloxacin perfusions.

To test the stability of moxifloxacin under perfusion conditions, control experiments were performed by adding the drug solution to the perfusion model in the absence of a liver. This did not result in any detectable degradation products of moxifloxacin for up to 60 min.

Determination of moxifloxacin and its metabolites in bile and perfusate

In order to study time-dependent first-pass metabolism, bile (30–50 μL) and perfusate (approximately 1.5 mL) samples were collected over 5 min intervals for up to 60 min. Samples were immediately frozen on dry ice, and stored at –80°C until analysis. Before analysis, bile samples were centrifuged (5000 *g* for 5 min). Aliquots (100 μL) of diluted bile samples (10 μL supernatant plus 990 μL distilled water, or perfusate supernatant were injected onto the HPLC column. HPLC analysis was performed with minor modifications of the previously described method (Fuhrmann et al 2004). Briefly, moxifloxacin and its metabolites were separated on a Hypersil BDS-C18 column (5 μm, 250×4.6 mm i.d., Astmoor, Runcorn, UK) preceded by a Hypersil BDS-C 18 precolumn (5 μm, 10×3.6 mm i.d.) at a column temperature of 35°C. The mobile phase consisted of a continuous gradient mixed from ion pair buffer, pH 3.0 (50 mM potassium phosphate with phosphoric acid and 5 mM heptane sulfonic acid) and methanol, at a flow rate of 1 mL min^{-1}. Moxifloxacin and its metabolites were monitored fluorimetrically at 296 nm (excitation) and 504 nm (emission). Linear calibration curves were calculated from the peak areas of moxifloxacin and its metabolites compared with the external standard moxifloxacin in samples created by spiking drug-free bile and perfusate with standard solutions of moxifloxacin to obtain a concentration range of 5 ng to 25 μg mL^{-1}) (average correlation coefficients > 0.99). For this method, the lower limit of quantification for moxifloxacin was 5 ng mL^{-1} in both bile and perfusate. Intra-day variability for moxifloxacin was 3.6–7.9% and inter-day variability was 3.9–8.7% at moxifloxacin concentrations of 0.1, 0.5, 1 and 10 μg (mL bile)$^{-1}$. The detection limit, defined as a signal-to-noise-ratio of 3, was 0.5 ng mL^{-1} for both bile and perfusate.

Structural identification of moxifloxacin metabolites

Bile samples (5 μL) were mixed with 85 μL 0.1 M sodium acetate buffer (pH 6.8) and incubated with 10 μL β-glucuronidase (10 U) or 10 μL sulfatase (5 U) at 37°C for 1 h. The reaction was stopped by placing the vials on ice for 3 min. The samples were centrifuged at 5000 *g* for 5 min and 80 μL of the clear supernatant was injected onto the HPLC column as described above. In control experiments, bile samples containing no moxifloxacin were incubated without β-glucuronidase or sulfatase under identical conditions. Liquid chromatography/mass spectrometry (LC/MS) measurements of moxifloxacin and its metabolites were performed using an HPLC system fitted with a 235C diode array detector and a 200 autosampler (Perkin Elmer, Sciex Instruments, Wellesley, MA, USA). The system was coupled in-line to an Api 150Ex mass selective detector (Perkin Elmer, Sciex Instruments, Wellesley, MA, USA) fitted with an atmospheric pressure ionization source for electrospray ionization in the negative mode. The operating conditions were as follows: capillary voltage +3.6 kV; orifice voltage 35 V; gas temperature 400°C. The column, mobile phase, gradient, flow rate and injection

volume were the same as in the analytical HPLC assay described above.

Determination of bromsulphthalein in bile

BSP concentration was measured after dilution of bile with 0.1 M NaOH by absorbance at 575 nm according to a standard protocol (Jäger et al 1997). Moxifloxacin (up to 100 μM) did not interfere with the assay.

Data analysis

The availability (F) of moxifloxacin in the perfusate was calculated using the equation $F = C_{out}/C_{in}$, where C_{in} is the administered dose of moxifloxacin applied to the liver over the 60 min perfusion and C_{out} is the amount of moxifloxacin excreted in the effluent perfusate over 60 min.

The hepatic Cl of moxifloxacin was calculated as: $Cl = E \times$ perfusate flow rate, where E is the hepatic excretion rate $(1 - F)$.

The amount of moxifloxacin and its metabolite recovered in bile during the 60 min perfusion, expressed as a percentage of the administered dose (f_{bile}), was calculated as:

$f_{bile} = [(C_c \times \text{bile flow} \times \text{liver weight})/C_{in}] \times 100$, where C_{in} is the amount of moxifloxacin applied to the liver during single-pass perfusion for 60 min and C_c is the cumulative concentration of moxifloxacin and its metabolites in bile.

The amount of moxifloxacin and its metabolite recovered in bile during the 60 min perfusion, expressed as a percentage of the administered dose ($f_{perfusate}$), was calculated as:

$f_{perfusate} = (C_c/C_{in}) \times 100$, where C_{in} is the amount of moxifloxacin delivered to the liver during the 60 min single-pass perfusion and C_c is the cumulative concentration of moxifloxacin and its metabolites in the perfusate.

Statistical analysis

Data are expressed as mean + s.d. of three individual experiments. The Mann–Whitney *U*-test was used to calculate the difference between the values in Wistar and TR^- rats (using SPSS for Windows, version 11.5.0; SPSS, Chicago, IL, USA). The threshold of significance was $P < 0.05$.

Results

Influence of moxifloxacin on bile flow

As shown in Figure 2, addition of moxifloxacin (10 μM) to the perfusion medium stimulated bile flow by 11.2% in perfused livers of Wistar rats (a non-significant increase). Bile flow reached peak values of 0.99 ± 0.062 mg (g liver)$^{-1}$ min^{-1} within 10 min, indicating moderate solvent drag by the excretion of moxifloxacin and its metabolites into bile. After this initial maximum, bile flow slowly decreased returning to initial values (0.89 ± 0.049 mg (g liver)$^{-1}$ min^{-1}) by the end of the 60 min perfusion. In contrast to Wistar rats, addition of moxifloxacin to the perfusion medium did not influence bile flow of perfused livers from TR^- rats (Figure 2).

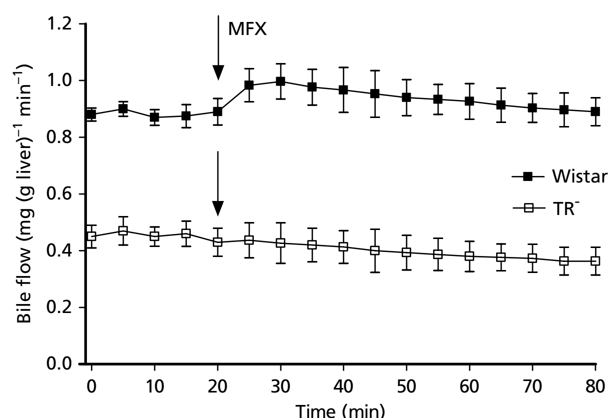

Figure 2 Influence of moxifloxacin on bile flow in Wistar and TR^- rats. Rat livers were perfused with Krebs–Henseleit buffer for 20 min before addition of 10 μM moxifloxacin to the perfusion medium. Data represent the mean ± s.d. of three independent experiments.

Metabolism and disposition of moxifloxacin in Wistar rats

The concentration of moxifloxacin and the formation of moxifloxacin metabolites in bile and perfusate samples during perfusion of livers with moxifloxacin (10 μM) was determined every 5 min for up to 60 min using an established HPLC assay. In addition to native moxifloxacin (retention time 14.60 min), two metabolites, M1 and M2, with retention times of 8.06 and 14.04 min, respectively, could be detected in bile.

Treatment of bile samples with β-glucuronidase led to the disappearance of M1 and a concomitant increase in moxifloxacin, whereas M2 remained unchanged. After analogous treatment with sulfatase, M2 disappeared and there was a concomitant increase in parent moxifloxacin. These results indicate that M1 is conjugated with sulfuric acid and M2 with glucuronic acid. Furthermore, LC/MS analysis of bile samples revealed stable molecular $[M+H]^+$ ions at m/z 402, m/z 578 and m/z 481 amu, in agreement with the molecular weights of moxifloxacin, moxifloxacin monoglucuronide (M1: moxifloxacin + 176 amu), and moxifloxacin monosulfate (M2: moxifloxacin + 80 amu).

The kinetics of the excretion of moxifloxacin and its two metabolites into bile after addition of 10 μM moxifloxacin to the rat liver are shown in Figure 3A. Biliary excretion of unconjugated moxifloxacin, M1 and M2 reached a plateau after 10 min and remained fairly constant until the end of the perfusion (maximum values: 74.0 ± 11.2, 50.5 ± 10.3 and 83.5 ± 15.7 pmol (g liver)$^{-1}$ min^{-1}, respectively). The total amounts of moxifloxacin, M1 and M2 in the bile during 60 min of perfusion were: 0.225 ± 0.042%, 0.149 ± 0.031% and 0.253 ± 0.043% of the applied dose, respectively.

Secretion of moxifloxacin, M1 and M2 into the effluent perfusate reached steady state after 20–30 min, with maximum values of $29\,656 \pm 1358$, 54.8 ± 15.0 and 223 ± 118 pmol (g liver)$^{-1}$ min^{-1}, respectively (Figure 3B). The total amounts of moxifloxacin, M1 and M2 excreted in perfusate during 60 min of perfusion were: 80.7 ± 5.04%,

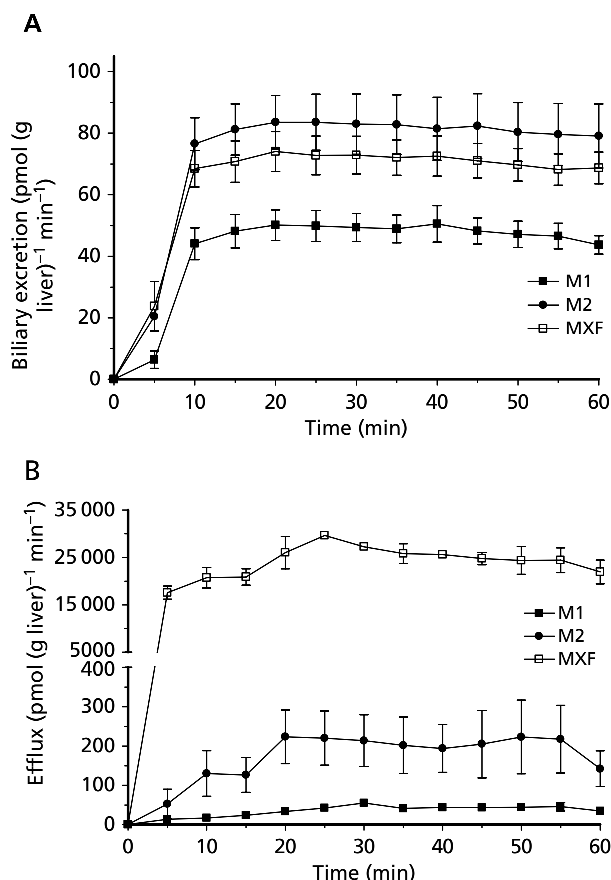

Figure 3 Time course of the biliary excretion (A) and efflux (B) of moxifloxacin (MXF) and its hepatically formed metabolites M1 (glucuronide) and M2 (sulfate) in Wistar rats following addition of 10 μM to the perfusate. Data represent mean + s.d. of three individual experiments.

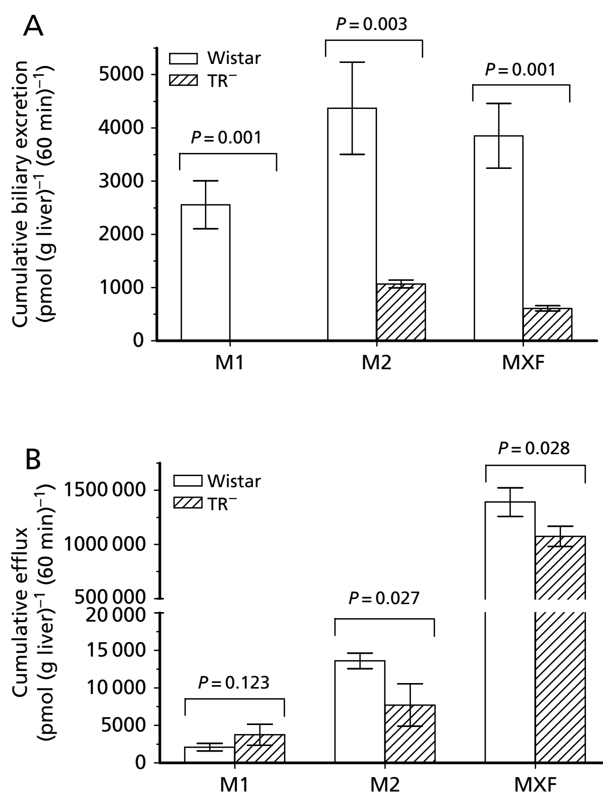

Figure 4 Cumulative biliary excretion of moxifloxacin (MFX) and its metabolites M1 (glucuronide) and M2 (sulfate) into bile (A) and effluent perfusate (B) of Wistar and TR^{-} rats. Data represent mean ± s.d. of three individual experiments. $P < 0.05$ Wistar vs TR^{-} rats.

0.121±0.025% and 0.597±0.323% of the applied dose, respectively.

Metabolism and disposition of moxifloxacin in TR^{-} rats

In mutant TR^{-} rats, moxifloxacin and its two conjugates could also be identified in bile and effluent perfusate. However, biliary excretion and efflux into perfusate during the 60 min perfusion differed greatly between the two strains of rat (Figure 4). Cumulative biliary excretion of moxifloxacin, M1 and M2 were significantly reduced to approximately 19.1%, 0.027% and 29.6% in TR^{-} rat livers compared with Wistar livers, whereas cumulative efflux of M1 was increased by 79%. Excretion of the parent drug and M2 into perfusate was decreased slightly, to 91.1% and 93.6% of values in Wistar rats, respectively.

Bioavailability and hepatic clearance of moxifloxacin

Pharmacokinetic parameters describing the disposition of moxifloxacin in livers of Wistar rats and TR^{-} rats were determined as described in the Methods section. Based on the low rate of metabolism in both rat strains, moxifloxacin has a high bioavailability, F (0.807±0.050 in Wistar rats; 0.755±0.050 in TR^{-} rats), which means that 19.3% and 14.5% of the parent drug is extracted by the livers of these rat strains during first pass through the liver (Table 1). The low extraction ratio also results in low Cl: 6.85±1.96 mL min^{-1} in Wistar rats and 8.73±1.82 mL min^{-1} in TR^{-} rats (perfusate flow rate 35.3±1.15 and 35.7±0.580 mL min^{-1}, respectively). The amounts of moxifloxacin and the M1 and M2 metabolites excreted into bile of Wistar rats (expressed as the percentage of the administered dose – f_{bile}) (0.225±0.042%, 0.149±0.031% and 0.0253±0.043%, respectively) differed significantly from values in TR^{-} rats (0.043±0.005%, 0.00004±0.00002% and 0.075±0.003%, respectively), again supporting the predominant role of Mrp2 in the biliary elimination of this drug and its conjugated biotransformation products. The total amount of moxifloxacin, M1 and M2 excreted into the effluent perfusate expressed as the percentage of dose ($f_{perfusate}$) was high and showed no significant differences between the two rat strains (80.7±5.05% vs 75.5±4.95% in Wistar and TR^{-} rats, respectively).

Effects of moxifloxacin on bromsulphthalein excretion

Two min after addition of BSP (1 μM) to the perfusion medium, the concentration increased to reach a steady-state

Table 1 Disposition of moxifloxacin (MXF) and its metabolites, M1 (glucuronide) and M2 (sulfate), in the isolated perfused rat liver of Wistar (control) and TR$^-$ (Mrp2-deficient) rats

Parameters	Wistar	TR$^-$
F	0.807 ± 0.050	0.755 ± 0.050
E	0.193 ± 0.050	0.245 ± 0.050
Cl (mL min^{-1})	6.85 ± 1.96	8.73 ± 1.82
f_{bile} (% dose)		
MXF	0.225 ± 0.042	0.043 ± 0.005*
M1	0.149 ± 0.031	0.00004 ± 0.00002*
M2	0.253 ± 0.043	0.075 ± 0.003*
$f_{perfusate}$ (% dose)		
MXF	80.7 ± 5.05	75.5 ± 4.95
M1	0.121 ± 0.025	0.265 ± 0.102
M2	0.597 ± 0.323	0.544 ± 0.206

Perfusions were performed with 10 μM moxifloxacin for 60 min using a single-pass model. Data represent mean ± s.d. of three individual experiments. *$P < 0.05$ Wistar vs TR$^-$ rats. F, bioavailability; E, hepatic excretion ratio; Cl, hepatic clearance; f_{bile}, amount excreted into bile; $f_{perfusate}$, amount excreted into perfusate (both as % of administered dose).

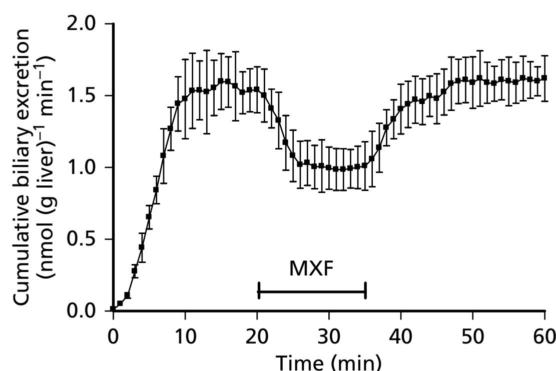

Figure 5 Effect of 10 μM moxifloxacin (MFX) on the biliary excretion of 1 μM bromsulphthalein in the isolated perfused liver of Wistar rats. After achieving a constant biliary excretion of bromsulphthalein (t = 20 min), 10 μM moxifloxacin was applied for 15 min.

excretion rate of 1.54 ± 0.16 nmol (g liver)$^{-1}$ min^{-1} (Figure 5). BSP is excreted into the bile of Wistar rats mainly as glutathione conjugate, which is a specific substrate of Mrp2 (Jäger et al 1997). The addition of moxifloxacin (10 μM) to the BSP-containing perfusion medium gradually decreased biliary excretion of BSP by 34.4%, to 1.01 ± 0.13 nmol (g liver)$^{-1}$ min^{-1} after 15 min. When moxifloxacin was removed from the perfusion medium, the biliary excretion of BSP recovered rapidly within 13 min, to reach a level of 1.60 ± 0.15 nmol (g liver)$^{-1}$ min^{-1}.

Discussion

In patients, biliary excretion is an important elimination pathway for moxifloxacin and its metabolites. However, to our knowledge, there is no in-vivo evidence that moxifloxacin is a substrate of MRP2 or that the hepatobiliary excretion of moxifloxacin is mediated by this efflux pump. The aim of the present study was therefore to investigate the role of the primary active transport system, termed 'canalicular multispecific organic anion transporter' (cmoat) in the secretion of moxifloxacin and its conjugated biotransformation products. Using an isolated perfused rat liver model of cmoat-deficient TR$^-$ rats, we found that the biliary excretion of moxifloxacin glucuronide (M1) was reduced to 0.027% compared with Wistar rats. This inability of TR$^-$ rats to excrete M1 indicates that M1 is almost exclusively eliminated into bile by cmoat in control (Wistar) rats. In contrast to moxifloxacin glucuronide, biliary secretion of unconjugated moxifloxacin and its sulfated metabolite M2 was partially maintained in the Mrp2-deficient rat livers (19.1% and 29.6%, respectively, compared with Wistar rats), indicating that there is a second transport system besides Mrp2 for moxifloxacin and its sulfate.

Different biliary excretion systems for glucuronides and sulfates have already been documented by other authors (Takenaka et al 1995; Zamek-Gliszczynski et al 2005). A likely candidate for the canalicular transport of moxifloxacin sulfate is the breast cancer resistant protein (BCRP, ABCG2), which is distributed in several tissues such as placenta, small intestine, colon and breast, and may play an important role in the biliary excretion of the sulfated conjugates of steroids and xenobiotics (Chandra & Brouwer 2004). This is in accordance with recent literature which showed high expression of this transport protein in the canalicular membrane of human liver cells (Maliepaard et al 2001).

The involvement of an active transport system is also supported by a recent clinical study in patients which showed that moxifloxacin is secreted extensively into the bile duct system, even in the presence of biliary obstruction (Schwab et al 2005). Efficient transport of moxifloxacin and its conjugates by cmoat is also suggested by the fact that the addition of moxifloxacin stimulates bile flow in control rats but not in TR$^-$ rats. This choleresis was maximum after 10 min (+11% at the peak value) and appears to result from the osmotic effects of parent moxifloxacin and its metabolites to the bile, where M1 reached concentrations of up to 74 μM, M2 109 μM and moxifloxacin 123 μM.

The total amount of moxifloxacin and its conjugates excreted into bile during 60 min of perfusion in Wistar rats was only 0.63% of the administered dose. In humans, however, the amount of parent moxifloxacin and its metabolites excreted into bile was about 61% (Stass & Kubitza 1999). This difference can be attributed to the fact that the isolated perfused rat liver cannot be maintained for longer than 60 min, as the bile flow decreases progressively during the perfusion process. Far higher amounts of moxifloxacin and its metabolites are therefore excreted into the bile beyond 60 min.

To test whether moxifloxacin and its conjugates compete with Mrp2-specific substrates, we studied the biliary secretion of the organic anion BSP and its modulation by moxifloxacin in Wistar rats. We found that 10 μM moxifloxacin reversibly inhibited Mrp2-mediated biliary elimination of BSP-reduced glutathione by 34%. After removal of moxifloxacin from the perfusion medium, the biliary excretion of conjugated BSP recovered rapidly within approximately

13 min to reach levels similar to those before the administration of moxifloxacin.

Inhibition of the Mrp2-mediated canalicular transport of BSP by moxifloxacin may have clinical relevance, as moxifloxacin elimination into bile might be decreased when other MRP2-specific drugs are co-administered during therapy. The observed overall reduced bile flow in mutant TR^- rats by more than 49%, however, is caused by a reduction of bile-acid-independent bile fraction seen in adult rats and is not initiated by moxifloxacin (Jansen et al 1985).

Our data also showed that acyl glucuronidation provides the main metabolic pathway for moxifloxacin and other fluoroquinolone-related antibiotic agents. It has been proposed that acyl glucuronides react with proteins via either a nucleophilic acyl substitution, which results in acylation of protein nucleophiles, or glycation mechanisms, in which the acyl glucuronide becomes covalently bound to the protein via an open-chain open-acyl migrated isomer (Bailey & Dickinson 2003; Tachibana et al 2005). Some of these acyl-glucuronide-forming drugs, including benoxaprofen, diflunisal, tolmetin and zomipirac, cause toxic idiosyncratic reactions, which are probably related to the formation of covalent adducts between the reactive acyl glucuronide and a functional protein (Shipkova et al 2003). Among the fluoroquinolone-related compounds, specific idiosyncratic adverse reactions have been reported for temafloxacin, trovafloxacin and tosufloxacin, such as temafloxacin-associated haemolytic uracaemic-like syndrome and temafloxacin-associated liver failure (Andersson & MacGowan 2003; Ball 2003). Whether moxifloxacin also causes acyl-glucuronide-mediated adverse reactions remains unknown at present. However, fluoroquinolone-associated anaphylactoid and anaphylactic reactions have been documented recently as potentially fatal events, not only for moxifloxacin but also for ciprofloxacin, ofloxacin, norfloxacin and levofloxacin (Aleman et al 2002; Ho et al 2003; Sachs et al 2006). Additional studies are therefore needed to further elucidate the chemical reactivity of the acyl glucuronide of moxifloxacin and the observed anaphylactoid and anaphylactic reactions.

Conclusions

Our data demonstrate that Mrp2 is the transporter associated with the biliary excretion of moxifloxacin and its glucuronidated and sulfated metabolites in rat liver. MRP2 may therefore play a key role in the excretion of moxifloxacin and its conjugates into bile of patients.

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