Gender Difference in the Urinary Excretion of Organic Anions in Rats

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ABSTRACT

This study was aimed at clarifying the gender differences in the urinary excretion of organic anions and the gene expression of organic anion transporters in rats. The renal clearance with regard to the plasma concentration (CL_{urine,p}) of taurocholate, dibromosulfophthalein (DBSP), and zenarestat, all substrates and/or inhibitors of organic anion transporting polypeptide 1 (Oatp1), was much higher in female than in male rats. The following results imply that the transport system(s) for the re-absorption of zenarestat across the luminal side exhibits a gender difference: 1) the renal uptake clearance assessed by an in vivo integration plot analysis of zenarestat from the blood side does not show any clear gender differences; 2) the renal clearance with regard to the kidney concentration (CL_{urine,k}) of zenarestat in female rats was approximately 30 times higher than in male rats; and 3) both CL_{urine,p} and CL_{urine,k} were increased in male rats by the coinfusion of DBSP, which is an inhibitor of organic anion transporters. Northern and Western blot analyses confirmed a previous finding that the gene expression of Oatp1, which is localized at the apical plasma membrane of the kidney, was much higher in the kidneys of male rats. Overall, a gender difference in urinary excretion is commonly observed for several organic anions, including Oatp1 substrates and inhibitors, and Oatp1 and/or transporters that have a similar substrate specificity to Oatp1 could be involved in such a phenomenon involving its substrates.

The kidney plays an important role in the elimination of therapeutic agents from the body, and the renal excretion of xenobiotics involves at least three processes, including glomerular filtration, secretion, and reabsorption via the renal tubules. Several types of xenobiotic transporters have been recently identified and are suggested to be primarily involved both in the renal secretion and reabsorption of organic anions and cations. These include the organic anion transporter (OAT) family, the organic anion transport polypeptide (OATP), and the organic cation transporter (Oct) family (Sekine et al., 2000; van Aubel et al., 2000; Dresser et al., 2001). As far as the OATP family in rats is concerned, Oatp1 (gene symbol, Slc22a8), Oatp3 (Slc21a7), Oat-K1 (Slc21a4), and Oat-K2 are expressed in the kidney (Bergwerk et al., 1996; Masuda et al., 1997, 1999; Tojo et al., 1999; Hasegawa et al., 2002). As ATP-dependent primary active transporters, P-glycoprotein (Abcb1), multidrug resistance-associated protein 1 (Mrp1) (Abcc1), and Mrp2 (Abcc2) are expressed in the kidney (Thiebaut et al., 1987; Schaub et al., 1997; Peng et al., 1999). Oligopeptide transporters (PEPT1 and PEPT2), which accept β-lactam antibiotics and angiotensin-converting enzyme inhibitors as substrates, are also expressed in the kidney (Fei et al., 1994; Saito et al., 1996).

Some xenobiotic transporters have been reported to exhibit gender differences in their expression. For example, the gene product of Oct2 (Slc22a2) in male rat kidney is higher than that in female rat kidney, whereas the mRNA expression of Oct1 (Slc22a1), Oct3 (Slc22a3), and Oat1 does not show any gender difference (Urakami et al., 1999, 2000). The expres-
sion of Oct2 in the kidney is possibly under hormonal regulation and is increased by testosterone treatment. Gender differences and hormonal regulation have recently been reported in the expression of Oat2 and Oat3 (Kobayashi et al., 2001). The expression of Oatp1 mRNA is also higher in male than in female rat kidney and regulated by testosterone (Lu et al., 1996), although the relevance of such gender differences in terms of the renal excretion of organic anions is still unclear.

Oatp1 is composed of 670 amino acids and has 12 membrane-spanning domains (Jacquemin et al., 1994). It has been shown that Oatp1 mRNA is localized in the liver, kidney, brain, lung, skeleton, muscle, and proximal colon (Jacquemin et al., 1994). Immunomorphological investigations have revealed that the gene product detected by Oatp1 antibody is localized at the apical plasma membrane of the kidney in the S3 segment of the proximal tubule of the outer medulla (Bergwerk et al., 1996). Oatp1 is thought to be the multispecific anion transporter that accepts many types of organic anions, including taurocholate (TCA) and other bile acids, as substrates (Eckhardt et al., 1999; Burckhardt and Wolff, 2000). The driving force is considered to be reduced glutathione (Li et al., 1998). Bergwerk et al. (1996) have reported that Oatp1 in the liver is present in the form of a 83-kDa protein, whereas that in the kidney is only 37 kDa under reducing conditions. Thus, the structure of the Oatp1 gene product might differ from organ to organ, although the difference between the liver and kidney in terms of the function of Oatp1 remains to be clarified.

In addition, some organic anions are reported to exhibit a gender difference in their urinary excretion in rats. These include zenarestat, torsemide, eugurane sodium, clentiazem, and nivaldinipine metabolites (Kling et al., 1991; Tanaka et al., 1991a; Nakamura et al., 1993; Terashita et al., 1994; Sato et al., 2000). The degree of urinary excretion of these compounds is much smaller in male than in female rats. A series of earlier studies has revealed that the renal clearance of zenarestat, an aldose reductase inhibitor for the treatment of diabetic neuropathy, in female rats is approximately 50 times higher than that in male rats (Tanaka et al., 1991a). Since its unbound fraction in plasma and glomerular filtration rate (GFR) are relatively similar in males and females, the reason for this gender difference cannot be due to a difference in glomerular filtration (Tanaka et al., 1993). Furthermore, the renal clearance of zenarestat in female rats is inhibited by probenecid, which is a typical inhibitor of the renal secretion of organic anions, whereas the renal clearance in male rats is unaffected (Tanaka et al., 1991a). Hypophysectomized male rats and normal female rats exhibit similar urinary excretion of zenarestat, and treatment of male and female hypophysectomized rats with testosterone resulted in the urinary excretion of zenarestat comparable with that by intact adult male rats (Tanaka et al., 1992), suggesting hormonal regulation of its excretion system(s).

In the light of the above findings, we formed the hypothesis that the gender difference in the urinary excretion of some organic anions may be due to a difference in the expression of Oatp1. Since Oatp1 can take up a variety of types of organic anions and is localized at the apical membrane to a higher degree in male rats, the higher urinary excretion of anionic compounds in female rats may be explained by a gender difference in the reabsorption process mediated by Oatp1. To obtain evidence for such a hypothesis, in the present study, we have investigated the existence of gender differences in the urinary excretion of the substrate and inhibitor of Oatp1, TCA, and dibromosulphophthalein (DBSP), respectively. We also report here that zenarestat has an affinity for Oatp1 and that the gender difference in its urinary excretion results from its transport across the apical membranes. Gender differences in the gene expression of other organic anion transporters were also examined in the present study to identify potential candidates that may be involved in the mechanism governing such gender differences in urinary excretion.

### Experimental Procedures

**Materials.** Zenarestat (FK-366) was donated by Fujisawa Yaku-hin Kogyo (Osaka, Japan). Inulin- and HPLC-grade acetonitrile were purchased from Wako Pure Chemical Industries (Osaka, Japan). DBSP was purchased from Société d’Etudes et de Recherches Biologiques (Paris, France). Isopropyl ether was purchased from Hayashi Pure Chemical Industries (Osaka, Japan). [3H]Estradiol 17β-d-glucuronide (E17β; 55 μCi/nmol) and [3H]TCA (3 μCi/nmol) were purchased from PerkinElmer Life Sciences (Boston, MA). All other chemicals and reagents were commercial products of analytical grade. Seven-week-old male and female Sprague-Dawley rats were purchased from Charles River Japan (Tokyo, Japan) and had free access to water and food. The studies reported on in this article have been carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health.

**Infusion Study.** The bladder was catheterized using polyethyl-ene tubing (no. 8; o.d. 2.33 mm; Hibi, Tokyo, Japan). The body temperature of the rats was maintained by keeping them under suitable lighting. Zenarestat dissolved in saline was administered via the femoral vein at a priming dose of 1 mg/kg and continued as a sustained infusion of 3 mg/kg in the volume of 6 ml/h. Inulin was administered at a priming dose of 10 mg/kg and continued as a sustained infusion of 20 mg/kg/h. Blood was collected at 30, 60, 90, 120, and 150 min. Urine specimens were collected at 0 to 30, 30 to 60, 60 to 90, 90 to 120, and 120 to 150 min. In the inhibition study, DBSP was injected after mixing it with zenarestat injection solution. The highest dose of DBSP exhibits a transport maximum in its biliary excretion (Goto et al., 2002). The urinary clearance with regard to the plasma concentration (CLurine,p) was calculated as the ratio of the cumulative excreted amount over 150 min to the area under the curve over 150 min (AUC0–150min). The AUC was calculated based on the trapezoidal rule where the initial plasma concentration (C0) was calculated by dividing the loading dose by V0. The urinary clearance with regard to the kidney concentration (CLurine,p) was calculated as the ratio of the urinary excretion rate from 120 to 150 min to the kidney concentration at 150 min. The GFR was assumed to be equal to the total clearance (CLtot) of inulin.

**Intravenous Bolus Injection.** Rats underwent bile duct cannulation using polyethylene tubing (PE10; i.d. 0.61 mm; BD Biosciences, Bedford, MA), and the bladder was catheterized as described for the infusion study. DBSP (2 mg/kg) or [3H]TCA (5 μCi/kg) was administered via the femoral vein. Zenarestat (1 mg/kg) was also administered via the femoral vein to rats without bile or bladder cannulation and, then, 1 and 3 min after administration, the animals were killed, and the liver and kidneys were removed immediately (integration plot analysis). The plasma concentration–time profile was fitted to a biexponential equation, and the AUC was estimated by integration. The CLtot was calculated as Dose/AUC0–∞. The CLurine,p was calculated as the ratio of the cumulative excreted amount over 180 min to the AUC0–180min. The V0 was calculated by dividing the dose by the C0 calculated from the biexponential equation. The initial slope in a plot (designated as the integration plot) of the amount of zenarestat in tissue/plasma concentration of zenares-
Determination of the Amount of Each Compound. For the determination of zenarestat, 0.1 M citrate buffer (pH 4.7; 100 μl) was added to 50 μl of rat plasma, followed by 0.5 N HCl (100 μl) and isopropl ether (400 μl). This sample was shaken for 5 min, then centrifuged for 2 min at 3000 rpm. The organic layer (300 μl) was added to 0.01 N NaOH (50 μl), shaken for 5 min, and then centrifuged for 2 min at 3000 rpm. The organic layer was removed and the aqueous layer (20 μl) was added to 1 M phosphate buffer (pH 5.5; 30 μl), and 20 μl were subjected to HPLC. Kidney was homogenated in 2.5 volumes of 0.1 M citrate buffer (pH 4.7). Then, 100 μl of urine or 300 μl of kidney homogenate were used instead of 50 μl of plasma. HPLC conditions were as follows: column, Nucleosil 5C18 (5 μm; GL Sciences, Tokyo, Japan), 4.0 mm i.d. × 15 cm; guard column, Nucleosil 5C18 (5 μm; GL Sciences), 4.0 mm i.d. × 1 cm; mobile phase, 20 mM phosphate buffer (pH 6.0)/acetonitrile (70/30); flow rate, 1 ml/min; column temperature, 40°C. Inulin in plasma and urine was measured and fitted to the following equation:

\[ V_{\text{o}} = \frac{V_{\text{inhibitor}}}{V_{\text{o-inhibitor}}} = \frac{1}{1 + IK_{\text{a}}} \]  

where \( V_{\text{o-inhibitor}} \) and \( V_{\text{inhibitor}} \) represent the net uptake values obtained by subtracting the uptake in vector-transfected LLC-PK₁ cells from that in Oat1-transfected LLC-PK₁ cells in the presence or absence of inhibitor, respectively, and \( I \) represents the zenarestat concentration.

Northern Blot Analysis. Northern blot analysis was performed as previously described (Ogawa et al., 2000). A 2-μg sample of mRNA, extracted from liver and kidney using ISOGEN (Nippon Gene, Tokyo, Japan) and Oligotex dT30 super (Takara, Tokyo, Japan) according to the manufacturer’s protocol, was electrophoresed on 1% agarose/formaldehyde gel and transferred to a nitrocellulose filter. The filter was hybridized at 42°C with a full-length cDNA of Oat2, Oat3, and Mrp2, and 2190 to 2700 bp of cDNA of Oat1 and 2700 to 3610 bp of cDNA of Oatp2 randomly labeled with \( ^{32} \)PdCTP. The filter was then washed with 0.1% standard saline citrate/0.1% SDS at 55°C. No cross-reaction was observed for this Oatp1 probe with cRNA of Oatp2 and Oatp3 or for this Oatp2 probe with cRNA of Oatp1 and Oatp3.

Western Blot Analysis. Anti-rat Oatp1 serum was previously raised against a fusion protein with the C-terminal 40 amino acids and maltose-binding protein as described in Eckhardt et al. (1999). Membrane fractions were prepared as previously described (Ogawa et al., 2000), diluted with the sample buffer, with or without reducing agent, and denatured at 95°C for 2 min before separation on 3.75% stacking and 10% resolving SDS-polyacrylamide gels. Proteins were transferred electrophotically to polyvinylidene difluoride membranes using a blotter (Trans-Blot; Bio-Rad, Richmond, CA) at 15 V for 1 h. The membrane was blocked with Trans-buffer saline containing 0.05% Tween 20 (TBST) and 5% bovine serum albumin for 1 h at room temperature. After washing with TBST (three times for 10 min), the membrane was incubated with anti-rat Oatp1 serum (dilution 1:500), allowed to bind \( ^{125} \)I-labeled sheep anti-rabbit IgG antibody diluted 1:200 in TBST containing 5% bovine serum albumin for 1 h at room temperature, and washed with TBST (three times for 5 min). Then, the membranes were exposed to Fuji imaging plates (FujiFilm, Kanagawa, Japan) for 3 h at room temperature and analyzed with an imaging analyzer (BAS 2000; FujiFilm).

Results

Gender Difference in the Urinary Excretion of Organic Anions. The plasma concentration profile of TCA and DBSP showed a minimal gender difference, whereas their urinary excretion over 180 min in female rats was 3.8 and 7.7 times higher than in males, respectively (Fig. 1). Neither the CL\text{out}, the \( f_u \), nor the \( R_t \) of TCA and DBSP showed any evidence of a gender difference (Table 1). The CL\text{urine,p} of TCA and DBSP in female rats was 4.4 and 7.7 times higher than in male rats, respectively (Table 1). During the constant infusion of zenarestat alone, its urinary excretion rate was...
also higher in female than in male rats (Fig. 2, C and D), the CL\textsubscript{urine,p} in female rats being 18 times higher than that in male rats (Table 2), whereas the plasma concentration profile showed only a minimal gender difference (Fig. 2, A and B).

**Gender Difference in the Transport of Zenarestat across the Apical Side.** To examine the gender difference in the transport of zenarestat across the antiluminal membranes, its renal uptake after 1 and 3 min following a bolus injection was examined (Fig. 3). The tissue-to-plasma concentration ratio was similar for the two genders (Fig. 3). The slope of the integration plot in male and female rats (0.0731 and 0.0892 ml/min/g tissue, respectively) was similar (Fig. 3).

Next, to analyze the transport clearance across the luminal side, the CL\textsubscript{urine,k} was examined when it was found that the clearance in female rats was 31 times higher than that in male rats (Table 2). The effect of DBSP on plasma zenarestat concentrations was not as marked (Fig. 2, A and B), whereas the CL\textsubscript{urine,k} was increased, whereas the CL\textsubscript{urine,k} at 150 \( \mu \)mol/h/kg DBSP being 2.6 and 5.7 times higher than in the control (Table 2). The CL\textsubscript{urine,p} was increased, whereas the CL\textsubscript{urine,k} was decreased by DBSP in female rats, although the change compared with the control was less than 50% (Table 2). Such a phenomenon might be explained by inhibition of efflux transport from tubular cells to the luminal and antiluminal spaces. Further studies are required to examine such a hypothesis. In the presence of 150 \( \mu \)mol/h/kg DBSP, the CL\textsubscript{urine,k} in female rats was 3.7 times higher than that in male rats (Table 2). Thus, the gender difference in CL\textsubscript{urine,k} in the presence of DBSP was not as marked compared with the control.

**Zenarestat Has an Affinity for Oatp1.** The uptake of [\(^3\)H]E217G, a typical substrate of Oatp1, by Oatp1-transfected LLC-PK1 cells was inhibited by zenarestat with a \( K_i \) value of 8.97 \( \mu \)M (Fig. 4).

**Gender Difference in the Expression of Organic Anion Transporters.** The mRNA expression of Oatp1 in the kidney was much higher in male than in female rats, whereas this gender difference was minimal in the liver (Fig. 5A). Such higher Oatp1 expression was also confirmed by Western blotting (Fig. 5C). These findings were compatible with previous findings (Lu et al., 1996; Goto et al., 2002). Bergwerk et al. (1996) have reported that renal Oatp1 migrates as 33- and 37-kDa peptides under reduced conditions, whereas liver Oatp1 migrates as 83-kDa protein. In the present study, the size of renal Oatp1 was also smaller than that of hepatic Oatp1, although multiple bands cannot be clearly seen in the kidney (Fig. 5B). Oudar et al. (1991) reported a gender difference in the relative volume of both the cortex and medulla with regard to the total kidney volume. The difference in the relative volume of the medulla was at most 2-fold greater in female than in male rats and could have a minor effect on the observation of higher Oatp1 expression in male than in female rats. The mRNA of Oatp2 was not detected in either male or female rat kidney (Fig. 5A). The mRNA of Mrp2 in the liver did not show any clear gender difference, whereas that in the kidney could not be detected under these conditions (Fig. 5A). The mRNA of Oat3 in the liver was higher in male than in female rats (Fig. 5A), and these results were compatible with recent findings (Kobayashi et al., 2001).

**Discussion**

Several reports have shown gender differences in the urinary excretion of certain types of organic anions in rats (Tanaka et al., 1991a; Nakamura et al., 1993; Terashita et al., 1994; Sato et al., 2000), although the mechanism for such gender differences is not yet fully understood. Considering that Oatp1 takes up a variety of types of organic anions into cells (Eckhardt et al., 1999) and that the gene expression of
Oatp1 in kidney is higher in male than in female rats (Lu et al., 1996), the urinary excretion of its substrates may also exhibit a gender difference if Oatp1 is mainly involved in the renal transport of the substrates. Actually, we have previously reported that the urinary excretion of E217βG, a typical substrate of Oatp1, is more than 250 times higher in female than in male rats (Goto et al., 2002). Therefore, here, we attempted to examine such a gender difference in urinary excretion for other substrates and inhibitors, including therapeutic agents. We found that the CLurine,p of TCA and DBSP, a substrate and inhibitor of Oatp1, respectively, is higher in female than in male rats (Fig. 1, Table 1). In addition, zenarestat, which exhibits a similar gender difference in its urinary excretion (Fig. 2; Tanaka et al., 1991a), has been shown to have an affinity for Oatp1 in the present study (Fig. 4). Therefore, at least some types of Oatp1 substrates or inhibitors commonly exhibit a gender difference in their urinary excretion in rats.

To examine the reason for such gender differences, further kinetic analysis was performed in vivo to investigate the excretion of zenarestat. Since the uptake of zenarestat by the kidney is not very different between males and females (Fig. 3), there may be only a minimal gender difference in the renal uptake of zenarestat. In addition, a gender difference may be present in zenarestat transport across the apical membrane since the CLurine,k of zenarestat was higher in female than in male rats (Table 2). This CLurine,k was increased by DBSP (Table 2), suggesting that such transport can be inhibited by DBSP. Considering both the GFR (Table 2) and fG of zenarestat (0.0033 and 0.0056 in male and female rats, respectively; Tanaka et al., 1993), the estimated glomerular filtration clearance for this compound (fGFR) is 1.2 to 2.2 ml/min/kg, which is much higher than the CLurine,p of zenarestat in control male rats (Table 2), suggesting extensive reabsorption of this compound in males.

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### Table 2

**Pharmacokinetic parameters of zenarestat**

Data shown in Fig. 2 were used to determine these parameters (mean ± S.E. of four or five rats). Rats received an intravenous priming dose of zenarestat (1 mg/kg) followed by an infusion at 3 mg/h/kg.

<table>
<thead>
<tr>
<th></th>
<th>CLurine,p</th>
<th>CLurine,k</th>
<th>GFR</th>
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</thead>
<tbody>
<tr>
<td><strong>Male</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>0.0432 ± 0.0061</td>
<td>0.247 ± 0.013</td>
<td>6.33 ± 0.16</td>
</tr>
<tr>
<td>+ DBSP 25 μmol/h/kg</td>
<td>0.0872 ± 0.0083</td>
<td>0.360 ± 0.027</td>
<td>6.33 ± 0.40</td>
</tr>
<tr>
<td>+ DBSP 75 μmol/h/kg</td>
<td>0.134 ± 0.087</td>
<td>1.62 ± 0.21</td>
<td>6.02 ± 0.15</td>
</tr>
<tr>
<td>+ DBSP 150 μmol/h/kg</td>
<td>0.112 ± 0.021</td>
<td>1.40 ± 0.45</td>
<td>6.35 ± 0.07</td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.771 ± 0.452</td>
<td>7.61 ± 0.45</td>
<td>6.38 ± 0.08</td>
</tr>
<tr>
<td>+ DBSP 25 μmol/h/kg</td>
<td>0.883 ± 0.088</td>
<td>7.14 ± 1.00</td>
<td>6.34 ± 0.20</td>
</tr>
<tr>
<td>+ DBSP 75 μmol/h/kg</td>
<td>0.943 ± 0.102</td>
<td>4.33 ± 0.28</td>
<td>6.16 ± 0.20</td>
</tr>
<tr>
<td>+ DBSP 150 μmol/h/kg</td>
<td>1.13 ± 0.03</td>
<td>5.22 ± 0.33</td>
<td>6.35 ± 0.01</td>
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*a* CLurine,p was obtained by dividing the cumulative urinary excretion by the AUC.

*b* CLurine,k was obtained by dividing the rate of urinary excretion at 150 min by the concentration in the kidney.

*c* p < 0.05; significantly different from male control.

### Fig. 3

Integration plot of the renal uptake of zenarestat. The time profiles of the plasma and tissue concentrations of zenarestat were measured after its intravenous bolus injection (1 mg/kg) in male (●) and female (▲) rats. Data are expressed as integration plots. The initial slope represents the CLuptake. Each point and the vertical and horizontal bars represent the mean ± S.E. of three independent experiments.

### Fig. 4

Inhibition of the uptake of [3H]E217βG by zenarestat in Oatp1-transfected LLC-PK1 cells. LLC-PK1 cells stably transfected with rat Oatp1 were incubated at 37°C for 2 min with [3H]E217βG (0.3 μCi/ml). The uptake in vector-transfected cells was subtracted from that in Oatp1-transfected cells and normalized by the substrate concentration in the medium. The solid line indicates the fitted line. Each point and vertical bar represent the mean ± S.E. of three independent experiments.
and 2) and that Oatp1 is expressed at a higher level in male rats (Fig. 5; Lu et al., 1996) on the apical membranes of tubular epithelial cells (Bergwerk et al., 1996), such a gender difference can be explained if we hypothesize that Oatp1 is involved in the reabsorption of its substrates. The urinary excretion in castrated male rats was increased to a level comparable with that found in female rats (Tanaka et al., 1991b), and treatment of male and female gonadectomized or hypophysectomized rats with testosterone resulted in urinary excretion of zenarestat that was characteristic of that in male rats. (Tanaka et al., 1991b, 1992). On the other hand, the mRNA for Oatp1 is reduced in the kidney of castrated male rats, and treatment of castrated male and female rats with testosterone increased its expression (Lu et al., 1996). The Oatp1 gene product is also reduced in the kidney of castrated male rats, whereas it is increased in female rats treated with testosterone (Goto et al., 2002). Urinary clearance of both zenarestat (Table 2) and E217/G9252 (Goto et al., 2002) is increased by the Oatp1 inhibitor DBSP. These findings are compatible with the hypothesis that the reabsorption of these compounds, which is possibly mediated by Oatp1 and/or transporters that have a similar substrate specificity to Oatp1, can be inhibited by DBSP. However, in the presence of the highest dose of DBSP (150 μmol/h/kg), the CL_u of zenarestat in female rats was still 4 times higher than in males (Table 2), whereas the CL_u of E217/G9252 showed only a minimal gender difference at the same DBSP dose (Goto et al., 2002). Since the CL_u of zenarestat at 150 μmol/h/kg of DBSP was still lower than the fGFR as calculated above (Table 2), reabsorption may not be completely inhibited by DBSP and/or other mechanisms may be involved in the gender difference in renal excretion. Interestingly, the urinary excretion of zenarestat is reduced by probenecid in female rats, whereas such inhibition is minimal in males (Tanaka et al., 1991a), suggesting that some probenecid-sensitive transporter, involved in the secretion of zenarestat, is expressed in the kidneys of female rats. Since probenecid is an inhibitor of Oatp1, the inhibition of Oatp1 by probenecid cannot be ruled out in the studies by Tanaka et al. (1991a). Nevertheless, considering their results together with the present findings, multiple transporters, including a probenecid-sensitive transporter and a male-specific reabsorption transporter, might be involved in the gender difference seen in zenarestat excretion. Further studies are needed to clarify this hypothesis.

Northern blot analysis revealed that Oatp1 gene expression is much higher in male rats than in females, whereas no clear gender difference can be detected for Oatp2 or Mrp2, although one was observed both in Oat2 in kidney and Oat3 in liver (Fig. 5). Lu et al. (1996) have also reported a similar gender difference in mRNA for Oatp1 where the probe for such Oatp1 detection corresponded to 254 to 1436 bp, which was located in the open reading frame of Oatp1. Considering that the OATP family shows a high degree of sequence homology (85 and 87% homology between Oatp1 and Oatp2, and between Oatp1 and Oatp3, respectively), in this study, we used a probe consisting of the noncoding region of Oatp1, which showed only a minimal cross-reaction with the cRNA of Oatp2 and Oatp3. Cattori et al. (2000) have reported another Oatp member, Oatp4, which exhibits liver-specific expression in male rats. Considering these results, the gene expression of Oatp1, apart from the other three clones in kidney, is higher in male rats than in females, although there is still a need to exclude the possibility that other, perhaps unknown, members of the OATP family may affect the Northern blot analysis.

In addition to Oatp1, the mRNA of Oat2 showed a gender
difference in the kidney (Fig. 5) as reported by Kobayashi et al. (2001). Oat2 takes up a variety of types of substrates, including salicylate, proglandin E2, indomethacin, and 3'-azido-3'-deoxythymidine (Sekine et al., 1998; Morita et al., 2001), because its substrate specificity is different from that of Oat1 to some extent. However, the present findings alone are not enough to rule out the possibility that Oat2 may be involved in the gender difference in the urinary excretion of certain types of organic anions. In fact, bromosulfophthalein, a structural analog of DBSP and TCA, has been reported to inhibit Oat2-mediated transport (Sekine et al., 1998; Morita et al., 2001). Therefore, further studies are needed to clarify whether this transporter is involved in the gender difference in the secretion and/or reabsorption of organic anions.

In conclusion, the urinary excretion of several Oatp1 substrates and inhibitors exhibits a gender difference in rats, and a DBSP-sensitive reabsorption process at the apical side is involved to some extent in the gender difference seen in the urinary excretion of zearalenest.

References


Bergwerk AJ, Shi X, Ford AC, Kanai N, Jacquemin E, Burk RD, Bai S, Novikoff PM, et al. (2001) Oat2 takes up a variety of types of substrates, including salicylate, prostaglandin E2, indomethacin, and 3'-azido-3'-deoxythymidine (Sekine et al., 1998; Morita et al., 2001). Therefore, further studies are needed to clarify whether this transporter is involved in the gender difference in the secretion and/or reabsorption of organic anions.

In conclusion, the urinary excretion of several Oatp1 substrates and inhibitors exhibits a gender difference in rats, and a DBSP-sensitive reabsorption process at the apical side is involved to some extent in the gender difference seen in the urinary excretion of zearalenest.


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