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# Sex-Dependent Expression and Activity of the ATP-Binding Cassette Transporter Breast Cancer Resistance Protein (BCRP/ABCG2) in Liver

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## ABSTRACT

The breast cancer resistance protein (BCRP/ABCG2) is an ATP-binding cassette drug efflux transporter present in the liver and other tissues that affects the pharmacological behavior of many compounds. To assess the possible role of BCRP in sex-dependent pharmacokinetics, we studied the in vivo disposition of several murine Bcrp1 substrates in male and female wild-type and Bcrp1 knockout mice. After oral administration of the antibiotic nitrofurantoin, the area under the plasma concentration-time curve in wild-type female mice was approximately 2-fold higher than in wild-type male mice. Moreover, after i.v. administration of nitrofurantoin, the antiulcerative cimetidine, the anticancer drug topotecan, and the carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), the plasma levels in wild-type female mice were all significantly higher than those in wild-type male mice. Analysis of the expression of murine Bcrp1 in several pharmacokinetically important tissues showed that only the hepatic Bcrp1 expression was higher in male mice compared with female mice. In line with this difference, the hepatobiliary excretion for nitrofurantoin and PhIP was, respectively, 9-fold higher and approximately 2-fold higher in male compared with female wild-type mice. No significant sex differences were observed in plasma levels or hepatobiliary excretion for any of the tested compounds in Bcrp1$^{-/-}$ mice, indicating that Bcrp1 was the main cause of the sex difference in wild-type mice. Analysis of hepatic expression of human BCRP also indicated a higher expression in men compared with women. In conclusion, sex-dependent expression of BCRP/Bcrp1 in the liver may be a cause of sex-specific variability in the pharmacokinetics of BCRP substrates, with potential impact on the clinical-therapeutic applications and toxicity risks of drugs.





The hepatocyte is a major player in transport, metabolism, and excretion of many endogenous and xenobiotic compounds. It is endowed with a variety of transporters of the ATP-binding cassette (ABC) family to mediate biliary excretion at the canalicular membrane, including the breast cancer resistance protein (BCRP/ABCG2), a multidrug transporter (Doyle et al., 1998; Allen and Schinkel, 2002). Apart from its presence in the biliary canalicular membrane of hepatocytes, BCRP is also localized in the apical membranes of intestinal, placental, and mammary alveolar epithelia, and several other epithelia. This transmembrane protein affects the pharmacological and toxicological behavior of many drugs and toxins. It transports a range of structurally diverse compounds, including anticancer drugs, dietary compounds, food carcinogens such as 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), antibiotics such as nitrofurantoin, and other drugs such as cimetidine (van Herwaarden et al., 2003; Burger et al., 2004; Jonker et al., 2005; Merino et al., 2005; Pavek et al., 2005). Several in vivo studies indicated that Bcrp1 mediates the hepatobiliary excretion and secretion into the milk of its (drug) substrates and limits their oral bioavailability (F) and fetal and brain penetration (Jonker et al., 2000, 2005; van Herwaarden et al., 2003; Cisternino et al., 2004).

Several anticancer drugs that are known BCRP substrates such as topotecan, methotrexate, and doxorubicin have displayed sex-dependent pharmacokinetics in humans (Wade et al., 1992; Dobbs et al., 1995; Godfrey et al., 1998; Gallo et al., 2000; Wall et al., 2000; Loos et al., 2003). Sex is one variable that contributes to interindividual dif-

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**ABBREVIATIONS:** ABC, ATP-binding cassette; BCRP, breast cancer resistance protein; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; mAb, monoclonal antibody; HPLC, high-performance liquid chromatography; AUC, area under the plasma concentration-time curve; F, oral bioavailability.

ferences in pharmacokinetics, including absorption, distribution, metabolism, and excretion (Morris et al., 2003), and a potentially important determinant for the clinical effectiveness of drug therapy. To assess the possible role of BCRP in the sex-dependent pharmacokinetics of its substrates, we studied the in vivo disposition of several known murine Bcrp1 substrates in male and female wild-type and Bcrp1 knockout mice. Sex-associated differences in transport processes for endogenous and exogenous substrates have been reported in various organs, including liver, in rats, mice, and humans (for reviews, see Meibohm et al., 2002; Morris et al., 2003). For this reason, we determined the expression of murine Bcrp1 in liver and other tissues in male and female mice. To extrapolate the data to humans, we further determined the expression of human BCRP in livers of men and women.

## Materials and Methods

**Animals and Human Tissues.** Mice were housed and handled according to institutional guidelines complying with Dutch legislation. Animals used were Bcrp1$^{-/-}$ and wild-type mice, all of >99% FVB genetic background between 9 and 14 weeks of age, unless indicated otherwise. Bcrp1$^{-/-}$ and wild-type mice of 129/Ola genetic background in the same age range were used when indicated. Animals were kept in a temperature-controlled environment with a 12-h light/dark cycle and received a standard diet (AM-II; Hope Farms, Woerden, The Netherlands) and acidified water ad libitum.

Normal human liver samples were obtained from the tissue cryobank of The Netherlands Cancer Institute from surgical specimens. No systematic difference between male and female donors was found regarding the following variables: age, disease, smoking, or drinking habits and treatments. All of the tissues were snap frozen in liquid nitrogen and subsequently stored at −70°C.

**Chemicals.** Nitrofurantoin, xylazine, and cimetidine were from Sigma-Aldrich (St. Louis, MO); PhIP and [$^{14}$C]PhIP (10 Ci/mol) were from Toronto Research Chemicals, Inc. (Toronto, ON, Canada); topotecan (Hycamtin) and [$^{14}$C]topotecan (56 Ci/mol) were from SmithKline Beecham (King of Prussia, PA); [*N*-methyl-$^{3}$H]cimetidine (20 Ci/mmol) was from Amersham Biosciences UK, Ltd. (Little Chalfont, Buckinghamshire, UK); ketamine (Ketanest-S) was from Parke-Davis (Hoofddorp, The Netherlands); and methoxyflurane (Metofane) was from Medical Developments Australia Pty, Ltd. (Springvale, VIC, Australia). All other compounds used were reagent grade.

**Western Blot Analysis.** Crude membrane fractions from tissues were prepared as described previously (Ogihara et al., 1996). Western blotting was performed as described previously (Jonker et al., 2001). For detection of murine Bcrp1, blots were probed with mAb BXP-53 (1:20), and mAb binding was detected by using peroxidase-conjugated rabbit anti-rat IgG (1:1000; DakoCytomation Denmark A/S, Glostrup, Denmark). For detection of human BCRP, blots were probed with mAb BXP-21 (1:150) (Maliepaard et al., 2001), and mAb binding was detected by using peroxidase-conjugated goat anti-mouse IgG (1:1000; DakoCytomation Denmark A/S). Equal protein loading was confirmed by Ponceau S and India ink staining.

**Pharmacokinetic and Gall Bladder Cannulation Experiments.** Pharmacokinetic and gall bladder cannulation experiments were performed as described previously (Merino et al., 2005). For nitrofurantoin experiments, drug levels in plasma and bile were determined by HPLC (Merino et al., 2005). In i.v. administration of [$^{14}$C]topotecan, [$^{3}$H]cimetidine, and [$^{14}$C]PhIP, levels of radioactivity in plasma were determined by liquid scintillation counting. For [$^{14}$C]PhIP, at the end of the experiment, intestinal contents (feces) were separated from intestinal tissue and subsequently homogenized in 4% bovine serum albumin. Radioactivity in homogenates was determined by liquid scintillation counting.

**Pharmacokinetic Calculations and Statistical Analysis.** The two-sided, unpaired Student's *t* test was used throughout to assess the statistical significance of differences between two sets of data. Results are presented as the means ± S.D. Differences were considered to be statistically significant when $P < 0.05$. The area under the plasma concentration-time curves (AUCs) were calculated with the PK Solutions (Ashland, OH) computer program (Farrier, 1997) using the trapezoidal rule and further extrapolated to infinity by dividing the last experimental plasma concentration by the terminal slope ($\beta$). The F value for nitrofurantoin was determined by ($AUC_{p.o.}$/dose p.o.)/($AUC_{i.v.}$/dose i.v.), assuming linearity of dose dependence.

## Results

**Plasma Pharmacokinetics of Nitrofurantoin in Male and Female Mice.** To assess whether Bcrp1 might mediate sex differences in pharmacokinetics of its substrates, we first determined the plasma concentration of nitrofurantoin, a known Bcrp1 substrate (Merino et al., 2005), as a function of time, after oral and intravenous administration in male and female wild-type and Bcrp1$^{-/-}$ mice.

After oral administration of nitrofurantoin (10 mg/kg) (Fig. 1A), the AUC in wild-type female mice was 2-fold higher than in wild-type male mice. In contrast, there was no significant sex difference in the Bcrp1$^{-/-}$ mice (Table 1). We showed earlier that plasma levels of orally administered nitrofurantoin are higher in male Bcrp1$^{-/-}$ than wild-type mice (Merino et al., 2005). We now show that the difference between wild-type and Bcrp1$^{-/-}$ plasma AUCs is higher in male mice (3.3-fold; $P < 0.01$) compared with female mice (1.6-fold; $P < 0.01$; Table 1).

Upon i.v. administration (5 mg/kg) (Fig. 1B), the AUC in wild-type female mice was 1.7-fold higher than in wild-type male mice. Again, however, in Bcrp1$^{-/-}$ mice, there was no significant sex difference (Table 1), indicating that the sex difference observed in the wild-type mice is most probably mediated by Bcrp1. In addition, although there was a significant difference between wild-type and Bcrp1$^{-/-}$ plasma levels in male mice (1.7-fold; $P < 0.01$), this was not the case in female mice (Table 1).

Regarding F (Table 1), although there was a marked difference (between 1.6- and 2-fold) between wild-type and Bcrp1$^{-/-}$ mice, this value was very similar between both sexes, also in the wild types, indicating that oral and intravenous pharmacokinetics are affected to the same degree by the sex difference.

**Plasma Pharmacokinetics of Other Known Bcrp1 Substrates in Male and Female Mice.** To investigate whether the pharmacokinetic sex differences observed for nitrofurantoin could also apply to other known Bcrp1 substrates, we determined the levels of radioactivity in plasma, 30 min after intravenous administration (1 mg/kg) of the antiulcerative [$^{3}$H]cimetidine, the anticancer drug [$^{14}$C]topotecan, and the dietary carcinogen [$^{14}$C]PhIP, in male and female wild-type and Bcrp1$^{-/-}$ mice (Fig. 2).

In all cases, the plasma levels of compounds in wild-type female mice were significantly higher than those in wild-type male mice. In contrast, there was no significant sex difference in any case in the Bcrp1$^{-/-}$ mice. Moreover, for cimetidine (Fig. 2A), a significant difference between wild-type and Bcrp1$^{-/-}$ mice was only observed in male mice. For the other

two compounds, topotecan and PhIP (Fig. 2, B and C), both male and female mice showed a significant difference between wild-type and Bcrp1$^{-/-}$ mice. Note that plasma levels of intravenously administered Bcrp1 substrates are generally higher in Bcrp1$^{-/-}$ mice because of decreased elimination (Jonker et al., 2002; van Herwaarden et al., 2003). Bcrp1-mediated pharmacokinetic sex differences thus affect a variety of substrates.

**Western Blot Analysis of the Expression of Mouse Bcrp1 and Human BCRP in Both Sexes.** To assess whether the sex difference observed in the pharmacokinetics of Bcrp1 substrates was caused by a sex difference in Bcrp1 expression, we performed Western blot analysis of the primary organs determining plasma pharmacokinetics (i.e., liver, small intestine, and kidney), in male and female mice (Fig. 3).

We did not observe any clear sex-dependent difference in Bcrp1 expression in the small intestine and kidney (Fig. 3A). However, there was a marked sex difference in the liver samples of adult mice from two different mouse strains (FVB and 129/Ola), with expression consistently higher in the male mice (Fig. 3B). A dilution series showed that the difference was on the order of 2- to 3-fold in FVB mice (data not shown). We also assessed whether the sex difference in hepatic Bcrp1 levels was dependent on age. At 10 days after birth there was no sex difference (Fig. 3C), whereas around 5 weeks (puberty) the sex difference was already similar to that in adults (data not shown).

Because the lack of difference in plasma levels of nitrofurantoin between wild-type and Bcrp1$^{-/-}$ female mice (Table 1) was not in accordance with the 5-fold lower plasma levels we observed previously in lactating wild-type versus Bcrp1$^{-/-}$ female mice (Merino et al., 2005), we studied whether lactation or pregnancy could influence Bcrp1 expression in liver, small intestine, and kidney. However, Western blot analysis did not show any difference between virgin, lactating, and pregnant female mice in Bcrp1 expression in any organ tested (Fig. 4).

**Hepatobiliary Excretion of Nitrofurantoin and PhIP in Male and Female Mice.** Because the liver was the only pharmacokinetically important tissue displaying a clear sex difference in Bcrp1 expression, we expected that hepatobiliary excretion would be a primary cause of the sex difference. We therefore studied whether there was a Bcrp1-dependent

TABLE 1
AUC and F of male and female wild-type and Bcrp1$^{-/-}$ mice after oral and intravenous administration of nitrofurantoin

Mice received nitrofurantoin orally (10 mg/kg) or intravenously (5 mg/kg). Plasma levels were measured by HPLC to calculate the AUC, and F was calculated as described under *Materials and Methods*. Each value represents the mean ± S.D. ($n$ = 3–6).

| | AUC oral | AUC i.v. | F |
|---|---|---|---|
| | *min · μg/ml* | | % |
| Wild type | | | |
| Male | 54.7 ± 6.8 | 96.0 ± 9.0 | 28.5 ± 7.6 |
| Female | 113.0 ± 18.5* | 159.9 ± 21.5* | 35.3 ± 12.5 |
| Bcrp1$^{-/-}$ | | | |
| Male | 181.3 ± 30.8†† | 167.4 ± 22.0†† | 54.2 ± 19.7† |
| Female | 182.5 ± 30.9†† | 166.4 ± 15.9 | 54.8 ± 19.3† |

* $P < 0.01$, comparing difference between male and female mice.
† $P < 0.05$, comparing difference between wild-type and Bcrp1$^{-/-}$ mice of the same sex.
†† $P < 0.01$, comparing difference between wild-type and Bcrp1$^{-/-}$ mice of the same sex.

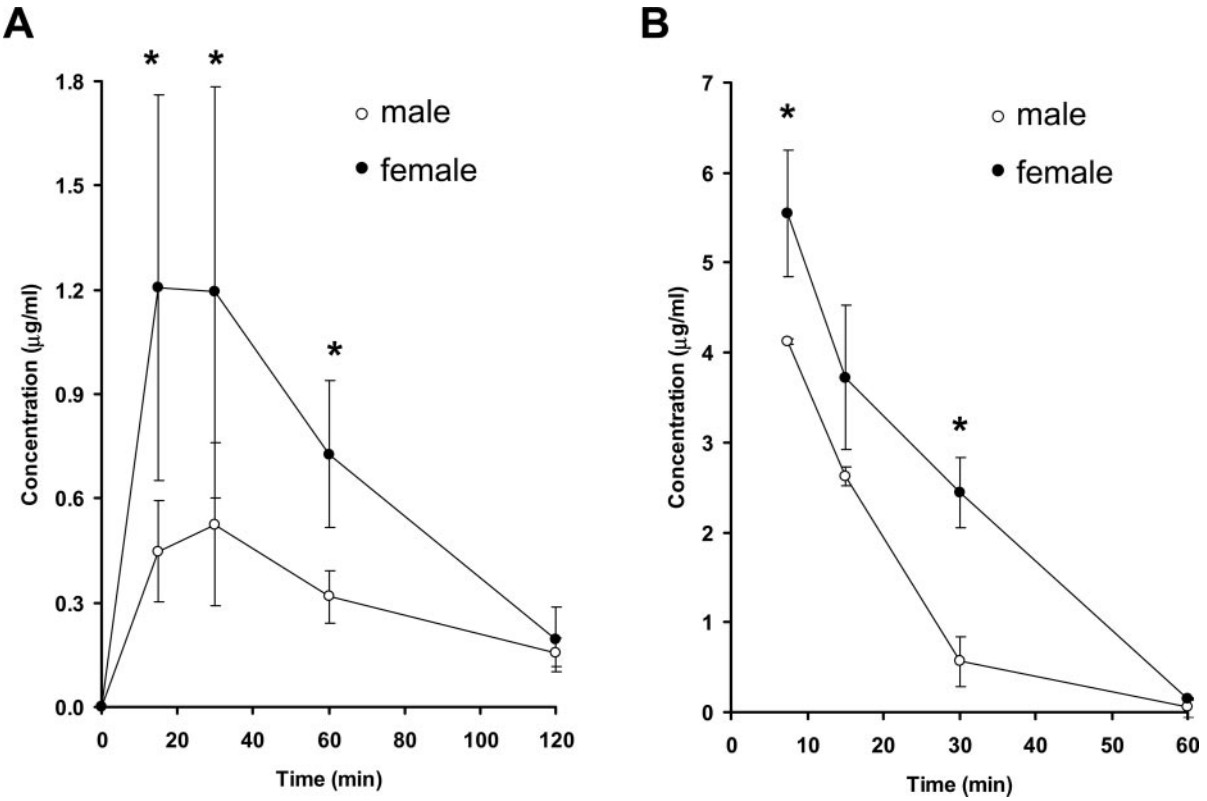


**Fig. 1.** Sex-dependent pharmacokinetics after oral (10 mg/kg) (A) and intravenous (5 mg/kg) (B) administration of nitrofurantoin. Plasma concentration versus time curve of male (○) and female (●) wild-type mice. Plasma levels of nitrofurantoin were determined by HPLC. Results are the means; error bars indicate S.D. ($n$ = 3–6; *, $P < 0.05$, comparing difference between male and female mice).

sex difference in hepatobiliary excretion of nitrofurantoin and PhIP.

We administered 5 mg/kg nitrofurantoin i.v. to male and female Bcrp1$^{-/-}$ and wild-type mice with a cannulated gall bladder and ligated common bile duct. Biliary excretion of nitrofurantoin was measured in fractions of 15 min during 1 h. All bile fractions from wild-type male mice showed a dramatically higher excretion of nitrofurantoin into bile compared with wild-type female mice (Fig. 5). At 1 h after i.v. administration, the cumulative nitrofurantoin excretion, as percentage of the dose, was 9.7 ± 3.2 versus 1.1 ± 0.5% in male and female wild-type mice, respectively. It thus seems that the higher expression of Bcrp1 in wild-type male liver correlates with markedly higher biliary excretion of nitrofurantoin. In addition, there was no sex difference in biliary nitrofurantoin excretion in Bcrp1$^{-/-}$ mice (0.17 ± 0.08 versus 0.12 ± 0.08% in male and female mice, respectively), which was virtually abolished in this type of mice, as demonstrated previously (Merino et al., 2005).

An indirect way of looking at biliary excretion is measuring small intestinal content after i.v. administration of the drug, although interference from the direct intestinal excretion can be present. In the case of PhIP, however, the

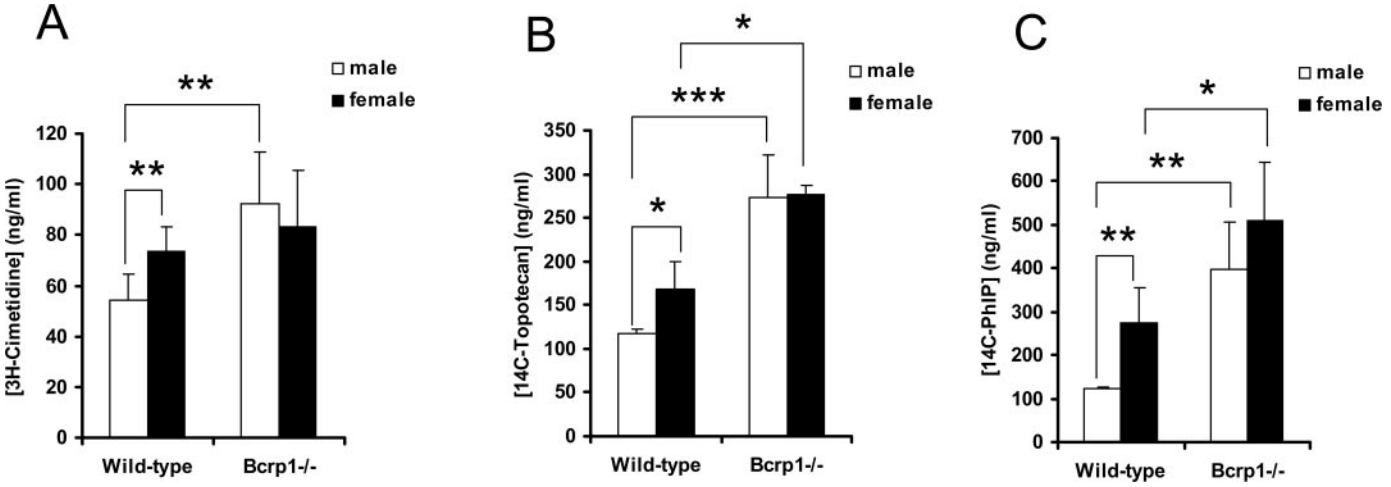


**Fig. 2.** Effects of sex and Bcrp1 genotype on plasma concentration 30 min after i.v. administration of 1 mg/kg [$^3$H]cimetidine (A), [$^{14}$C]topotecan (B), and [$^{14}$C]PhIP (C) in male and female wild-type and Bcrp1$^{-/-}$ mice. Results are the means; error bars indicate S.D. ($n$ = 4–12; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, comparing difference between male and female mice).

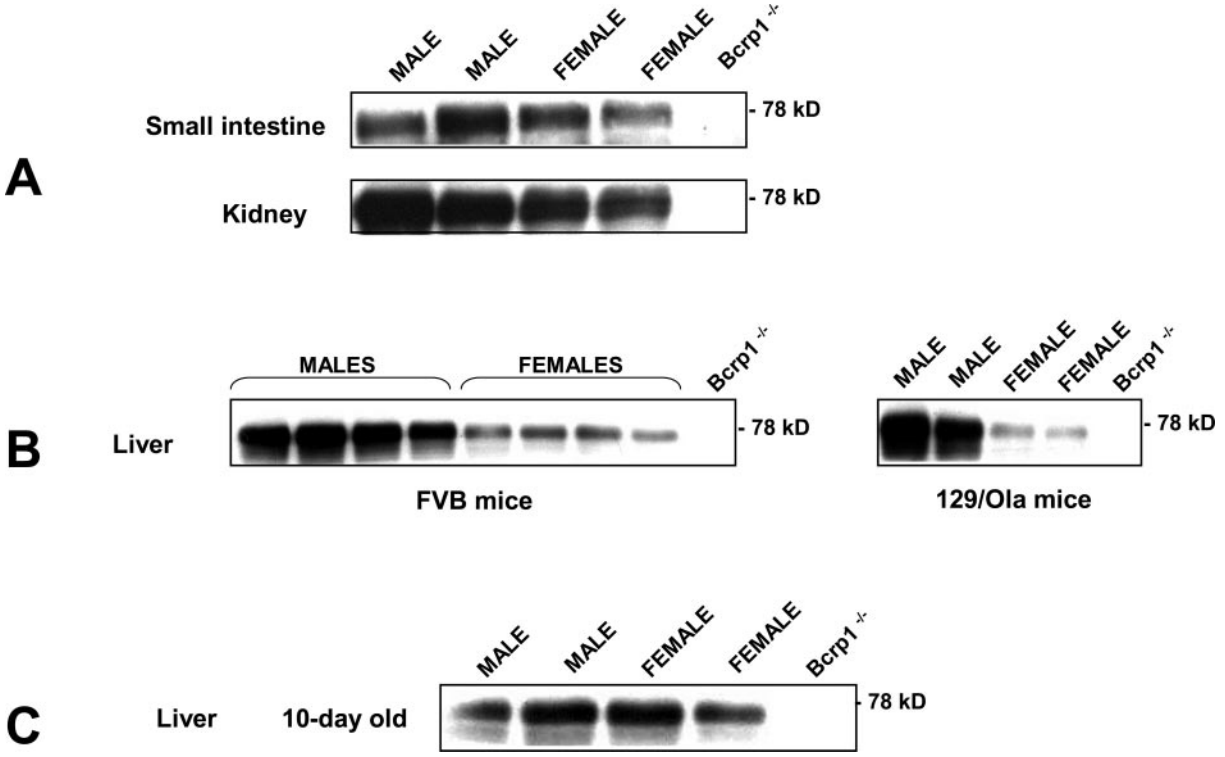


**Fig. 3.** Western blot analysis of murine Bcrp1 expression in male and female mice using crude membrane fractions (10 $\mu$g of protein per lane) from adult mouse small intestine and kidney (A), adult mouse liver of FVB and 129/Ola strains (B), or 10-day-old mouse liver of FVB strain (C). Equal protein loading was verified by Ponceau S and India ink total protein staining (not shown).

direct intestinal excretion is very low: approximately 4% in wild-type and 2% in Bcrp1$^{-/-}$ male mice (van Herwaarden et al., 2003). We administered 1 mg/kg [$^{14}$C]PhIP i.v. to male and female Bcrp1$^{-/-}$ and wild-type mice, and, after 30 min, small intestinal contents (feces) were separated from small intestinal tissue. Radioactivity measured in the small intestinal content was almost 2-fold higher in wild-type male mice (31.0 ± 2.5% of the dose) compared with female mice (16.5 ± 3.9% of the dose) (Fig. 6), indicating that the hepatobiliary excretion of PhIP was approximately 2-fold higher in male mice. Small intestinal content levels of [$^{14}$C]PhIP in Bcrp1$^{-/-}$ mice were still lower (7–8%), and no difference was observed between male and female mice. Subtracting the non-Bcrp1-dependent excretion, the Bcrp1-dependent PhIP excretion in male mice was more than 2.5-fold higher than in female mice. This is in line with the sex difference in hepatic Bcrp1 level as the primary cause for the male/female difference in intestinal PhIP content in wild-type mice.

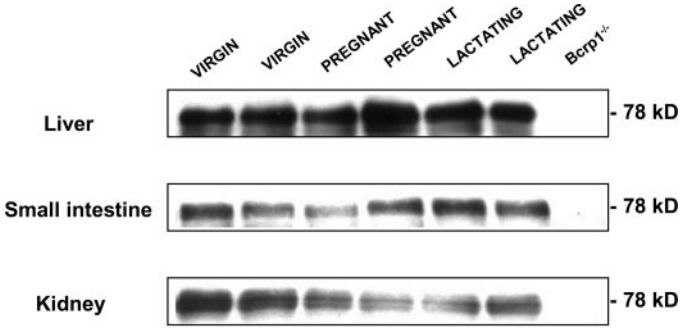


**Fig. 4.** Western blot analysis of murine Bcrp1 expression in virgin, pregnant, and lactating female mice using crude membrane fractions (10 $\mu$g of protein per lane) from liver, small intestine, and kidney. Equal protein loading was verified by Ponceau S and India ink total protein staining (data not shown).

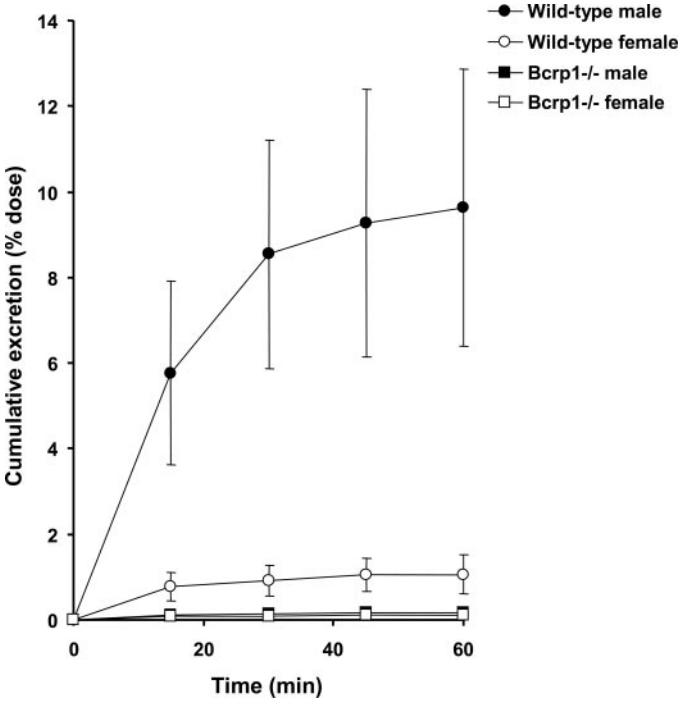


**Fig. 5.** Cumulative hepatobiliary excretion of nitrofurantoin in male and female wild-type and Bcrp1$^{-/-}$ mice. Nitrofurantoin (5 mg/kg) was administered i.v. to mice with a cannulated gall bladder. Levels of nitrofurantoin were measured in bile fractions by HPLC. Data are expressed as percentage of the dose. Results are the means; error bars (sometimes smaller than the symbols) indicate S.D. ($n$ = 5–9). At all time points, $P <$ 0.001, comparing male and female wild-type mice.

**Western Blot Analysis of the Expression of Human BCRP.** To extrapolate the data we obtained in mice to the human situation, we analyzed human BCRP expression in a limited series of livers of male and female humans (Fig. 7). The expression of BCRP was consistently higher in men compared with women, suggesting that the sex difference observed in mouse hepatic Bcrp1 may also apply to human BCRP.

## Discussion

Our data clearly show that the hepatic protein expression of mouse Bcrp1 is higher in male mice compared with female mice (Fig. 3B) and that the sex-dependent pattern of expression occurs during pubertal development (Fig. 3C). From the pharmacokinetic studies, we conclude that the sexual dimorphism in the hepatic expression of Bcrp1 causes the sex difference observed in the pharmacokinetics of several known Bcrp1 substrates: higher plasma levels in female mice after oral and i.v. administration (Figs. 1 and 2) and lower biliary excretion compared with male mice (Figs. 5 and 6). Our expression data further indicate that also in humans hepatic BCRP levels are higher in men than in women (Fig. 7).

Physiological differences between male and female exist in a variety of tissues, including some not usually considered to be sexually dimorphic. The mammalian liver is one example. Sexual differences have been described for hepatic transport as well as for enzymatic activities, drug detoxification, and lipid metabolism (Simon et al., 1999). Many enzymes belonging to the cytochrome P450 superfamily are expressed in the liver in unique, sex-dependent

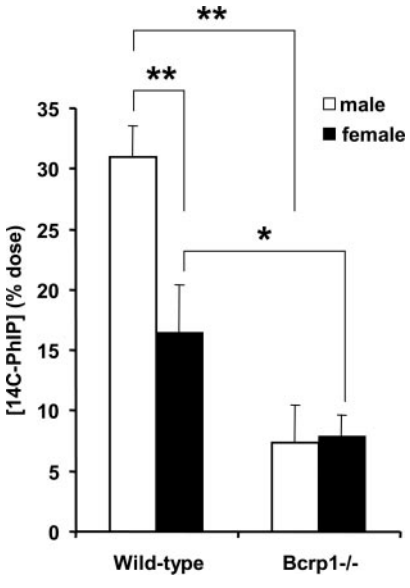


**Fig. 6.** Radioactivity measured in small intestinal content 30 min after i.v. administration of 1 mg/kg [$^{14}$C]PhIP to male and female wild-type and Bcrp1$^{-/-}$ mice. Data are expressed as percentage of the dose. Results are the means; error bars indicate S.D. ($n$ = 4–5; *, $P < 0.01$; **, $P < 0.001$).

patterns during postnatal and pubertal development (Shapiro et al., 1995). These sex differences are most dramatic in rodents, but they may occur in humans as well, where they have an impact on cytochrome P450-catalyzed drug metabolism and pharmacokinetics (Jaffe et al., 2002; Meibohm et al., 2002). It is interesting that the hepatic expression of the ABC transporter P-glycoprotein also seems to be higher in men compared with women (Schuetz et al., 1995).

The hepatic expression of murine Bcrp1 is influenced by sex but not by some sex-dependent physiological states like pregnancy or lactation, because no difference in Bcrp1 expression was observed between virgin, pregnant, and lactating female mice in liver (Fig. 4). In contrast, Bcrp1 expression in the mammary gland is highly induced during late pregnancy and especially lactation (Jonker et al., 2005), and Bcrp1 plays a prominent role in the secretion of several of its substrates, including nitrofurantoin, into milk (Jonker et al., 2005; Merino et al., 2005). Even Bcrp1-mediated milk excretion could affect nitrofurantoin plasma pharmacokinetics. Indeed, the 80-fold higher excretion of nitrofurantoin into the milk of the wild-type compared with the Bcrp1$^{-/-}$ lactating female mice, observed in the latter study, might be responsible for the early observed reduction in the plasma levels (5-fold) of the wild-type compared with the Bcrp1$^{-/-}$ lactating female mice after i.v. administration (Merino et al., 2005). This difference in plasma levels between wild-type and Bcrp1$^{-/-}$ lactating female mice contrasts with the lack of difference between wild-type and Bcrp1$^{-/-}$ virgin female mice (Fig. 1).

During the preparation of this manuscript, Tanaka et al. (2004) showed a male-predominant expression of Bcrp mRNA in mouse liver and rat kidney, but not in rat liver, further supporting our mouse protein expression data, and showing species-dependent sex differences in the tissue expression of Bcrp mRNA. Their results also suggested that male-predominant expression of Bcrp mRNA in mouse liver seems to be regulated by the inductive effect of testosterone. Imai et al. (2005) demonstrated that estradiol can down-regulate BCRP levels in several cell lines at a post-transcriptional level. There may thus be many levels at which sex hormones affect BCRP expression, and the effect can differ between species. To assess whether the sex difference observed in the hepatic expression of mouse Bcrp1 could be extrapolated to the human situation, we performed Western analysis of human samples. Our data suggest that also in humans, men have higher hepatic expression of BCRP compared with women (Fig. 4D). This is in agreement with the sex difference observed in the pharmacokinetics of several known BCRP substrates in humans. Significantly higher plasma clearance was noted in men compared with women for topotecan (Gallo et al., 2000; Loos et al., 2003), methotrexate (Godfrey et al., 1998; Wall et al., 2000), doxorubicin (Dobbs et al., 1995), and epirubicin (Wade et al., 1992). This sex difference can thus have important pharmacological and toxicological implications. Inappropriate dosing may, for example, lead to suboptimal treatment in some patients and expose others to the risk of unacceptable toxicity. For methotrexate, it has been suggested that higher effective exposure to the drug because of lower clearance in women could contribute to their better chemotherapeutic overall outcome (Wall et al., 2000). Along the same lines, it has been suggested that women are at higher risk for methotrexate toxicity; therefore, lower dosing may be appropriate in female patients (Godfrey et al., 1998).

We cannot exclude the possibility that other factors can also be involved in the sex difference observed in the pharmacokinetics of these compounds in humans. For example, both topotecan and methotrexate are (albeit modest) P-glycoprotein substrates, and the hepatic expression of P-glycoprotein is higher in men compared with women. However, clearance of P-glycoprotein substrates in general seems to be similar in men and women (Schwartz, 2003). Another confounding factor could be the possible sex difference in metabolism. However, for topotecan, metabolism is a minor route of elimination (Loos et al., 2000) and for methotrexate, it has been reported that its metabolism is below 6% of the dose in rats (Fahrig et al., 1989). In conclusion, it may well be that BCRP is involved in the sex difference observed in the pharmacokinetics of several known BCRP substrates in humans.

Until 1993, women were excluded from clinical phase I and early phase II trials based on the possible risks of studying women with child-bearing potential. Thus, information about pharmacokinetics in women, and the possibility of sex differences in the relationship between dose and efficacy or adverse drug reactions, has historically been limited. Complete evaluation of sex differences in clinical trials is often complicated by providing a sufficient sample size with enough statistical power and an optimal study design that excludes confounders such as age, race, menstrual cycle, or comedications. Nevertheless, there is no doubt that potential sex-specific differences should be evaluated during the clinical drug developmental process of all newly developed compounds (Beierle et al., 1989). Insights from this study further support the necessity to evaluate such sex-dependent differences and may help to identify drugs (BCRP substrates) that have an increased likelihood of displaying sex-dependent pharmacokinetics.

Intra- or interindividual differences in BCRP activity in humans have been attributed to pharmacological or dietary induction, stimulation, or inhibition of BCRP or because of known genetic BCRP polymorphisms (Mizuarai et al., 2004; Sparreboom et al., 2004; Kobayashi et al., 2005). In this study, we clearly show another likely source of interindividual difference in pharmacokinetics of BCRP substrates (i.e., the sex-dependent expression and activity of BCRP in the liver) with possible impact on the clinical-therapeutic applications and toxicity risks of drugs.


**Fig. 7.** Western analysis of human BCRP expression in men and women using crude membrane fractions (10 $\mu$g of protein per lane) from human liver. Equal protein loading was verified by Ponceau S and India ink total protein staining (not shown).

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