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Distribution of breast cancer resistance protein (BCRP/ABCG2) mRNA expression along the human GI tract

Heike Gutmann [a,c], Petr Hruz [a,b,c], Christian Zimmermann [a,c], Christoph Beglinger [b,c], Juergen Drewe [a,c,*]

[a] *Clinic for Pharmacology and Toxicology, University Clinic Basel, Hebelstr 2, CH-4031 Basel, Switzerland*
[b] *Department of Gastroenterology, University Clinic Basel, CH-4031 Basel, Switzerland*
[c] *Department of Research, University Clinic Basel, CH-4031 Basel, Switzerland*

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Abstract

Human breast cancer resistance protein (BCRP/ABCG2) is an ABC-transporter that is present on the luminal membrane of intestinal epithelial cells and restricts absorption of anticancer drugs such as methotrexate, topotecan, mitoxantrone, and doxorubicin. The exact anatomic distribution of BCRP along the gastrointestinal (GI) tract, however, has not been determined before. The aim of this study was, therefore to investigate BCRP mRNA expression pattern along the GI tract in 14 healthy subjects. Furthermore, BCRP duodenal mRNA expression was compared with MDR1/ABCB1 mRNA. Additionally, BCRP mRNA expression was investigated in two human intestinal cell lines (Caco-2 and LS180). Since previous animal studies have suggested sex specific differences in BCRP expression, we analyzed intestinal BCRP expression with respect to sex. Biopsies were taken from different gut segments (duodenum, terminal ileum and ascending, transverse, descending and sigmoid colon). Gene expression was assessed by quantitative real-time PCR (Taqman). BCRP mRNA expression was maximal in the duodenum and decreased continuously down to the rectum (terminal ileum 93.7%, ascending colon 75.8%, transverse colon 66.6%, descending colon 62.8%, and sigmoid colon 50.1% compared to duodenum, respectively). BCRP expression in the duodenum was comparable to MDR1/ABCB1 gene expression. Caco-2 cells showed a comparable expression of BCRP as human duodenal tissue. Gender specific differences in BCRP expression were not observed. These findings represent the first systematic site-specific analysis of BCRP expression along the GI tract. This information might be helpful to develop target strategies for orally administered anticancer drugs.

Keywords: BCRP; ABCG2; MDR1; ABCB1; Intestinal mapping; Human; Caco-2 cells; LS180 cells

1. Introduction

BCRP/ABCG2 is a half-transporter that belongs to the *white* subfamily of ATP-binding cassette (ABC) transporters. BCRP was originally cloned from multidrug resistant tumor cells [1–3] and displays a wide substrate specificity. It mediates the energy dependent translocation of various anticancer drugs such as methotrexate [4], topoisomerase inhibitors (such as topotecan [5]), mitoxantrone, and doxorubicin [6] across cellular membranes. BCRP knock-out mice were found to be healthy and showed no major pathological alterations. When fed with a chlorophyll rich diet containing the chlorophyll degradation product, the phototoxic phenophorbide a, the BCRP knock-out mice developed phototoxic skin lesions [7]. Its localization indicates an important role in the protection of tissues against xenobiotics. BCRP expression was detected mainly in excretory organs, e.g. in canalicular membranes of the liver, in epithelial cells of the small intestine, colon, kidney and lung, as well as in the blood–brain barrier and the placenta [8,9].

The expression of BCRP in epithelial cells of the intestine implies that BCRP might be an important transporter limiting the absorption of orally administered anticancer drugs and ingested toxins [9–11]. Due to its broad substrate specificity, BCRP might influence the pharmacokinetics of many unrelated substances including anticancer drugs,

Abbreviations: BCRP, human breast cancer resistance protein; ABCG2, human breast cancer resistance protein; PCR, polymerase chain reaction

* Corresponding author. Tel.: +41 61 265 3848; fax: +41 61 265 8581.
E-mail address: juergen.drewe@unibas.ch (J. Drewe).

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HIV drugs, and endogenous compounds [12,13]. Up to now, there is little knowledge about the expression pattern of BCRP along the human intestine. This information however might be helpful for the development of specific galenical targeting approaches, which may be utilized to improve intestinal absorption of anticancer drugs. Therefore, the expression of BCRP was investigated in the human intestine of 14 healthy subjects and its duodenal expression was compared with that of MDR1.

In vitro screening of drug absorption is commonly done in human intestinal cell lines (such as Caco-2 [14,15] and LS180 [16]). Therefore, it is of interest to compare the expression level of BCRP in these cell lines with the human duodenal BCRP mRNA expression.

In addition, membrane transport differences of endogenous and xenobiotic compounds associated with sex have been reported previously for several transport proteins [17–23]. Recently sex associated differences for BCRP, the BCRP analogue in rat and mice has been described by Tanaka et al. [24]. They observed a higher expression of BCRP mRNA of male rats in the kidney and of male mice in the liver compared to females. These sex differences were attributed to the suppressive effect of estradiol in rats and to the inductive effect of testosterone in mice, respectively. Intestinal expression of rat and mouse BCRP seems not be influenced by sex. However, data about intestinal rat and mouse BCRP expression exhibited high interspecies differences and were restricted to duodenum, jejunum and ileum. We therefore wanted to determine, whether there are sex-related differences in human BCRP mRNA expression along the intestinal tract that might lead to pharmacokinetic variations in drug absorption.

2. Materials and methods

2.1. Cell cultures

Caco-2 cells (passage 42) and LS180 cell line (passage 36) were purchased from ATCC (Manassas, USA). Both cell lines were cultured in Dulbecco's MEM with Glutamax-I, supplemented with 10% (v/v) fetal calf serum, 1% nonessential amino acids, 1% sodium pyruvate, 50 μg/ml gentamycin. All cultures were maintained in a humified 37 °C incubator with a 5% carbon dioxide in air atmosphere.

2.2. Biopsies

Intestinal biopsies were obtained from 14 healthy subjects (7 females, 7 males, age 43–75 years, average age 59.8 years, no medication), which served as a control group in a clinical study. This study systematically investigated the regional expression of different genes in patients with inflammatory bowel disease. The study protocol included specifically the investigation of drug transporting proteins and it was approved by the local Ethical Committee. Informed consent was obtained from subjects prior to inclusion. Indications for a combined gastroscopy and colonoscopy in these control patients were cancer screening, irritable bowel syndrome and unspecific abdominal pain. No macroscopically pathological findings were observed during endoscopies in these subjects. Three to four biopsies each were obtained from duodenum, terminal ileum, ascending colon, transverse colon, descending colon and sigmoid colon.

2.3. Preparation of samples

For isolation of total RNA from cell cultures cells, medium was removed and RNA was extracted from Caco-2 and LS180 cell line using the RNeasy Mini Kit (Qiagen, Hilden, Germany).

The biopsy samples were immediately submerged in a tube with RNAlater (Ambion) and stored at −80 °C until further processing. For RNA isolation two biopsies from each intestinal region were homogenized for 30 s (Polytron PT 2100, Kinematika AG, Switzerland) and RNA was extracted using the RNeasy Mini Kit (Qiagen GmbH, Hilden, Germany) following the instructions provided by the manufacturer.

RNA was quantified with a GeneQuant photometer (Pharmacia, Uppsala, Sweden). After DNase I digestion (Gibco, Life Technologies, Basel, Switzerland) 1.5 μg of total RNA was reverse-transcribed by Superscript (Gibco Life Technologies) according to the manufacturer's protocol using random hexamers as primers. TaqMan analysis was carried out on a 7900HT Sequence Detection System (Applied Biosystems, Rotkreuz, Switzerland). PCR conditions were 10 min at 95 °C followed by 40 cycles of 15 s 95 °C and 1 min at 60 °C. Each TaqMan reaction contained 10 ng of cDNA in a total volume of 10 μl. TaqMan Universal PCR Mastermix from Applied Biosystems was used. The concentrations of primers and probes were 900 nM and 225 nM, respectively. Primers and probes were designed according to the guidelines of Applied Biosystems with help of the Primer Express 2.0 software (BCRP probe: 5′-CCATTGCATCTTGGCTGTCATGGCTT-3′, BCRP forward primer: 5′-CAGGTCTGTTGGTCAATCTCACA-3′, BCRP reverse primer: 5′-TCCATATCGTGGAATGCTGAAG-3′, villin probe: 5′-TCATCAAAGCCAAGCAGTACCCACCAAG-3′, villin forward primer: 5′-CATGAGCCATGCGCTGAAC-3′, villin reverse primer: 5′-TCATTCTGCACCTCCACCTGT-3′). Primers and probes were synthesized by Invitrogen (Basel, Switzerland) and Eurogentec (Seraing, Belgium), respectively. All samples were run in triplicates and were quantified using a standard curve. Standards were generated by a serial dilution of PCR products of the appropriate gene. For each sample the number of BCRP transcripts and the number of villin transcripts were determined. By calculating the ratio of

BCRP/villin mRNA, the transporter gene expression was normalized. This approach has previously been established to account for variations in the enterocyte content of biopsies [25,26]. Not reverse-transcribed RNA served as a negative control.

2.4. Statistical analysis

BCRP gene expression was compared between the different intestinal segments by analysis of variance. In the case of significant differences between intestinal segments, all segments were compared with the expression in duodenum using Dunnett's *t*-test using sex as a covariate. Comparison of BCRP and/or MDR1 mRNA expression was performed by unpaired two-sided *t*-test. The level of significance was $p = 0.05$. All statistical comparisons were performed using SPSS for Windows software (version 12.0).

3. Results

The expression pattern of BCRP from the duodenum to the sigmoid colon is shown in Fig. 1. Maximal BCRP mRNA expression was found in the duodenum. In the colonic segments BCRP mRNA expression is continuously decreasing from proximal to distal. In ascending colon the BCRP level is significantly reduced to 75.8% of the duodenum, in transverse colon to 66.6%, in descending colon to 62.8%, and in sigmoid colon to 50.1%, respectively. In the terminal ileum BCRP mRNA expression is slightly but not significantly reduced compared to duodenum (93.7%). The expression of BCRP mRNA was not significantly different between males and females, neither in the duodenum and the terminal ileum, nor in the different colonic segments of the human GI tract (Fig. 1). BCRP mRNA expression was normalized to villin to account for variation in enterocyte content (ratio of BCRP/villin mRNA) as suggested in the literature [25,26]. This was justified, since the mRNA expression of villin was not significantly different between the different parts of the intestine. The variability of BCRP mRNA measurement was determined by repetitive determination ($N = 10$) and amounted to 4.3% (coefficient of variation).

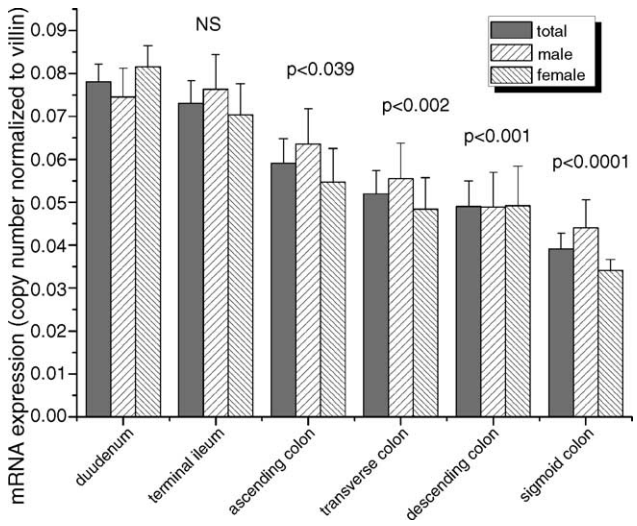

Fig. 1. Expression of BCRP/ABCG2 mRNA in different gut segments. Data represent mean (±S.E.M.) of biopsies from 14 healthy subjects (7 males, 7 females), except terminal ileum, where biopsies from 13 subjects (6 males, 7 females) were used.

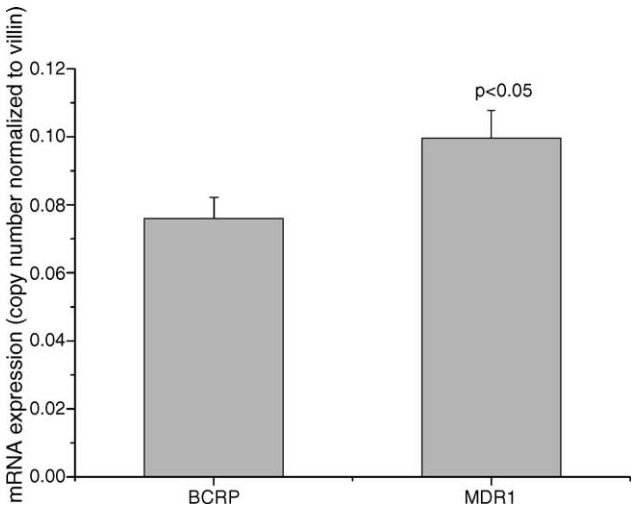

Fig. 2. Expression of BCRP/ABCG2 mRNA and MDR1/ABCB1 mRNA in human duodenum normalized to the expression of villin. Data represent mean (±S.E.M.) of biopsies from 14 healthy subjects.

Since MDR1 and BCRP share some of their substrates, duodenal mRNA expression of these genes was compared. MDR1 mRNA and BCRP mRNA expression was in the same range in the duodenum, with a slightly but significantly ($p < 0.05$) lower expression of BCRP (Fig. 2).

The duodenal mRNA expression of BRCP was comparable to the expression in Caco-2 cells, which are reported to exhibit duodenal-like transporter expression [15]. However, the mRNA expression of BCRP in LS180 cells was almost 100-fold lower ($p < 0.001$; Fig. 3).

4. Discussion

Previous studies had reported that cellular BCRP is localized in the apical membranes of small intestinal and colonic epithelia [9,10], where it limits the bioavailability of toxic compounds. There is some information about tissue distribution of BCRP in animal species such as rat and mice or BCRP expression in isolated parts of the intestine [24,26]. However, only limited information is available about the site-specific localization of BCRP along the GI tract in humans, which might be important for the development of specific galenic formulations of anticancer drugs. Here, we present for the first time a systematic analysis of the site-specific expression of BCRP along the GI tract. BCRP mRNA expression decreased continuously from the duodenum to the sigmoid colon. In

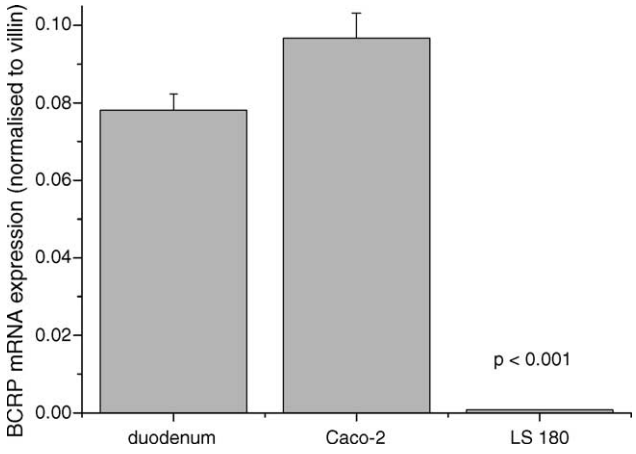

Fig. 3. Expression of BCRP/ABCG2 mRNA in human duodenum ($N = 14$); Caco-2 cells ($N = 3$) and LS180 cells ($N = 3$) normalized to the expression of villin. BCRP mRNA expression was significantly lower in LS180 cells than in Caco-2 cells and duodenal tissue ($p < 0.001$). Data represent mean (±S.E.M.).

human jejunum, a part which was not investigated in our study due to ethical reasons, Taipalensuu et al. [26] found a high level of BCRP mRNA expression. The BCRP gene expression exhibited even a 3.4-fold higher expression than the MDR1 gene. Data in rat [24] have shown that the level of BCRP gene expression is higher in the jejunum compared to duodenum. However, it is not trivial to extrapolate animal data to humans, because species differences have been described even between rodents. Whereas rats expressed high levels of BCRP in the ileum, the ileal level of BCRP mRNA in mice was rather low. Nevertheless one is tempted to speculate that BCRP expression levels might be maximal in the jejunum. Since expression of BCRP is still high in the terminal ileum, which is close to the jejunum, these data are not in contrast to our findings.

Differences in the membrane transport of xenobiotics and endogenous compounds caused by different levels of sexual hormones such as testosterone and estradiol have been previously described in several studies [27,28]. This sex related differences in membrane transport includes several membrane transporters such as organic cation transporters [17,18], organic anion transporters [19–21], and multidrug resistance proteins MDR1a, MDR1b and MDR2 [22,23]. Recently, Tanaka et al. reported sex-related differences of BCRP expression levels in rodents [24]. We found no significant differences in the level of BCRP mRNA expression between males and females, neither in the duodenum and the terminal ileum, nor in the different colonic segments of the human GI tract. We therefore conclude that sexual hormones have most probably no effect on the expression pattern of BCRP in the adult human intestine.

The importance of MDR1 and MRPs for the protection from enteral absorption of potentially toxic xenobiotics and their limiting effects on enteral drug absorption has become more and more aware. BCRP shows some degree of substrate overlapping with these transporters and is also expressed in the small and large intestine [29]. To estimate the potential impact of BCRP for detoxification and drug absorption, we compared the level of BCRP mRNA expression in the duodenum with the level of MDR1 mRNA, another important ABC-transporter of xenobiotics in the intestine. We showed comparable mRNA expression of MDR1 and BCRP, with a slightly but significantly ($p < 0.05$) lower expression of BCRP. In jejunum, BCRP mRNA expression was found to be even higher as MDR1mRNA expression [26]. Taken together these findings indicate that BCRP might play an important role for limiting the absorption of orally administered anticancer drugs and ingested toxins.

The comparable mRNA expression of BRCP in human duodenum and the colonic carcinoma derived cell line Caco-2 may indicate its usefulness for in vitro studies of BRCP mediated transport of drugs. In addition, another colonic adenocarcinoma-derived cell line, LS180, was investigated for BCRP mRNA expression. This cell line is commonly used for the assessment of gene induction [15,16,30]. However, due to their low expression of BRCP mRNA, LS180 cells do not seem to be suitable for investigation of BRCP function.

We have to admit that our study results represent only mRNA expression, which may not correlate with protein expression and/or function. However, due to ethical reasons we were limited with the number and volumes of tissue biopsies taken from our patients. Therefore, for this mapping study only mRNA expression experiments could be performed. To assess functional expression of BRCP in different gut segments, further dedicated studies are needed.

5. Conclusion

These findings represent the first systematic site-specific analysis of BCRP expression along the GI tract and shows that its expression significantly decreased in all colonic segments compared with the small intestine. This knowledge might be important to develop target strategies for orally administered anticancer drugs.

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References

[1] Allikmets R, Schriml LM, Hutchinson A, Romano-Spica V, Dean M. A human placenta-specific ATP-binding cassette gene (ABCP) on

chromosome 4q22 that is involved in multidrug resistance. Cancer Res 1998;58(23):5337–9.
[2] Doyle LA, Yang W, Abruzzo LV, Krogmann T, Gao Y, Rishi AK, et al. A multidrug resistance transporter from human MCF-7 breast cancer cells. Proc Natl Acad Sci USA 1998;95(26):15665–70.
[3] Miyake K, Mickley L, Litman T, Zhan Z, Robey R, Cristensen B, et al. Molecular cloning of cDNAs which are highly overexpressed in mitoxantrone-resistant cells: demonstration of homology to ABC transport genes. Cancer Res 1999;59(1):8–13.
[4] Volk EL, Farley KM, Wu Y, Li F, Robey RW, Schneider E. Overexpression of wild-type breast cancer resistance protein mediates methotrexate resistance. Cancer Res 2002;62(17):5035–40.
[5] Kruijtzer CM, Beijnen JH, Rosing H, ten Bokkel Huinink WW, Schot M, Jewell RC, et al. Increased oral bioavailability of topotecan in combination with the breast cancer resistance protein and P-glycoprotein inhibitor GF120918. J Clin Oncol 2002;20(13):2943–50.
[6] Doyle LA, Yang W, Abruzzo LV, Krogmann T, Gao Y, Rishi AK, et al. A multidrug resistance transporter from human MCF-7 breast cancer cells. Proc Natl Acad Sci USA 1998;95(26):15665–70.
[7] Jonker JW, Smit JW, Brinkhuis RF, Maliepaard M, Beijnen JH, Schellens JH, et al. Role of breast cancer resistance protein in the bioavailability and fetal penetration of topotecan. J Natl Cancer Inst 2000;92(20):1651–6.
[8] Scheffer GL, Maliepaard M, Pijnenborg AC, van Gastelen MA, de Jong MC, Schroeijers AB, et al. Breast cancer resistance protein is localized at the plasma membrane in mitoxantrone- and topotecan-resistant cell lines. Cancer Res 2000;60(10):2589–93.
[9] Maliepaard M, Scheffer GL, Faneyte IF, van Gastelen MA, Pijnenborg AC, Schinkel AH, et al. Subcellular localization and distribution of the breast cancer resistance protein transporter in normal human tissues. Cancer Res 2001;61(8):3458–64.
[10] Pavek P, Merino G, Wagenaar E, Bolscher E, Novotna M, Jonker JW, et al. Human breast cancer resistance protein (BCRP/ABCG2): interactions with steroid drugs, hormones, the dietary carcinogen PhIP, and transport of cimetidine. J Pharmacol Exp Ther 2004;13:13.
[11] Jonker JW, Buitelaar M, Wagenaar E, Van Der Valk MA, Scheffer GL, Scheper RJ, et al. The breast cancer resistance protein protects against a major chlorophyll-derived dietary phototoxin and protoporphyria. Proc Natl Acad Sci USA 2002;99(24):15649–54. Epub 2002 Nov 12.
[12] van Herwaarden AE, Jonker JW, Wagenaar E, Brinkhuis RF, Schellens JH, Beijnen JH, et al. The breast cancer resistance protein (Bcrp1/Abcg2) restricts exposure to the dietary carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine. Cancer Res 2003;63(19):6447–52.
[13] Polli JW, Baughman TM, Humphreys JE, Jordan KH, Mote AL, Webster LO, et al. The systemic exposure of an *N*-methyl-D-aspartate receptor antagonist is limited in mice by the P-glycoprotein and breast cancer resistance protein efflux transporters. Drug Metab Dispos 2004;32(7):722–6.
[14] Hilgers AR, Conradi RA, Burton PS. Caco-2 cell monolayers as a model for drug transport across the intestinal mucosa. Pharm Res 1990;7(9):902–10.
[15] Pfrunder A, Gutmann H, Beglinger C, Drewe J. Gene expression of CYP3A4 ABC-transporters (MDR1 and MRP1–MRP5) and hPXR in three different human colon carcinoma cell lines. J Pharm Pharmacol 2003;55(1):59–66.
[16] Thummel KE, Brimer C, Yasuda K, Thottassery J, Senn T, Lin Y, et al. Transcriptional control of intestinal cytochrome P-4503A by 1alpha 25-dihydroxy Vitamin D3. Mol Pharmacol 2001;60(6):1399–406.
[17] Urakami Y, Nakamura N, Takahashi K, Okuda M, Saito H, Hashimoto Y, et al. Gender differences in expression of organic cation transporter OCT2 in rat kidney. FEBS Lett 1999;461(3):339–42.
[18] Urakami Y, Okuda M, Saito H, Inui K. Hormonal regulation of organic cation transporter OCT2 expression in rat kidney. FEBS Lett 2000;473(2):173–6.
[19] Buist SC, Cherrington NJ, Choudhuri S, Hartley DP, Klaassen CD. Gender-specific and developmental influences on the expression of rat organic anion transporters. J Pharmacol Exp Ther 2002;301(1):145–51.
[20] Buist SC, Klaassen CD. Rat and mouse differences in gender-predominant expression of organic anion transporter (Oat1–3; Slc22a6–8) mRNA levels. Drug Metab Dispos 2004;32(6):620–5.
[21] Kobayashi Y, Hirokawa N, Ohshiro N, Sekine T, Sasaki T, Tokuyama S, et al. Differential gene expression of organic anion transporters in male and female rats. Biochem Biophys Res Commun 2002;290(1):482–7.
[22] Piquette-Miller M, Pak A, Kim H, Anari R, Shahzamani A. Decreased expression and activity of P-glycoprotein in rat liver during acute inflammation. Pharm Res 1998;15(5):706–11.
[23] Salphati L, Benet LZ. Modulation of P-glycoprotein expression by cytochrome P450 3A inducers in male and female rat livers. Biochem Pharmacol 1998;55(4):387–95.
[24] Tanaka Y, Slitt AL, Leazer TM, Maher JM, Klaassen CD. Tissue distribution and hormonal regulation of the breast cancer resistance protein (Bcrp/Abcg2) in rats and mice. Biochem Biophys Res Commun 2004;326(1):181–7.
[25] Lown KS, Mayo RR, Leichtman AB, Hsiao HL, Turgeon DK, Schmiedlin-Ren P, et al. Role of intestinal P-glycoprotein (mdr1) in interpatient variation in the oral bioavailability of cyclosporine. Clin Pharmacol Ther 1997;62(3):248–60.
[26] Taipalensuu J, Tornblom H, Lindberg G, Einarsson C, Sjoqvist F, Melhus H, et al. Correlation of gene expression of ten drug efflux proteins of the ATP-binding cassette transporter family in normal human jejunum and in human intestinal epithelial Caco-2 cell monolayers. J Pharmacol Exp Ther 2001;299(1):164–70.
[27] Lu R, Kanai N, Bao Y, Wolkoff AW, Schuster VL. Regulation of renal oatp mRNA expression by testosterone. Am J Physiol 1996;270(2, Part 2):F332–7.
[28] Cerrutti JA, Brandoni A, Quaglia NB, Torres AM. Sex differences in *p*-aminohippuric acid transport in rat kidney: role of membrane fluidity and expression of OAT1. Mol Cell Biochem 2002;233(1–2):175–9.
[29] Allen JD, Schinkel AH. Multidrug resistance and pharmacological protection mediated by the breast cancer resistance protein (BCRP/ABCG2). Mol Cancer Ther 2002;1(6):427–34.
[30] Collett A, Tanianis-Hughes J, Warhurst G. Rapid induction of P-glycoprotein expression by high permeability compounds in colonic cells in vitro: a possible source of transporter mediated drug interactions? Biochem Pharmacol 2004;68(4):783–90.