Effects of Organic Anions and Bile Acid Conjugates on Biliary Excretion of Pravastatin in the Rat

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Purpose. Biliary organic anion excretion is mediated by an ATP-dependent primary active transporter, a so-called canalicular multispecific organic anion transporter (cMOAT). As there appear to be many canalicular organic anion transports, we examined the effects of various organic anions and bile acid conjugates on the biliary excretion of pravastatin in rats.

Methods. [^{14}C]pravastatin was intravenously injected into rats with bile drainage in the presence and absence of the continuous infusion of organic anions and bile acids, and radioactivity of its biliary excretion was studied.

Results. Biliary excretion of [^{14}C]pravastatin was markedly inhibited by sulfobromophthalein-glutathione, taurolithocholate-3-sulfate, ursodeoxycholate-3,7-sulfate, and ursodeoxycholate-3-O-glucuronide. In contrast, dibromosulfophthalein only slightly inhibited biliary pravastatin excretion, and cefpiramide did not affect biliary pravastatin excretion.

Conclusions. These findings further support the multiplicity of canalicular organic anion transport, and pravastatin is considered to be excreted through a canalicular transporter which is absent in EHBR in addition to through cMOAT.

KEY WORDS: organic anions; bile acids; pravastatin; canalicular multispecific organic anion transporter (cMOAT); Eisai hyperbilirubinemic rats (EHBR).

INTRODUCTION

Biliary excretion of organic anions has been shown to be mediated by an ATP-dependent primary active transporter (1–3), a so-called canalicular multispecific organic anion transporter (cMOAT), which is defective in mutant hyperbilirubinemic rats, TR$^-$/GY rats (4,5) and EHBR (6). Recently, cDNA cloning of cMOAT has been reported and the absence of cMOAT and abnormalities of cMOAT mRNA in TR$^-$/GY rats and EHBR have been identified (7–9).

Pravastatin (PS) is an anionic HMG-CoA reductase inhibitor (10) and is taken up by the liver by a Na^+-independent active transport system (11). Biliary PS excretion is markedly delayed in EHBR (12,13), but its ATP-dependent transport by canalicular membrane vesicles is present in EHBR, suggesting that PS is excreted through cMOAT and an ATP-dependent canalicular transporter which is absent in EHBR (13,14). On the other hand, a multiplicity of canalicular organic anion transport has been suggested (12,15–20). Therefore, we examined the effects of various organic anions and bile acid conjugates, which biliary excretion is defective in EHBR (6,12,15,21–23) on PS excretion in control rats and EHBR. Furthermore, as a control, biliary taurocholate (C-tau) excretion was simultaneously examined to confirm whether any organic anions or bile acid sulfate and glucuronide influence biliary C-tau excretion.

MATERIALS AND METHODS

Materials

[^{14}C]PS (10 mCi/mmol) and unlabeled PS were kindly provided by Sankyo Pharmaceutical Co. (Tokyo), and [^{3}H]C-tau (2.6 Ci/mmol, DuPont NEN) was purchased from Dai-ichi Chemical Co. (Tokyo). Sulfobromophthalein (BSP) was purchased from Dai-ichi Pharmaceutical Co. (Tokyo), and dibromosulfophthalein (DBSP) from Societe d'Etudes et de Recherche Biologique (Paris). Cefpiramide (CPM) was obtained from Yamanouchi Pharmaceutical Co. (Tokyo). Taurolithocholate-3-sulfate (3-sul-LC-tau) was purchased from Calbiochem (San Diego, CA). Ursodeoxycholate-3,7-disulfate (3,7-sul-UDC) and ursodeoxycholate-3-O-glucuronide (3-glcA-UDC) were generously provided by Tokyo Tanabe Co. (Tokyo).

Experimental Procedures

Male Sprague-Dawley rats (SDR) were purchased from Japan Laboratory Animals Inc. (Saitama, Japan), and male EHBR were obtained from Sankyo Labo Service Co. (Tokyo, Japan). All experiments were performed using rats each weighing approximately 270 g after overnight fasting. All animals received humane care in compliance with the "Principles of Laboratory Animal Care" (NIH publication #85-23). Animals were anesthetized with an intraperitoneal injection of pentobarbital (5 mg/100 g) and the common bile duct was cannulated with a PE-10 tubing (Becton Dickinson Primary Care Diagnostics, Sparks, MD) after laparotomy. The femoral vein was cannulated with a 3-F venous catheter and infused with 3% human serum albumin in 5% glucose solution (standard solution) at the rate of 2 ml/hr. Bile was collected in preweighed tubes. During the experiments, the rats were kept in fixable cages and body temperature was maintained at 37°C.

Thirty minutes after bile duct cannulation, a mixture of tracer amounts of [^{14}C]PS (3×10^5 dpm) and [^{3}H]C-tau (2×10^5 dpm) dissolved in the standard solution (50 μl) was injected into the femoral vein, and bile was collected every 10 minutes for 50 minutes. An aliquot of bile sample was counted for the radioactivity by a liquid scintillation counter. According to a report by Yamazaki et al. (14), about half of [^{14}C]PS in the bile is considered to be metabolized to hydrophilic compounds, probably glutathione conjugate and dihydrodiol (24).

The infusion via the femoral vein of BSP, DBSP, CPM, 3-sul-LC-tau, 3,7-sul-UDC and 3-glcA-UDC at the rate of 0.2 μmol/min/100 g b.wt. was started just after bile duct cannulation and continued during the experiments.

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ABBREVIATIONS: cMOAT, canalicular multispecific organic anion transporter; EHBR, Eisai hyperbilirubinemic rats; PS, pravastatin; C-tau, taurocholate; BSP, sulfobromophthalein; DBSP, dibromosulfophthalein; CPM, cefpiramide; 3-sul-LC-tau, taurolithocholate-3-sulfate; 3,7-sul-UDC, ursodeoxycholate-3,7-disulfate; 3-glcA-UDC, ursodeoxycholate-3-O-glucuronide; SDR, Sprague-Dawley rats.

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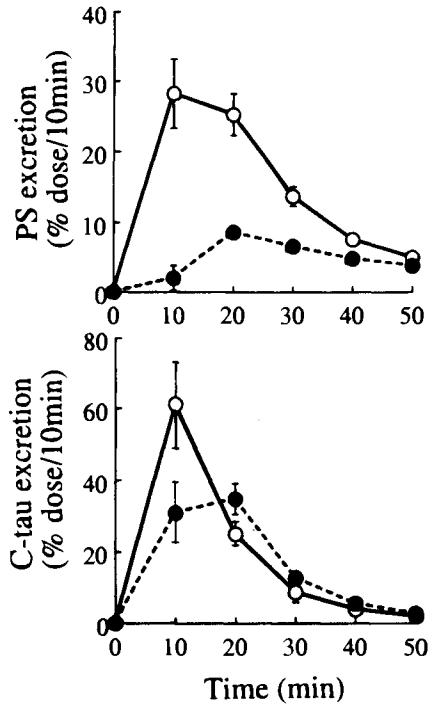

Fig. 1. Biliary excretion of [^{14}C]PS and [^{3}H]C-tau in SDR and EHBR. A mixture of tracer amounts of [^{14}C]PS and [^{3}H]C-tau was injected as a bolus into the femoral vein. Data are the means ± SD for control rats (n = 4, open circles) and EHBR (n = 3, closed circles).

All data were expressed as the mean±SD. A statistical analysis was performed by the Mann-Whitney U test, and $P < 0.05$ was considered to indicate a significant difference.

RESULTS

Biliary [^{14}C]PS excretion was markedly delayed in EHBR (Fig. 1), and cumulative [^{14}C]PS excretion in 50 minutes in EHBR was about one-third of that in the control rats (Table 1). Although there was some delay in biliary [^{3}H]C-tau excretion in EHBR, cumulative excretion in 50 minutes was similar to that of SDR. This was partly due to a significantly decreased bile flow in EHBR to 62% of that in SDR (data not shown), as has been previously reported (6).

Table 1. Summary of Cumulative Excretion of PS and C-tau in SDR and EHBR

	n	PS excretion	C-tau excretion
SDR			
Control	4	79.7 ± 7.0	100.8 ± 7.0
BSP	4	43.5 ± 0.9*	92.3 ± 3.7
DBSP	3	60.8 ± 4.8*	87.6 ± 10.7
CPM	3	77.5 ± 1.2	95.2 ± 2.1
3-sul-LC-tau	5	22.0 ± 6.1*	83.6 ± 7.0
3,7-sul-UDC	3	57.3 ± 7.1*	78.9 ± 3.1
3-glcA-UDC	4	26.1 ± 4.2*	87.9 ± 1.5
EHBR			
Control	3	25.5 ± 0.7	86.9 ± 12.3
BSP	4	17.3 ± 2.3*	87.9 ± 1.5
DBSP	3	20.4 ± 5.4	82.7 ± 5.6
CPM	3	22.4 ± 0.7*	86.8 ± 1.7

Data are the mean ± SD of % dose per 50 minutes. *$P < 0.05$ vs control experiments.

Effects of organic anions on biliary [^{14}C]PS excretion in SDR are shown in Fig. 2. Among these organic anions, CPM significantly increased bile flow to 131% of that in the control rats (data not shown). BSP and DBSP significantly decreased biliary [^{14}C]PS excretion, but CPM did not (Table 1). The extent of the inhibition of [^{14}C]PS excretion was more prominent by BSP than by DBSP.

Effects of organic anions on biliary [^{14}C]PS excretion in EHBR are shown in Fig. 3. Bile flow was not changed by the infusion of any organic anions (data not shown). BSP and CPM slightly, but significantly, decreased biliary [^{14}C]PS excretion (Table 1).

The effects of bile acid conjugates on biliary [^{14}C]PS excretion in SDR are shown in Fig. 4. Among these bile acids, 3,7-sul-UDC significantly increased bile flow to 148% of that in control rats (data not shown). 3-Sul-LC-tau, 3,7-sul-UDC, and 3-glcA-UDC significantly decreased biliary [^{14}C]PS excretion. Biliary [^{14}C]PS excretion which was inhibited by 3-sul-LC-tau and 3-glcA-UDC was similar to that observed in EHBR (Fig. 1).

All organic anions and bile acid conjugates did not influence biliary [^{3}H]C-tau excretion in either SDR or EHBR (Figs. 2–4, Table 1).

DISCUSSION

The multiplicity of biliary organic anion excretion has been suggested (15–19). Furthermore, we previously reported that biliary lithocholate-3-sulfate excretion was inhibited by both BSP and DBSP (12), whereas biliary estradiol-17β-glucuronide excretion was inhibited by BSP, but not by DBSP (20). Since biliary excretion of all these compounds is markedly decreased in EHBR (6,12,16,17,20), the degree of involvement of cMOAT on biliary excretion of these compounds is considered to be different.

Biliary excretion of a tracer dose of PS was delayed in EHBR (Fig. 1). The extent of the delay of PS excretion in EHBR was similar to that with a dose of 10 μmol/100 g b.wt. (12) and not so marked as the excretion of a tracer dose of lithocholate-3-sulfate or estradiol-17β-glucuronide (12,20). This suggests the presence of another excretory pathway of PS other than cMOAT, as has been considered from results using canalicular membrane vesicles (13,14).

In the present study, we found that some organic anions and bile acid conjugates inhibited overall biliary PS excretion in SDR. PS is reported taken up by the liver by a Na^{+}-independent active transport system as other organic anions (11), and bile acid sulfates and glucuronides are also considered to be taken up by a Na^{+}-independent active transport system (25). Accordingly, a possibility remains that the infused organic anions and bile acid conjugates may have delayed PS excretion by inhibiting its hepatic uptake. However, such effect is considered to be minor according to the following reasons; although DBSP infusion is reported to inhibit PS uptake by rat liver (26), DBSP infusion only sightly inhibited biliary PS excretion in the present study, and the infusion of 3-sul-LC-tau and 3-glcA-UDC, which markedly inhibit PS excretion, did not change the blood PS levels 2–20 min after its injection (Takikawa et al. unpublished data). Furthermore, we did not examine the PS metabolites in

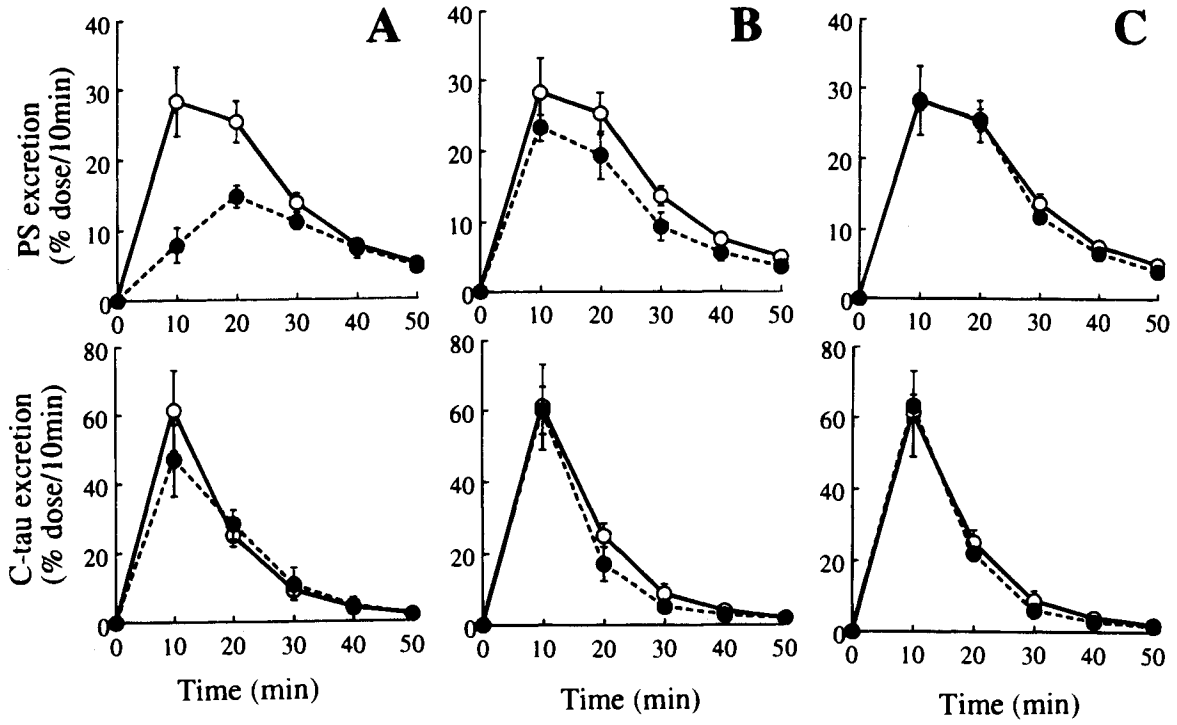

Fig. 2. Effects of organic anions on biliary excretion of [^{14}C]PS and [^{3}H]C-tau in SDR. A mixture of tracer amounts of [^{14}C]PS and [^{3}H]C-tau was injected as a bolus into the femoral vein and BSP (A), DBSP (B), CPM (C) were infused at the rate of 0.2 μmol/min/100 g b.wt. Data are the means ± SD for control rats (n = 4, open circles) and experiments with organic anions (n=3–5, closed circles).

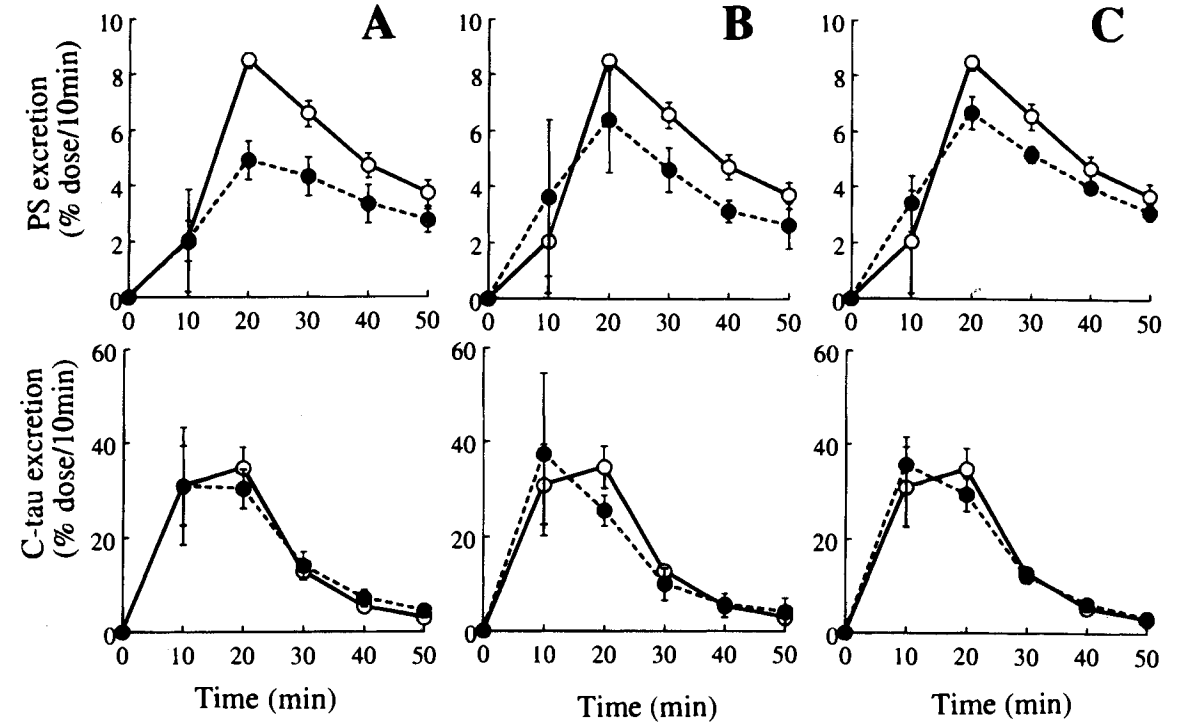

Fig. 3. Effects of organic anions on biliary excretion of [^{14}C]PS and [^{3}H]C-tau in EHBR. A mixture of tracer amounts of [^{14}C]PS and [^{3}H]C-tau was injected as a bolus into the femoral vein and BSP (A), DBSP (B), CPM (C) were infused at the rate of 0.2 μmol/min/100 g b.wt. Data are the means ± SD for control rats (n = 3, open circles) and experiments with organic anions (n=3–4, closed circles).

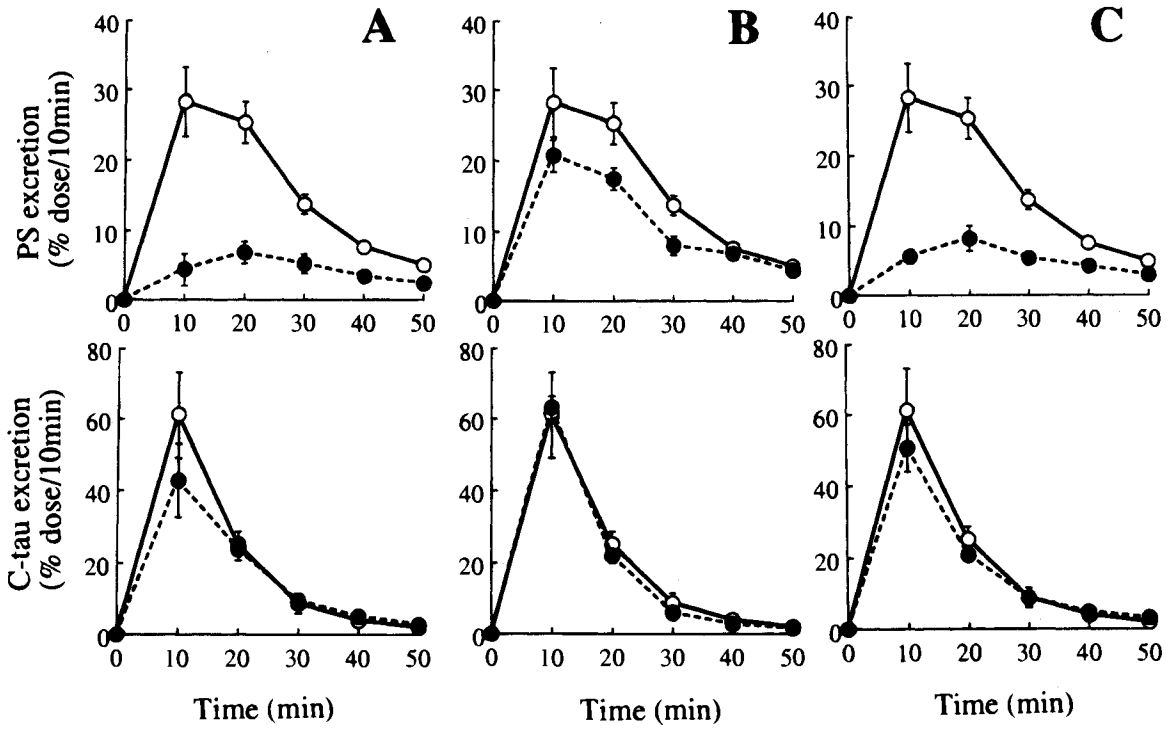

Fig. 4. Effects of bile acid conjugates on biliary excretion of [^{14}C]PS and [^{3}H]C-tau in SDR. A mixture of tracer amounts of [^{14}C]PS and [^{3}H]C-tau was injected as a bolus into the femoral vein and 3-sul-LC-tau (A), 3,7-sul-UDC (B) and 3-glcA-UDC (C) were infused at the rate of 0.2 μmol/min/100 g b.wt. Data are the means ± SD for control rats (n=4, open circles) and experiments with bile acid conjugates (n=3–4, closed circles).

the bile since referred substrates were not available. Accordingly, the present findings measuring the total radioactivity in the bile cannot exclude the possibility that the observed findings are not due to the canalicular PS transport phenomena; for example, it is possible that the infused compounds inhibit the metabolism of PS, thus reducing the excretion of the total radioactivity in the bile.

The inhibition of biliary PS excretion by BSP was more marked than that by DBSP (Fig. 2). In these experiments, BSP was mainly excreted as the glutathione conjugate (BSP-SG). However, the extent of the inhibition by BSP of PS excretion was not so marked as that observed of lithocholate-3-sulfate or estradiol-17β-glucuronide (12,20). These findings further suggest the existence of a canalicular PS transporter other than cMOAT.

Biliary PS excretion was not affected by CPM (Fig. 2). These data are in agreement with our previous report that biliary excretion of BSP and lithocholate-3-sulfate was not, or only slightly, inhibited by CPM whereas CPM excretion was inhibited by BSP and lithocholate-3-sulfate (23).

BSP and CPM only slightly inhibited biliary PS excretion in EHBR (Fig. 3). These findings suggest that with the absence of cMOAT the excretory pathway(s) for BSP or CPM are almost independent from that of PS. Such an hypothesis may be true in SDR, but it cannot be excluded that excretory pathway(s) less important in the presence of cMOAT in SDR may be compensatorily overexpressed in EHBR.

In contrast to the effect of BSP and DBSP, the reduction of biliary PS excretion by 3-sul-LC-tau and 3-glcA-UDC was more prominent (Fig. 4), suggesting that the excretory pathway(s) for these bile acid conjugates are extensively overlapped to that of PS. Previously, we reported that 3-sul-LC-tau and 3,7-sul-UDC are excreted into bile without biotransformation, whereas a part of 3-glcA-UDC is further conjugated with taurine (21,25). Furthermore, the excretory pathway(s) for PS seems not completely identical to that of estradiol-17β-glucuronide, since biliary estradiol-17β-glucuronide excretion was similarly inhibited by BSP, 3-sul-LC-tau, and 3-glcA-UDC, and was not inhibited by DBSP (20,27). In contrast, 3,7-sul-UDC had only a little effect on biliary PS excretion (Fig. 4), although biliary 3,7-sul-UDC excretion was markedly decreased in EHBR (22). These findings suggest that excretory pathways for PS and 3,7-sul-UDC are not the same.

Recently, Yamazaki et al. (28) reported that biliary PS excretion is mediated mainly by cMOAT in normal rats according to in vivo and in vitro studies using canalicular membrane vesicles. Especially, they used ^{3}H-labeled PS in the in vitro studies with a specific activity 3 orders of magnitude higher than that of ^{14}C-labeled PS previously used in in vitro studies (12,13), by which precise kinetic studies became possible. According to their findings, the presence of PS excretion even in EHBR (Fig. 1) and relatively slight inhibition of PS excretion by organic anions (Fig. 2), suggest that the contribution of cMOAT is different for the excretion of PS and its metabolites.

The fact that biliary C-tau excretion was not affected by any of the organic anions or bile acid sulfates and glucuronide in SDR and EHBR (Figs. 2–4) further supports that the pathway of biliary C-tau excretion (27) is completely different from that of organic anions, bile acid sulfates, and glucuronides.

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