Regional Expression and Activity of Breast Cancer Resistance Protein (Bcrp/Abcg2) in Mouse Intestine: Overlapping Distribution with Sulfotransferases

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ABSTRACT:

Breast cancer resistance protein (Bcrp/Abcg2) is a member of the ATP-binding cassette transporter family with the ability to transport a variety of sulfate conjugates. In the present study, the regional expression and activity of Bcrp and sulfotransferases (SULTs/Sults) were investigated in mouse intestine. Western blotting analysis revealed the highest expression of Bcrp in the ileum over the duodenum, jejunum, and colon. Functional analysis of Bcrp was performed in everted intestinal sacs using 4-methylumbelliferone glucuronide (4MU). The mucosal secretion clearance of 4MU sulfate formed in the enterocytes was markedly reduced in the jejunum, ileum, and colon of Bcrp (−/−) mice in comparison with wild-type mice, whereas a slight and nonsignificant reduction was observed in the duodenum. The reduction in the mucosal secretion clearance was most marked in the ileum followed by the colon and jejunum. In addition, the mucosal secretion clearance of minoxidil sulfate, an active metabolite of minoxidil, was also significantly reduced in the intestine of Bcrp (−/−) mice. The sulfation activity of 4MU was higher in the colon than in the small intestine where glucuronidation activity was somewhat higher than the sulfation activity. Real-time polymerase chain reaction analysis showed that the expression of sulfotransferases, such as Sult1a1/2, Sult1b1, and Sult1d1, was also highest in the colon. These results suggest that Bcrp activity is higher in the mid to lower intestine and that the cooperation of Bcrp and SULT provides an important detoxification pathway, particularly in the colon.

The intestine is an important tissue acting as a barrier to orally administered xenobiotics. Numerous metabolizing enzymes and transporters are expressed in the intestinal epithelial cells and act as a barrier to foreign materials (Suzuki and Sugiyama, 2000; Kaminsky and Zhang, 2003; Chan et al., 2004). Cytochrome P-450s, carboxylesterases, UDP-glucuronosyltransferases (UGTs), sulfotransferases (SULTs/Sults), and carboxylesterases are major intestinal metabolizing enzymes (Kaminsky and Zhang, 2003). In addition, ABC transporters, such as P-glycoprotein (P-gp), multidrug resistance-associated protein 2 (MRP2), and breast cancer resistance protein (BCRP/Bcrp), are expressed in the apical membrane of enterocytes and can restrict the intestinal absorption of xenobiotics by extruding them into the intestinal lumen (Chan et al., 2004). These metabolizing enzymes and transporters are expressed and function in a region-dependent manner, and, in addition to their independent role, they are mutually involved in the detoxification of xenobiotics in some cases. For instance, CYP3A and P-gp, which have overlapping substrate specificities (Kivistö et al., 2004), have different locations in the intestine; CYP3A is highly expressed and exhibits high activity in the upper intestine (Zhang et al., 1999; Gotot et al., 2000; Mottino et al., 2000), whereas P-gp is highly expressed in the lower intestine (Mouly and Paine, 2003; Valenzuela et al., 2004). Therefore, the intestinal absorption of their common substrates can be limited throughout the entire intestinal tract (Tamura et al., 2003).

Bcrp is characterized by its broad substrate specificity, e.g., anticancer drugs, HMG-CoA reductase inhibitors, carcinogens, and sulfate conjugates (Doyle and Ross, 2003; Suzuki et al., 2003; Hirano et al., 2005; van Herwaarden et al., 2006). BCRP has been reported to limit the oral availability of topotecan and ME3277, which is produced intracellularly from its ester-type prodrug (Jonker et al., 2002; Kondo et al., 2005). Furthermore, BCRP accepts a variety of sulfate conjugates of xenobiotics as substrates, leading to the hypothesis that Bcrp is involved in the efflux of sulfate conjugates formed intracellularly by SULT. Indeed, it has been demonstrated that Bcrp is involved in the efflux of sulfate conjugates in the kidney (Mizuno et al., 2004), liver (Zamek-Gliszczynski et al., 2006; Enokizono et al., 2007), and jejunum (Adachi et al., 2005).

SULT is an enzyme that transfers the sulfate group from 3'-
phosphoadenosine 5'-phosphosulfate to substrates (Nagata and Yamazoe, 2000). In mice, Sults consist of two major subfamilies, Sult1 and Sult2. Generally, Sult1 isoforms mediate sulfation of phenolic compounds, such as p-nitrophenol, and Sult2 isoforms mediate sulfation of hydroxysteroids, such as dehydroepiandrosterone (Nagata and Yamazoe, 2000). Sulfation is usually a detoxification pathway; however, in some cases, it produces bioactive metabolites (Coughtrie et al., 1998). Minoxidil sulfate (MNXS) is one example of a biologically active sulfate conjugate. Minoxidil (MNX) is used to treat baldness and hypertension, and its pharmacological activity has been thought to be due to the activity of MNXS as a K⁺-ATPase channel opener (Buhl et al., 1990). In this case, the disposition of the sulfate conjugates is important for the pharmacological/toxicological response.

Previously, Adachi et al. (2005) demonstrated that 4MUS was actively excreted into the lumen by Bcrp in the jejunum using the in situ perfusion technique. The aim of the present study was to investigate this excretion throughout the whole intestine using the everted sac method in mice. The regional expression of Bcrp from the duodenum to the colon was investigated by Western blotting, and Bcrp activity was evaluated by comparing the mucosal efflux clearance of 4MUS between wild-type and Bcrp (−/−) mice. Sulfation and glucuronidation activities were estimated on the basis of the total amounts of 4MUS and 4-methylumbelliferone glucuronide (4MUG) produced during incubation. In addition to 4MUS, the production and efflux transport of MNXS was also investigated in the everted intestinal sacs.

Materials and Methods

**Chemicals.** 4MU, 4MUS, and 4MUG were purchased from Sigma-Aldrich (St. Louis, MO). MNX and MNXS were purchased from Wako Pure Chemical Industries (Osaka, Japan) and Toronto Research Chemicals (Toronto, Ontario, Canada), respectively. All the other chemicals were from Sigma-Aldrich.

**Animals.** Mice from a previously established Bcrp (−/−) line were used in this study (Jonker et al., 2002). Male Bcrp (−/−) and wild-type FVB mice (15–18 weeks old) were maintained under standard conditions with a light/dark cycle. Food and water were available ad libitum.

**Preparation of the Crude Membrane Fraction of Enterocytes.** Wild-type FVB mice were anesthetized with ether and sacrificed by exsanguination from the femoral artery and vein. Immediately after sacrifice, the duodenum (1–8 cm from the stomach), jejunum (10–18 cm from the stomach), ileum (8 cm above the cecum), and colon (5 cm below the cecum) were dissected. Each intestinal segment was ligated at one end and then everted. The open end of the everted sacs was ligated after the insertion of a polyethylene tube (SP-45; Natsume, Tokyo, Japan). The length of the prepared everted sacs was 3 cm for the colon and 5 cm for the other three segments. Krebs-Ringer buffer (KRB), pH 6.4 was added to the serosal side of the everted sacs through a cannula. The added volume of KRB was 240 μl for the colon and 400 μl for the other three segments. The everted sacs were incubated in KRB for 10 min at 37°C, then they were transferred to KRB containing 10 μM 4MU and incubated at 37°C. Aliquots (200 μl) were collected from the mucosal solution at 0, 10, 20, and 30 min after incubation. At the last sampling point, the serosal solution was collected via the cannula. The sacs were washed with ice-cold PBS, weighed, and homogenized with a 9-fold volume of PBS to obtain 10% tissue homogenates. For the study of minoxidil sulfate, minoxidil (10 μM) was added to the mucosal solution of the sacs. Other procedures were the same as those used in the 4MU study.

**LC/MS Analysis.** Mucosal solution (100 μl) was mixed with 30% trichloroacetic acid (10 μl) and centrifuged at 15,000 g for 10 min. The supernatant was subjected to LC/MS analysis. Intestinal homogenate was mixed with an equal volume of acetonitrile (4MUS and 4MUG) or acetonitrile containing 0.05% formic acid (MNXS) and centrifuged at 15,000 g for 10 min. The supernatant was diluted with a 4-fold volume of water (4MUS and 4MUG) or 0.05% formic acid (MNXS) and subjected to LC/MS. Samples were analyzed on a ZQ micromass instrument (Waters, Milford, MA) equipped with an Alliance 2695 LC system (Waters). Chromatographic separation was performed on a CAPCELL PACK C18, MGII column (3 μm, 3.0 × 50 mm; Shiseido, Tokyo, Japan) in gradient mode. The column temperature and flow rate were 40°C and 0.4 ml/min, respectively. For the analysis of 4MUS and 4MUG, 10 mM ammonium acetate (pH 6.0) and acetonitrile were used as the mobile phase. The acetonitrile concentration was 2% at 0 min, 60% at 5 min, and 2% from 5.01 to 9 min. 4MUS and 4MUG were detected at m/z 255 and 351, respectively, under electrospray negative ionization conditions. The desolvation temperature, capillary voltage, and cone voltage were 350°C, 3200 V, and 40 V, respectively. For the analysis of MNXS, 0.05% formic acid and acetonitrile were used as the mobile phase. The acetonitrile concentration was 10% at 0 min, 50% at 4 min, and 10% from 4.01 to 7 min. MNXS was detected at m/z 288 under electrospray negative ionization conditions. The desolvation temperature, capillary voltage, and cone voltage were 350°C, 3200 V, and 30 V, respectively. The lower limits of quantitation in mucosal solution were 0.03 μM (4MUS), 0.1 μM (4MUG), and 0.003 μM (MNXS), corresponding to secreted amounts of 0.06, 0.2, and 0.006 nmol/cm of tissue, respectively. The lower limits of quantitation in the intestine were 0.3 nmol/g of tissue (4MUS), 3 nmol/g of tissue (4MUG), and 0.1 nmol/g of tissue (MNXS).

**Kinetic Analysis in the Everted Sac Studies.** The amounts of 4MUS and MNXS secreted to the mucosal side were calculated by multiplying the concentrations of 4MUS and MNXS by the volume of the mucosal solution. The amounts of 4MUS and MNXS secreted to the mucosal side were plotted against time, and the efflux rates to the mucosal side (Rmucosal) were estimated from the slope. The secretion clearances to the mucosal side (Clmucosal) were calculated by dividing Rmucosal by the intestinal concentrations of 4MUS and MNXS.

**mRNA Quantification.** The mRNA levels of Sult and villin were quantified by the real-time polymerase chain reaction (PCR) method. Epithelial cell-enriched fractions were collected from the duodenum, jejunum, ileum, and colon as described above. Total RNA was isolated from the epithelial cell-enriched fractions using ISOGEN (Wako Pure Chemicals). Real-time PCR was performed with a Quantitect SYBR Green PCR Kit (QIAGEN, Valencia, CA).
and a LightCycler system (Roche Diagnostics, Mannheim, Germany). The primers used in the quantification are listed in Table 1. Because of the high homology, primers designed for common regions were used for the detection of Sult1a1 and 1a2, and Sult2a1 and 2a2, and their mRNA expressions were not determined individually. An external standard curve was generated by dilution of the target PCR product, which was purified by agarose gel electrophoresis. The absolute concentration of the external standard was measured with PicoGreen dsDNA Quantification Reagent (Molecular Probes, Eugene, OR).

**Statistical Analysis.** Statistical analysis for significant differences was performed using a two-tailed Student’s t test. P < 0.05 was considered to be statistically significant.

**Results**

**Regional Expression of Bcrp.** The protein expression of Bcrp was compared in the duodenum, jejunum, ileum, and colon. Because Bcrp is localized only in epithelial cells of the intestine (Maliepaard et al., 2001), the protein expression of Bcrp was normalized by that of villin to correct for intersample variations in the enrichment of epithelial cells. Villin was chosen because it is specifically expressed in epithelial cells, and its use as a correction marker was established in earlier reports (West et al., 1988; Lown et al., 1997). Bcrp/villin ratios represent the protein levels of Bcrp in epithelial cells. The protein levels of Bcrp and villin in each intestinal segment were quantified by Western blotting. The crude membrane fraction obtained from the jejunum was analyzed by Western blotting with different amounts of loaded protein (Fig. 1a). Good linear relationships were observed between the band density and the amounts of loaded protein prepared from the jejunum for both Bcrp and villin (Fig. 1b). The crude membrane fractions of duodenum, ileum, and colon were analyzed with appropriate amounts of protein to bring the band densities within the range of the calibration curves (Fig. 1a). The protein levels of Bcrp and villin relative to those in the jejunum were estimated from the band densities for the duodenum, ileum, and colon. The ratios of the protein levels of Bcrp to those of villin (Bcrp/villin ratio) were compared among the four intestinal segments (Fig. 1c). In the comparison, the Bcrp/villin ratio of the jejunum was set at 1. The highest Bcrp/villin ratio was observed in the ileum, and the Bcrp/villin ratios for the other three segments were similar. The Bcrp/villin ratio of the ileum was about twice that of the other three segments.

**Regional Bcrp Function.** The mucosal transport of 4MUS was measured by using everted sacs and compared in the four intestinal segments between wild-type and Bcrp (−/−) mice. 4MU was added to the mucosal side, and the mucosal efflux of intracellularly formed 4MUS was determined. The mucosal efflux of 4MUS was almost linear up to 30 min (Fig. 2a), suggesting that the initial efflux rates were maintained until 30 min. That result allowed us to calculate CL_{mucosal} from the mucosal efflux rate and tissue concentrations at 30 min. Compared with wild-type mice, in Bcrp (−/−) mice the mucosal efflux of 4MUS was reduced and, concomitantly, the tissue concentrations of 4MUS were increased in the jejunum, ileum, and colon. The CL_{mucosal} of 4MUS is summarized in Fig. 2c. Significant reductions in CL_{mucosal} were observed in the jejunum, ileum, and colon in Bcrp (−/−) mice. The ratios of CL_{mucosal} in wild-type mice to those in Bcrp (−/−) mice (CL_{mucosal} ratio) were 6.8, 13, and 9.2 for the jejunum, ileum, and colon, respectively. The ileum exhibited the highest CL_{mucosal} ratio. In the duodenum, the CL_{mucosal} ratio was slightly reduced in Bcrp (−/−) mice (CL_{mucosal} ratio = 1.9), but the difference was not statistically significant.

**Regional Metabolism of 4MU.** The amounts of 4MUS and 4MUG formed during the experiments in the four intestinal segments are shown for both wild-type and Bcrp (−/−) mice (Fig. 3). It was found that there was no significant change between wild-type and Bcrp (−/−) mice in the amounts of 4MUS and 4MUG formed (−/−). In both groups of animals, the amount of 4MUS produced was greater in the lower intestine, especially the colon, whereas the amount of 4MUG was greater in the upper intestine (duodenum and jejunum), and lowest in the colon. The ratio of the amount of 4MUS to 4MUG was unchanged when the everted sac was incubated at a concentration of 1 μM 4MU, suggesting that metabolic saturation did not occur at a concentration of 10 μM 4MU (data not shown).

**Regional Expression of Sults.** The mRNA expressions of Sults (Sult1a1/2, 1b1, 1d1, 1e1, 2a1/2, and 2b1) were compared among the four intestinal segments. The mRNA expression of Sult1c1 was not measured because Sult1c1 has been reported to be olfactory-specific (Tamura et al., 1998). The mRNA levels of Sults were normalized by those of villin (Sult/villin ratio) for the same reason with Bcrp. mRNA expression of all Sults, except Sult1e1 and Sult2a1/2, were detected in the intestine. For all of the Sults detected in the intestine, there was no significant difference in the Sult/villin ratios between wild-type and Bcrp (−/−) mice. The Sult/villin ratios of Sult1a1/2, 1b1, and 1d1 were significant and markedly higher in the colon (Fig. 4). In the other three segments, the Sult/villin ratios of 1b1 and 1d1 gradually decreased from the duodenum to the ileum (Fig. 4). The mRNA of Sult1a1/2 could be detected in the duodenum, jejunum, and ileum; however, the mRNA levels were below the lower limit of quantification (2000 copies/μg of RNA), corresponding to a Sult/villin ratio of <0.01. The cycle number at the threshold in real-time PCR analysis was similar for the duodenum, jejunum, and ileum, suggesting that the mRNA levels of Sult1a1/2 in these three segments were not largely different. The Sult/villin ratios of Sult2b1 were similar for all four intestinal segments of wild-type mice although the ratio was significantly reduced in the colon of Bcrp (−/−) mice in comparison with the other three segments (Fig. 4).

**Production and Transport of MNXS in Intestine.** The sulfation of MNX and the transport of MNXS were investigated in everted ileum sacs (Fig. 5). After incubation of MNX in everted ileum sacs, MNXS appeared in both the mucosal solution and tissue. The mucosal efflux of MNXS was similar in wild-type and Bcrp (−/−) mice; however, the tissue concentration of MNXS was increased in Bcrp (−/−) mice. The CL_{mucosal} of MNXS was significantly reduced in Bcrp (−/−) mice and was about half the CL_{mucosal} in wild-type mice.

**TABLE 1**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward</th>
<th>Reverse</th>
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<tbody>
<tr>
<td>Sult1a1/2</td>
<td>GGAATCCGCTTCCCTTGGAATTTC</td>
<td>CAATGGGCTTCTCCCCGGATTT</td>
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<tr>
<td>Sult1b1</td>
<td>TGAAGATGTTCCCAAAGCA</td>
<td>GAATTCTTGGAGGCTAAAACAAA</td>
</tr>
<tr>
<td>Sult1c1</td>
<td>GAGGTGAGTTAGTGGATGTTT</td>
<td>TCACTGAGGCAAGGTTGTTT</td>
</tr>
<tr>
<td>Sult2a1/2</td>
<td>TGGTGCAGAAACGAAAAGAC</td>
<td>TGCCTGATATTAGCAGATCA</td>
</tr>
<tr>
<td>Sult2b1</td>
<td>CAAGTCTGAGTTGAGAAAGACAT</td>
<td>TTCAAGGTAATTACAGACAGAT</td>
</tr>
<tr>
<td>villin</td>
<td>CAGGTTGAGGTCCGAGAT</td>
<td>GAATCTGAAACTTTGCACTCCTGTT</td>
</tr>
</tbody>
</table>
Discussion

In the present study, regional expression and function were investigated for both Bcrp and SULTs in mouse intestine. The highest Bcrp protein expression was observed in the ileum, and the Bcrp levels in the other three intestinal regions were similar (Fig. 1c). This result is very similar to the distribution of Bcrp mRNA in mouse intestine (Tanaka et al., 2005). In the functional analysis of Bcrp, the $\text{CL}_\text{mucosal}$, obtained by dividing the efflux rate by the tissue concentration of 4MUS, represents the intrinsic efflux activity on the brush border membrane per unit length. A significant reduction in the $\text{CL}_\text{mucosal}$ of 4MUS was observed in the jejunum, ileum, and colon of Bcrp ($+/−$) mice, whereas in the duodenum the $\text{CL}_\text{mucosal}$ of 4MUS exhibited only a slight reduction in Bcrp ($+/−$) mice, which was not statistically significant ($P > 0.05$) (Fig. 2c). Accordingly, Bcrp plays a major role in the mucosal efflux of 4MUS in the mid to lower intestine. The absolute value of $\text{CL}_\text{mucosal}$ was almost 2-fold greater in the jejunum than that in the other segments although the Bcrp expression corrected by villin was somewhat lower in the jejunum than in the ileum. The difference in the surface area per unit length may account for this discrepancy because the surface area per unit length in the jejunum has been reported to be 1.7-fold greater than that in the ileum (Mayhew, 1988). Indeed, the ratio of $\text{CL}_\text{mucosal}$ in wild-type and Bcrp ($+/−$) mice, representing the relative importance of Bcrp to the net efflux, was greatest in the ileum (13) followed by the colon (9.2) and jejunum (6.8). Unlike in other segments of the intestine, in the duodenum Bcrp makes a minimal contribution to the net efflux.

Although Mrp2 is highly expressed in the duodenum, Adachi et al. (2005) excluded any involvement of Mrp2 in the mucosal efflux of 4MUS. For other Mrps, Mrp3 is localized at the basolateral membrane of the enterocytes (Rost et al., 2002). Immunoblotting using Mrp4 antibody detected its abundant expression in the duodenum and colon where staining showed that Mrp4 is primarily localized to the basal cytoplasmic region of the enterocytes (Johnson et al., 2006). Therefore, the mucosal efflux of 4MUS is mediated by other, as yet unknown, transporter(s) in the duodenum.

The amount of 4MUS formed in the colon was greater than that in the segments of small intestine (Fig. 3a), whereas the amounts of 4MUG formed in the duodenum, jejunum, and ileum were greater than those of 4MUS in the corresponding segments (Fig. 3b). Because the reaction was measured under linear conditions (data not shown), these values represent regional differences in the enzymatic activities of sulfation and glucuronidation in the intestine. Namely, sulfation makes a greater contribution in the ileum than in the upper intestine and becomes the major conjugation pathway in the colon, whereas glucuronidation is the major conjugation pathway for 4MU in the small intestine. The SULT isoform(s) responsible for 4MU sulfation in the intestine remains to be determined. The mRNA distribution of Sult1 isoforms was consistent with the net enzymatic activity of sulfation, whereas the mRNA levels of Sult2b1, which mediates sulfation of hydroxysteroids and cholesterol, were similar through the intestine (Fig. 4). Because Sult1 isoforms are known to be phenol sulfotransferase, the sulfation activity of phenolic compounds will be
markedly higher in the colon than in the small intestine. Very recently, Alnouti and Klaassen (2006) quantified the mRNA expression of Sult isoforms in various mouse tissues including the intestine. The expression profiles of Sult isoforms were consistent with those observed in this study except for Sut1b1, 1d1, and 2b1, the expression of which in the colon was comparable to or lower than that in the small intestine. This discrepancy may be due to the difference in methods of RNA preparation and correction. Because Alnouti and Klaassen prepared mRNA from whole intestinal tissue and normalized the expression by the total RNA content, it is possible that they underestimated the mRNA expression of Sut1b1 and 1d1 in the colon because the colon has no villi and a smaller number of epithelial cells than other segments. The intestinal distribution of UGT mRNAs in mice has not been reported, whereas that in rats has (Grams et al., 2000). Among UGT1 isoforms, UGT1A1, 1A2, 1A6, and 1A7 mRNAs were detected in rat intestine, and the mRNA level of UGT1A7, an ortholog of human UGT1A7 that has been shown to accept 4MU as substrate (Uchaipichat et al., 2004), was higher in the upper intestine. The total mRNA levels of UGT1 isoforms evaluated using the primers designed for the common region were higher in the upper intestine (Grams et al., 2000). These results are consistent with the regional glucuronidation activity of 4MU. In the upper intestine, MRP2 is highly expressed (Gotoh et al., 2000; Mottino et al., 2000). Because MRP2 is well known to accept various glucuronide conjugates, e.g., estradiol 17β-glucuronide, bilirubin glucuronide, and SN-38 glucuronide (Konig et al., 1999), it can be involved in the luminal efflux of glucuronide conjugates formed in the enterocytes by UGTs. Indeed, MRP2 partly accounts for the luminal efflux of E3040 glucuronide in the jejunum; however, as for 4MUG, other transporter(s) play a major role because there was no change in the luminal efflux of 4MUG between normal and Mrp2-deficient mutant rats (Adachi et al., 2005).

MNXS is the active metabolite of MNX. The pharmacological
activity of MNX is thought to be mainly due to the activity of MNXS. Therefore, the disposition of MNXS is crucial for the pharmacological activity of MNX. The present study revealed that the Bcrp/Sults system is involved in the intestinal disposition of MNXS (Fig. 5). This result indicates that the intestinal Bcrp/Sults system also plays an important role in the pharmacological activity of MNX. Besides MNXS, there are many biologically active sulfates. Troglitazone, developed as an antidiabetic agent, was withdrawn from the market because of its liver toxicity, and there are several possible mechanisms for the liver toxicity of this drug. Cholestasis is one possible mechanism (Gitlin et al., 1998). Troglitazone sulfate, the major metabolite of troglitazone, has been shown to be a potent inhibitor of the bile salt excretion pump (Funk et al., 2001). Currently, it is proposed that inhibition of bile salt excretion pump function by the sulfate conjugate is the mechanism of troglitazone-induced cholestasis. Pregnenolone sulfate and dehydroepiandrosterone sulfate are endogenous sulfate conjugates. They can act as modulators of N-methyl-D-aspartate and GABA receptors and, thus, are referred to as neuroactive steroids.
(Stoffel-Wagner, 2003). Some carcinogens, such as 2-aminono-1-methyl-6-phenylimidazo[4,5-b]pyridine and 7,12-dimethylbenz[a]anthracene, are bioactivated by sulfation. The sulfate conjugates of these carcinogens exhibit a more potent DNA adduction ability than the parent forms (Glatt, 2000). In particular, 2-aminono-1-methyl-6-phenylimidazo[4,5-b]pyridine induces cancer in the colon where the interplay between Bcrp and Sult is thought to be most crucial. Our present results suggest the possibility that the intestinal Bcrp/Sult system plays an important role in the activities of such biologically active sulfate conjugates.

In conclusion, our results showed that Bcrp activity is higher in the mid to lower intestine and the sulfation activity of 4MU and mRNA expression of Sult1 isoforms is high in the colon. The cooperation of Bcrp and SULT will provide an important detoxification pathway in the colon for phenolic compounds.

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References

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