Involvement of Bile Salt Export Pump in Flutamide-Induced Cholestatic Hepatitis

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The non-steroidal antiandrogen flutamide is widely used for treatment of prostatic cancer, but causes side effects, including cholestatic hepatitis and fulminant hepatitis. We investigated the pathogenesis of flutamide-induced cholestatic hepatitis, focusing on the bile salt export pump (BSEP; ABCB11), which exports bile salts to the bile. We examined the inhibitory effects of flutamide and its active metabolite, hydroxyflutamide, on the transport of taurocholic acid (TCA) by membrane vesicles derived from hBSEP-expressing Sf9 cells. Flutamide inhibited the transport of TCA by hBSEP (IC50 value, about 50 μM), while hydroxyflutamide had no effect at up to 100 μM. When flutamide was administered to rats as a single oral dose of 100 mg/kg, the biliary excretion rate of bolus-injected [3H]TCA was decreased and the liver tissue concentration of flutamide exceeded 50 μM. Repeated doses of flutamide for 5 d (10 mg/kg/d) also decreased the biliary excretion rate of bolus-injected [3H]TCA. In this case, the liver tissue concentration of flutamide was below 0.1 μM. In both cases, no change in the mRNA level of rat Bsep was detected by RT-PCR. These results suggest that flutamide itself, but not its major metabolite, may cause cholestasis by inhibiting BSEP-mediated bile salt excretion.

Key words flutamide; bile salt export pump (BSEP); cholestasis; bile salt; taurocholic acid

Flutamide (2-methyl-N-[4-nitro-3-(trifluoromethyl)phenyl]propanamide) is a nonsteroidal antiandrogen used for the treatment of prostate carcinoma. However, its therapeutic activity is compromised because of potential liver toxicity. Among 1091 patients treated for prostate cancer with flutamide, the incidence of liver toxicity (defined as >4-fold increase in serum transaminase activity) was 0.36%.1 In addition, Wada et al. reported that the incidence of liver disorder (defined as >1.5-fold increase in alanine aminotransferase levels) was 26%.2 Furthermore, between February 1989 and December 1994, the Food and Drug Administration received reports of 20 deaths and 26 hospitalizations due to hepatotoxicity secondary to flutamide.3 Flutamide-induced hepatitis may be cholestatic and/or cytolytic, and fulminant hepatitis can occur.4–8 Fau et al. proposed that cytochrome P450-mediated generation of electrophilic metabolites may play a role in flutamide-induced cytolytic hepatitis, based on studies in isolated rat hepatocytes.9 Furthermore, Wang et al. reported that flutamide and hydroxyflutamide (a major metabolite of flutamide) are cytotoxic to primary cultured rat hepatocytes at concentrations of approximately 40 μM and 170 μM, respectively.10 However, little is yet known about the mechanism leading to cholestatic hepatitis. Therefore, this was the focus of the present study.

Cholestasis, defined as impairment of bile formation, is accompanied by retention of bile salts, and the resulting intracellular accumulation of bile salts causes cell damage through a direct effect on trafficking of Fas to the cell surface, leading to apoptosis.11,12 It was reported that mutation of the human bile salt export pump (BSEP, ABCB11) gene causes progressive intrahepatic cholestasis.13,14 Several drugs such as troglitazone, bosentan, and cyclosporine A induce cholestatic hepatitis, probably by inhibiting BSEP-mediated taurocholic acid transport.15–17 BSEP is the transport protein primary responsible for biliary excretion of unconjugated bile salts, which are more hepatotoxic than their amino acid (i.e. taurine and glycine) conjugated metabolites.18 It is known that flutamide-induced hepatotoxicity is ameliorated by ursodeoxycholic acid (UDCA).19 Since UDCA is a ligand of farnesoid X receptor (FXR) and FXR up-regulates BSEP activity, it was proposed that the action of UDCA is associated with a change in the expression level of BSEP.20 Furthermore, Fickert et al. reported that protein level of Bsep was increased in UDCA treated mice.21 Therefore, we examined the role of BSEP in flutamide-related cholestatic hepatitis. We also examined the effect of hydroxyflutamide, since hydroxyflutamide is cytotoxic and its plasma concentration is much higher than that of flutamide.22 Flutamide and hydroxyflutamide are metabolized to FLU-1 and FLU-3, and their glucuronides,23 but flutamide-induced cholestasis may not involve these metabolites, since no consistent differences were found in serum levels of these metabolites, including the glucuronides, between patients with normal function and those suffering hepatic dysfunction.24 Therefore, in the present study, we focused on the effects of flutamide itself and hydroxyflutamide.

MATERIALS AND METHODS

Chemicals [3H]Taurocholic acid (TCA; 1.92 TBq/mmol) was purchased from PerkinElmer Life Sciences (Boston, MA, U.S.A.). Flutamide, hydroxyflutamide and cyclosporine A were purchased from Wako Pure Chemical Industries (Osaka, Japan). Rat Bsep- and human BSEP-expressing Sf9 membranes (rBsep and hBSEP, respectively) were obtained from GenoMembrane, Inc. (Kanagawa, Japan). All other reagents were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.), Wako Pure Chemical Industries, Kanto Chemicals (Tokyo, Japan), Nacalai Tesque (Kyoto, Japan), and Kishida Chemicals (Osaka, Japan).

Uptake Study by Membrane Vesicles Cell membranes from hBSEP- or rBsep-expressing SF9 cells were suspended in the transport buffer (10 mM HEPES, 100 mM KNO3, 10 mM...
Mg(NO$_3$)$_2$ and 50 mM sucrose, pH 7.4), and passed through a 25-gauge needle (30 times). Flutamide, hydroxyflutamide and cyclosporine A were each dissolved in dimethylsulfoxide (DMSO; final concentration 1%), and DMSO without inhibitors was included as a control. After preincubation of the membrane vesicles for 2 min, uptake of [${}^3$H]TCA was initiated by the addition of a 90 μl aliquot of transport buffer containing [${}^3$H]taurocholic acid, 5 mM adenosine triphosphate (ATP) or 5 mM adenosine monophosphate (AMP), an ATP-regenerating system (10 mM creatine phosphate and 100 μg/ml creatine phosphokinase), and inhibitors to 10 μl of membrane vesicles (10 μg protein) at 37°C. At designated times, the uptake was terminated by the addition of 1 ml of ice-cold stop buffer (10 mM Hepes, 150 mM KNO$_3$, 10 mM Mg(NO$_3$)$_2$, 50 mM sucrose, 0.1 mM TCA, pH 7.4). To terminate the reactions, the mixture was filtered through a HA filter (0.45 μm; Millipore Corp., Bedford, MA, U.S.A.), and then washed twice with 5 ml of ice-cold stop buffer. Specific uptake by BSEP was evaluated as ATP-dependent uptake, obtained as the difference between the uptakes in the presence of ATP and of AMP. The radioactivity retained on the filter and in the reaction mixture was measured with a liquid scintillation counter (Perkin Elmer, Boston, MA, U.S.A.). A 250×4.6-mm i.d. Mightyseal RP-18 GP Aqua column (Kanto Chemical, Tokyo, Japan) was used for analysis. The mobile phase consisted of 50% water and 50% acetonitrile and was delivered at a flow rate of 1 ml/min at 40°C. Calibration curves were linear from 0.1 to 100 μM for both flutamide and hydroxyflutamide, and the coefficients of variation were less than 15%.

**HPLC Analysis of Flutamide and Hydroxyflutamide**

For the quantitation of flutamide and hydroxyflutamide, aliquots of plasma and liver homogenate were mixed with 2 volumes of methanol (v/v for plasma, w/v for liver), and left on ice for 30 min. The precipitated proteins were removed by centrifugation (13000g, 10 min), and a 10 μl aliquot of the supernatant was directly injected to the HPLC instrument. HPLC analyses were carried out on an Alliance system (Waters, Milford, MA, U.S.A.) consisting of the 2690 separation module, and the 2487 dual absorbance detector (300 nm), controlled with Empower software (Waters, Milford, MA, U.S.A.). A 250×4.6-mm i.d. Mightyseal RP-18 GP Aqua column (Kanto Chemical, Tokyo, Japan) was used for analysis. The mobile phase consisted of 50% water and 50% acetonitrile and was delivered at a flow rate of 1 ml/min at 40°C. Calibration curves were linear from 0.1 to 100 μM for both flutamide and hydroxyflutamide, and the coefficients of variation were less than 15%.

**Hepatic RNA Extraction and Semi-Quantitative PCR**

mRNAs of rBsep and Na$^+$-taurocholate cotransporting polypeptide (Ntcp) in liver were quantitated by means of a semi-quantitative PCR method. Total RNA was prepared from the liver using Isogen (Wako Pure Chemical Industries, Tokyo, Japan). The total RNA content was determined by measuring the absorbance at 260 nm. mRNA level in liver was analyzed by means of semi-quantitative reverse transcription polymerase chain reaction (RT-PCR). Single-stranded cDNAs were constructed using an oligo(dT) primer (Invitrogen Corp., Carlsbad, CA, U.S.A.) and Improm-II reverse transcriptase (Promega). These cDNAs provided templates for PCR using specific primers (rBsep: 5′-GAATTCCAGCAGCCATGGCTGACAGCAGC-A-3′; 5′-ACGCTGACTCCACACAGCTGACTGACAGC-CAC-3′, rNtcp: 5′-GGAGACATTGAAGGACAGGTTTG-3′; 5′-ATGCTGATTGGTCGTGCAGCCTCT-G-3′, respectively). The PCR conditions were: denaturation at 94°C for 30 s, annealing at 38—62°C for 30—60 s, and elongation at 72°C for 30 s in the presence of deoxynucleotides (dNTPs) and Ex Taq polymerase (Takara Shuzo Co. Ltd., Tokyo). Annealing time and temperature were changed as required, depending on the genes. The PCR cycle numbers were titrated for each primer pair to confirm that amplification was performed within a linear range. PCR products were analyzed by 2% agarose gel (w/v) electrophoresis and the gels were stained with ethidium bromide for visualization. mRNA levels were quantified by using light capture (Atto Co., Tokyo). PCR amplification data were normalized with respect to glyceraldehyde-3-phosphate dehydrogenase (G3PDH).

**Statistical Analysis**

Statistical significance was determined with Student’s t test or by analysis of variance (ANOVA) followed by Dunnett’s test, and a p value less than 0.05 was considered statistically significant.
RESULTS

Inhibitory Effect of Flutamide and Hydroxyflutamide on BSEP-Mediated Taurocholic Acid Transport in Vitro

To examine the involvement of BSEP in flutamide-induced cholestasis, the inhibitory effects of flutamide and hydroxyflutamide on BSEP were investigated. The ATP-dependent uptake of [3H]TCA by hBSEP-expressing membrane vesicles increased over 10 min, and the uptake at 2 min was taken as the initial uptake, since the increase was linear up to this time point (data not shown). Uptake of TCA was inhibited by cyclosporine A (1 μM), the positive control, showing that specific uptake of TCA by BSEP can be evaluated by the method used in the present study. Flutamide inhibited ATP-dependent [3H]TCA uptake in a concentration-dependent manner from 1 to 100 μM, and the estimated IC\textsubscript{50} value was 48.3±9.1 μM (Fig. 1). On the other hand, hydroxyflutamide was not inhibitory up to 100 μM. Similar results were obtained in rBsep-expressing membrane vesicles (38% of control at 100 μM flutamide, data not shown). Furthermore, we checked the reversibility of the inhibitory effect of flutamide and hydroxyflutamide on BSEP by examining the influence of pretreatment with them upon TCA transport. The inhibitory effect of flutamide on uptake of [3H]TCA by hBSEP-expressing membrane vesicles that had been preincubated with flutamide for 30 min was not significantly different from that in the case of membrane vesicles that had not been preincubated with flutamide (Fig. 2). There was also no pretreatment effect in the case of hydroxyflutamide (Fig. 2).

Effect of Flutamide on Biliary Excretion of Taurocholic Acid in Vivo

The cholestatic effect of a single dose and repeated doses of flutamide was examined in rats. Figure 3 shows the time course of cumulative biliary excretion and the biliary excretion rate of bolus-injected [3H]TCA. In both the single-dose group (100 mg/kg flutamide) and the repeated-dose group (10 mg/kg flutamide for 5 d), the biliary excretion rate of [3H]TCA was significantly decreased for the initial 10 min. Subsequently, however, the cumulative biliary excretions of [3H]TCA in both the single-dose group and the repeated-dose group were comparable with that of the control group. There was no significant difference in plasma concentration profiles of [3H]TCA among the 3 groups (Fig. 4), and the values of area under the curve (AUC), distribution volume, and total clearance showed no significant inter-group differences from that in the presence of ATP (AMP added instead of ATP) from that in the presence of ATP (AMP added instead of ATP) from that in the absence of ATP (AMP added instead of ATP).

Fig. 1. Effects of Flutamide and Hydroxyflutamide on [3H]Taurocholic Acid Uptake by hBSEP-Expressing Membrane Vesicles

Initial ATP-dependent [3H]TCA (0.5 μCi) uptake was determined in the absence (DMSO 1%) , presence of cyclosporine A (Cys A), flutamide, or hydroxyflutamide at 2 min. Concentrations used are shown in the figure. The ATP-dependent uptake was obtained by subtracting the value in the absence of ATP (AMP added instead of ATP) from that in the presence of ATP. Control samples contained 1% DMSO without inhibitors. The results are shown as mean±S.E.M. (n=3). An asterisk (*) indicates a significant difference from the control value by Dunnett’s test (p<0.05).

Fig. 2. Effect of Flutamide and Hydroxyflutamide Pretreatment on [3H]Taurocholic Acid Uptake by hBSEP-Expressing Membrane Vesicles

ATP-dependent initial uptake of [3H]TCA (0.5 μCi) was determined after preincubation of hBSEP-expressing membrane vesicles with (closed bar) or without (open bar) inhibitors for 30 min. The ATP-dependent uptake was obtained by subtracting the value in the absence of ATP (AMP added instead of ATP) from that in the presence of ATP. Control samples contained 1% DMSO without inhibitors. The results are shown as mean±S.E.M. (n=3). An asterisk (*) indicates a significant difference from the control value by Dunnett’s test (p<0.05).

Fig. 3. Effects of Flutamide on Cumulative Biliary Excretion and Biliary Excretion Rate of [3H]Taurocholic Acid in Rats

A bolus of 80 nmol/kg TCA containing 1 μCi/ml [3H]TCA was injected into the portal vein of rats with (triangle: single-dose; square: repeated-dose) or without (circle) flutamide treatment as described in Materials and Methods. The closed, and open symbols show cumulative biliary excretion, biliary excretion rate of [3H]TCA at 10 min intervals, respectively. The results are shown as mean±S.E.M. (n=3). An asterisk (*) indicates a significant difference from the control value (t-test, p<0.05).

Fig. 4. Effect of Flutamide on Plasma Concentration Profile of [3H]Taurocholic Acid in Rats

A bolus of 80 nmol/kg TCA containing 1 μCi/ml [3H]TCA was injected into the portal vein of rats with (triangle: single-dose: square: repeated-dose) or without (circle) flutamide treatment as described in Materials and Methods. The results are shown as mean±S.E.M. (n=3). An asterisk (*) indicates a significant difference from the control value (t-test, p<0.05).
Selves cause hepatic injury and cholestasis. Several drugs portal blood into the liver (Figs. 5A, B).

In addition, there was no significant change in the mRNA levels not significantly different among the groups (Figs. 5A, B). In flutamide were determined. The mRNA levels of rBsep were rNtcp in Rats

The effect of flutamide on mRNA levels of rBsep in rats treated with a single dose or repeated doses of flutamide were determined. The mRNA levels of rBsep were lower than the quantitation limit (less than 0.1 μM in plasma and 0.1 nmol/g of liver) at 1 h after [3H]TCA injection (25 h after the last dose of flutamide).

**Effect of Flutamide on mRNA Levels of rBsep and rNtcp in Rats** The effect of flutamide on mRNA levels of rBsep in rats treated with a single dose or repeated doses of flutamide were determined. The mRNA levels of rBsep were not significantly different among the groups (Figs. 5A, B). In addition, there was no significant change in the mRNA levels of Ntcp, which is expressed at the basolateral membrane of hepatocytes, and is involved in the transport of TCA from portal blood into the liver (Figs. 5A, B).

**DISCUSSION**

Cholestasis, defined as impairment of bile formation, is accompanied with retention of bile salts, which can themselves cause hepatic injury and cholestasis.11,12 Several drugs such as troglitazone, bosentan, and cyclosporine A induce cholestatic hepatitis, probably by inhibiting BSEP-mediated taurocholic acid transport.15–17 Fattinger et al. concluded that in vitro screening for BSEP inhibition can adequately predict the effects of drugs on bile salt serum levels in in vivo animal studies, and that both BSEP inhibition and increased serum bile salt levels in preclinical animal studies are indicative for the development of drug-induced cholestatic liver injury in humans. Therefore, we evaluated the inhibitory effects of flutamide and its major metabolite, hydroxyflutamide, on BSEP-mediated taurocholic acid (TCA) transport.

By in vitro transport experiments, we found that flutamide inhibited BSEP-mediated TCA transport, with an IC_{50} of approximately 50 μM (Fig. 1), while hydroxyflutamide was not inhibitory up to 100 μM. Since the inhibition constant (K_i) value of flutamide and hydroxyflutamide for androgen receptor is 1450 and 205 nm, respectively,25 and plasma concentration of hydroxyflutamide is higher than that of flutamide,24 the pharmacological effect of flutamide can be largely attributed to hydroxyflutamide. Therefore, the pharmacological effect of flutamide can be maintained without the inhibitory effect on Bsep. Next, we examined whether the inhibitory effect of flutamide on BSEP-mediated TCA transport is relevant in the clinical situation. It was reported that the plasma concentration of flutamide in rats reached approximately 1 μM after administration of flutamide orally at a dose of 15 mg/kg,26 and the liver-to-plasma concentration ratio (K_i) value of flutamide is approximately 5 (basic product information of flutamide Odyne, Nippon Kayaku Co., Ltd., Tokyo, Japan). Based on these reports, we administered

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**Table 1. Effects of Flutamide on AUC_{0—40 min}, Distribution Volume, and Total Clearance of [3H]Taurocholic Acid in Rats**

<table>
<thead>
<tr>
<th>Dose (Flutamide)</th>
<th>AUC_{0—40 min} (pmol·min/ml)</th>
<th>CL_{tot} (ml/min/kg)</th>
<th>CL_{bile} (ml/min/kg)</th>
<th>V_{dss} (ml/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single group</td>
<td>2099±166</td>
<td>36.1±4.0</td>
<td>35.9±4.0</td>
<td>346±61</td>
</tr>
<tr>
<td>Repeated group</td>
<td>2155±161</td>
<td>35.1±1.8</td>
<td>31.6±1.9</td>
<td>326±72</td>
</tr>
<tr>
<td></td>
<td>2113±92</td>
<td>36.0±1.7</td>
<td>34.3±0.7</td>
<td>334±36</td>
</tr>
</tbody>
</table>

A bolus of 80 nmol/kg TCA containing 1 μCi/ml [3H]TCA was injected into the portal vein of rats with or without flutamide treatment as described in Materials and Methods. The results are shown as mean±S.E.M. (n=3).

**Table 2. Plasma and Liver Concentrations of Flutamide and Hydroxyflutamide in Rats**

<table>
<thead>
<tr>
<th>Dose</th>
<th>Plasma (μM)</th>
<th>Liver (nmol/g liver)</th>
<th>K_i value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flutamide</td>
<td>OH-flutamide</td>
<td>Flutamide</td>
</tr>
<tr>
<td>Single</td>
<td>100 mg/kg</td>
<td>16.8±6.0</td>
<td>28.3±5.0</td>
</tr>
<tr>
<td>Repeated</td>
<td>10 mg/kg</td>
<td>BQL</td>
<td>0.3±0.0</td>
</tr>
<tr>
<td></td>
<td>(5 d)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Wistar rats were orally administered flutamide, and plasma and liver samples were obtained at 1 h after bolus injection of [3H]TCA as described in Materials and Methods. The results are shown as means±S.E.M. (n=3). BQL: below the quantitation limit (quantitation limit; 0.1 nmol in plasma, 0.1 nmol/g liver).
100 mg/kg flutamide as a single dose in order to obtain a liver concentration of flutamide close to the IC$_{50}$ value for BSEP, even though this dose is significantly higher than the clinical dose. In addition, to model the clinical situation, we administered 10 mg/kg of flutamide daily for 5 d (repeated-dose group), since this is close to the clinical dose (375 mg/d). In the flutamide-treated rats, the biliary excretion rate of [3H]TCA was decreased in the first 10 min in both the single-dose group and repeated-dose group (Fig. 3). Since the cumulative biliary excretion of [3H]TCA up to 60 min after injection of [3H]TCA showed no significant difference among the 3 groups (Fig. 3), the decrease of biliary excretion rate of [3H]TCA in the first 10 min can be ascribed to the decrease of transport of [3H]TCA from plasma into bile by flutamide. Furthermore, since there was no apparent difference in the plasma concentration profile of [3H]TCA among the 3 groups (Fig. 4, Table 1), it was suggested that the biliary excretion rate of [3H]TCA was decreased due to a change in the efflux process from hepatocytes into bile, but not in the uptake process from blood into hepatocytes. Therefore, it was demonstrated that flutamide may cause a decrease in the biliary excretion rate of [3H]TCA by inhibiting BSEP. For further understanding of flutamide-induced cholestasis, total bile acids concentrations in plasma, bile, and liver should be measured after flutamide treatment.

In the single-dose group, the flutamide concentration in liver (approx. 60 nmol/g of tissue) reached a level close to the IC$_{50}$ value for BSEP-mediated TCA uptake (approx. 50 μM) at 4 h after flutamide administration (Fig. 1, Table 2). However, in the repeated-dose group, the apparent flutamide concentration in liver was not high enough to inhibit BSEP (below 0.1 nmol/g of tissue), even though cholestasis was similar to that in the single-dose group. This apparent observation might be explained by irreversible inhibition by flutamide. There are reports of transporters being inhibited by such as 4,4’-disothiocyanato-stilbene-2,2’-disulphonic acid (DIDS), which is an irreversible inhibitor of monocarboxylate transporter in rat erythrocytes.

When erythrocytes were preincubated with DIDS, t-lactate transport into rat erythrocytes was still reduced, even when DIDS was washed out by replacing the uptake medium with DIDS-free medium. So, it is considered in the case of irreversible inhibitors that their inhibitory effects will be observed when the apparent inhibitor concentration in tissues is lower than IC$_{50}$ value obtained without preincubation. Therefore, we investigated the preincubation effect of flutamide and hydroxyflutamide on BSEP. However, neither flutamide nor hydroxyflutamide was an irreversible inhibitor of BSEP (Fig. 2). Accordingly, the observation that the retention of biliary excretion of [3H]TCA was observed at the apparent concentration lower than IC$_{50}$ value against BSEP was not explained by irreversible inhibition.

Flutamide-induced hepatotoxicity is ameliorated by ursodeoxycholic acid (UDCA), which is a ligand of farnesoid X receptor (FXR). The FXR plays an important role in maintaining bile acid homeostasis by regulating key genes involved in bile acid synthesis, metabolism and transport, such as CYP7A1, UGT2B4, BSEP, NTCP, apical sodium bile acid cotransporter (ASBT), and organic solute transporter (OST) alpha-OSTbeta in humans. Therefore, it may be possible that changes in the expression levels of these genes are involved in flutamide-induced cholestasis. In this study, since we measured the biliary excretion of bile acids after bolus injection of [3H]TCA, enzymes which are involved in synthesis and/or metabolism of bile acids, such as CYP7A1 and UGT2B4, are irrelevant. Furthermore, mRNA level of rBsep and rat Ntcp were not changed (Fig. 5). Accordingly, although the change of their protein levels cannot be denied, flutamide is not likely to affect at least the mRNA levels of these transporters. Therefore, Bsep might not play a major role in flutamide-induced cholestasis in repeated-dose group.

In this study, we focused on the effect of flutamide itself and hydroxyflutamide, but not other metabolites such as FLU-1, FLU-3, and their glucuronides, since no consistent differences were found in serum levels of these metabolites between patients with normal function and those suffering hepatic dysfunction. Furthermore, a similar pattern of the profile of flutamide metabolites was observed in the case of treatment with or without UDCA, which is known to be beneficial for flutamide-induced hepatotoxicity. However, it is possible that these metabolites inhibit Bsep-mediated bile acids transport. Therefore, these metabolites might be involved in flutamide-induced cholestasis in repeated-dose group. For further understanding of flutamide-induced cholestasis, inhibitory effect of these metabolites on Bsep should be evaluated.

The plasma concentration of flutamide reached 0.4 μM after administration of 250 mg three times a day for 9 d in normal geriatric volunteers. Since the K$_p$ value of flutamide is 5.9 in liver (Table 2), the hepatic concentration would be approx. 2.4 μM, assuming that the K$_p$ value of flutamide in human liver is similar to that in rat liver. At this concentration, flutamide would inhibit BSEP-mediated bile acid transport by about 5%. However, if the metabolism of flutamide was reduced, e.g., by SNPs, drug–drug interactions, or altered expression of related genes, the flutamide concentration in liver might become high enough to exert an inhibitory effect on BSEP, since flutamide is extensively metabolized in the liver.

In conclusion, we found that flutamide, but not its major metabolite hydroxyflutamide, inhibits BSEP-mediated TCA transport. In addition, flutamide did not affect the mRNA level of rBsep. Accordingly, though the IC$_{50}$ value is lower than normal clinical concentration of flutamide in liver, the direct inhibition of BSEP may contribute in part to the occurrence of flutamide-induced cholestatic hepatitis.

REFERENCES


