Ostα depletion protects liver from oral bile acid load

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Ostα depletion protects liver from oral bile acid load. Am J Physiol Gastrointest Liver Physiol 301: G574–G579, 2011. First published June 30, 2011; doi:10.1152/ajpgi.00141.2011.—Bile acid homeostasis is tightly maintained through interactions between the liver, intestine, and kidney. During cholestasis, the liver is incapable of properly clearing bile acids from the circulation, and alternative excretory pathways are utilized. In obstructive cholestasis, urinary elimination is often increased, and this pathway is further enhanced after bile duct ligation in mice that are genetically deficient in the heteromeric, basolateral organic solute transporter alpha-beta (Ostα-Ostβ). In this study, we examined renal and intestinal function in Ostα-deficient and wild-type mice in a model of bile acid overload. After 1% cholic acid feeding, Ostα-deficient mice had significantly lower serum ALT levels compared with wild-type controls, indicating partial protection from liver injury. Urinary bile acid excretion of Ostα-deficient mice was responsible for almost all of the bile acid load in Ostα-deficient mice, suggesting that intestinal losses of bile acids accounted for the protection from liver injury. Thus, fecal loss of bile acids after bile acid overload reduced the need for the kidney to filter and excrete the excess bile acids. In conclusion, Ostα-deficient mice efficiently eliminate excess bile acids via the feces. Inhibition of intestinal bile acid absorption might be an effective therapeutic target in early stages of cholestasis when bile acids are still excreted into bile.

Bile acids; kidney; intestine; bile acid homeostasis

Bile acids are synthesized in the liver and are a major component of bile. They are secreted into the small intestine where they function to solubilize dietary fats and lipids for absorption. Bile acids undergo an enterohepatic circulation where ~95% are reabsorbed in the ileum through the activity of the apical sodium-dependent bile acid transporter (Asbt) and then transported across the basolateral membrane into the portal circulation by the organic solute transporter alpha-beta (Ostα-Ostβ) (1, 13). This enterohepatic circulation is completed by the re-uptake of bile acids into the liver primarily by the sodium taurocholate cotransporting peptide, Ntcp. The bile acids that are not reabsorbed in the ileum travel to the colon where they can be deconjugated by bacterial flora and reabsorbed by the colonocyte or excreted into the feces. In addition, ~10–50% of bile acids (depending on the species) can escape hepatic reabsorption and remain in the peripheral circulation and subsequently get filtered at the renal glomerulus (8). Normally bile acids do not appear in the urine since they are reabsorbed in the proximal tubule of the kidney by renal Asbt (5, 6) and transported back into the circulation by basolateral Ostα-Ostβ and Mrp3. However, during cholestasis, bile acids are excreted in the urine as a result of impaired uptake by Asbt and enhanced expression of the apical efflux transporters Mrp2 and Mrp4. Thus, homeostatic regulation of bile acids depends on the intestine and kidney, as well as the liver, both in normal physiological conditions and in cholestasis.

Adaptive upregulation of OST/Ostα-OST/Ostβ in the liver of cholestatic humans and rodents is believed to aid in the basolateral elimination of toxic bile acids from the liver (3, 19). Much of our knowledge about adaptive regulation of membrane transporters, nuclear receptors, and regulatory enzymes has been acquired through animal models of cholestasis (for review, see Ref. 2). In addition, mice in which specific genes have been genetically knocked out have also added greatly to our understanding of the role these genes play in bile acid homeostasis in the liver as well as in the intestine and kidney. For example, use of mice with tissue-specific knockdown of Fxr have clarified the role of hepatic Fxr and intestinal Fgf15 in bile acid homeostasis in the mouse (10). Jung et al. (9) have shown that bile acid malabsorption in Abst−/− mice can be ameliorated by administration of Fxrg agonists and Fgf15. Mice genetically deficient in Ostα were found to have a small bile acid pool and increased fecal excretion of cholesterol (1, 13). This disruption in bile acid homeostasis was due to alterations in intestinal Fxr, Shp, and Fgf15, and hepatic Cyp7a1 (13). When Ostα-deficient mice are subjected to bile duct ligation, they are partially protected from liver injury despite the upregulation of bile acid synthesis (17). Urinary excretion of bile acids was significantly increased due to adaptive changes in uptake and efflux transporters of the kidney (17). This work highlighted the essential role the kidney plays in protection from bile acid toxicity in obstructive cholestasis and confirms previous studies in cholestatic humans (7, 12, 18).

The present study examines the role of alternative pathways of bile acid elimination in protection from liver injury after bile acid overload. Ostα-deficient mice were fed a 1% cholic acid diet, and renal and intestinal function and clearance of bile acids were determined. Our data indicate that the renal clearance of the bile acid load is not upregulated sufficiently to account for the protection from liver injury. Because bile secretion is maintained, the Ostα-deficient mice efficiently eliminate the additional circulating bile acids via the intestine rather than the kidney. These findings suggest that the intestine might be a preferred target in inhibition of bile acid transport, particularly in early stages of cholestasis where bile acids are still excreted by the liver into bile.

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MATERIALS AND METHODS

Male Ost$\alpha$-deficient and age-matched wild-type C57Bl mice 25–30 wk of age were used for this study. Ost$\alpha$−/− mice were generated as previously described (1, 11). All animals were housed in a temperature- and humidity-controlled environment under a constant light cycle where they had free access to water and food (Teklad 2018 Global Rodent Diet with or without 1% cholic acid, Harlan Laboratories, Madison WI). All experimental protocols were approved by the local Animal Care and Use Committee, according to criteria outlined in the “Guide for the Care and Use of Laboratory Animals” prepared by the National Academy of Sciences, as published by the National Institutes of Health (NIH publication 86-23, revised 1985).

Determination of glomerular filtration rate and urinary bile acid clearance. Mice on a basal or 1% cholic acid diet (Harlan Laboratories) were fasted for 12 h before experimentation. Food intake and body weight were monitored for the 5 days of diet, and feces were collected each day for determination of bile acid concentration and output. Surgery was carried out by the George M. O’Brien Kidney Center, Yale University School of Medicine. Mice were anesthetized (isoflurane 1–3% in oxygen to effect) via a nose cone assembly and a precision vaporizer. Body temperature was maintained throughout the surgical procedure by placing on a table heated with recirculating water. The bladder was accessed via a 0.5-mm midline incision in the surgical procedure by placing on a table heated with recirculating water. The bladder was accessed via a 0.5-mm midline incision in the lower abdominal area and catheterized for urine collection. The liver and kidney tissue were also obtained for routine histology as previously described (17).

Analytical assays. Hepatic and renal tissues and feces were extracted with 75% ethanol (100 mg/ml) for bile acid determination. Quantitation of 3α-hydroxy bile acids from plasma, urine, bile, and tissue extracts was done with a commercial kit (Diazyme Laboratories, Poway, CA). Assay for plasma alanine aminotransferase (ALT) was performed using a commercial kit (Thermo Scientific), and alkaline phosphatase (ALP) was determined by the Analytical Core of the Mouse Metabolic Phenotyping Center, Yale University School of Medicine. Liver and kidney tissue were also obtained for routine histology as previously described (17).

Western blotting. Protein expression was determined in total membrane fractions prepared as previously described (17). Polyclonal antibody to Asbt (gift of Paul Dawson, Wake Forest University, Winston-Salem, NC) and villin (C-19, Santa Cruz Biotechnology, Santa Cruz, CA) were incubated overnight at 4°C. Horseradish peroxidase-conjugated secondary antibodies were from Sigma (St. Louis, MO), and SuperSignal West Pico Chemiluminescent substrate was from Thermo Scientific (Rockford, IL). Densitometry was performed using the Fudotye System (FudoDyne, Hartland, WI).

Statistics. All data represent means ± SD based on Student’s t-test for 3–6 animals per group.

RESULTS

In basal fed mice, the Ost$\alpha$-deficient mice had significantly longer length of the small intestine and slightly higher liver-to-body weight ratios, whereas the kidney-to-body weight ratios were similar to the wild-type, age-matched controls (Table 1). The bile acid concentration was not significantly different in serum, bile, feces, or urine between the Ost$\alpha$-deficient and wild-type mice (Table 1). However, the hepatic bile acid concentration was significantly lower in the Ost$\alpha$-deficient mice (Table 1), reflecting the lower bile acid pool in these animals (1, 13, 17).

Renal function and bile acid clearance in Ost$\alpha$-deficient mice under basal and 1% cholic acid fed conditions. Examination of renal function and endogenous bile acid clearance in basal fed conditions revealed that the urine flow rate was higher in the Ost$\alpha$-deficient mice compared with the wild-type controls, although it was not quite statistically significant (4.13 ± 1.08 vs. 2.81 ± 0.60 μl/min·100 g kidney$^{-1}$; P = 0.0523). [3H]inulin clearance, a measure of the GFR, was not significantly different between Ost$\alpha$-deficient and wild-type mice, although it also had a tendency to be increased in the knock out animals (785 ± 173 vs. 594 ± 145 μl/min·100 g kidney$^{-1}$). Urinary bile acid clearance was not significantly different in the Ost$\alpha$-deficient and wild-type animals under basal conditions when the plasma and urine bile acid concentrations were low. However, when animals were fed a 1% diet of cholic acid for 5 days, bile acid clearance in the urine was significantly higher in the Ost$\alpha$-deficient mice compared with the wild-type mice (Table 1).

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<th>Table 1. Bile acid concentrations and fecal output in wild-type and Ost$\alpha$-deficient mice</th>
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Values are means SD. WT, wild-type mice; KO, knockout mice; BW, body weight; BA, bile acid; Int length, intestinal length; ND, not done. Significant difference (P < 0.05): *basal vs. cholic acid; †basal vs. basal; ‡cholic acid vs. cholic acid.
Ostα-deficient mice are partially protected from liver injury after cholic acid feeding. Our previous work suggested that differences in renal output of bile acids are revealed when the Ostα-deficient mice are subjected to obstructive cholestasis (17). Under the conditions of increased serum bile acids during bile duct obstruction, Ostα-deficient mice had threefold higher concentrations of urinary bile acid than the wild-type bile duct obstructed controls, despite the smaller bile acid pool (17). In the present study, plasma levels of bile acids were increased by administering a 1% cholic acid diet for 5 days before determination of the GFR. Both the Ostα-deficient and wild-type mice consumed equal amounts of the food over the 5-day period, and both groups lost ~15% body weight over those 5 days (including ~5% lost in the overnight fasting). Both groups had increased bile acid concentrations in plasma, hepatic, biliary, and fecal compartments after the cholic acid diet, but the concentrations were consistently lower in all compartments except the feces in the Ostα-deficient mice compared with the wild-type controls (Table 1). As previously reported in bile duct-obstructed Ostα-deficient mice, the cholic acid fed Ostα-deficient mice had lower serum ALT and ALP levels, suggesting that they were again partially protected from cholestatic liver injury (Fig. 2). However, although the Ostα-deficient mice had higher urinary bile acid clearance than the wild-type mice, the rate was too low to account for this protective effect. Furthermore, the protection was not due to differences in bile acid synthesis because Cyp7a1 mRNA levels are equally decreased in Ostα-deficient and wild-type mice after cholic acid feeding (data not shown).

Therefore, fecal output of bile acids was examined in more detail. When fecal bile acid concentrations were determined daily for the 5 days, it was apparent that the Ostα-deficient mice excreted higher amounts of bile acids immediately after the cholic acid diet was initiated (Fig. 3). One day after beginning the diet, Ostα-deficient mice had fourfold higher fecal bile acid concentrations than the wild-type controls. However, after 5 days, the concentration of fecal bile acids was similar between the two groups (Fig. 3). When the total bile acid clearance (K) was determined by the equation K = (CU × Q)/CP, where CU is the urinary bile acid concentration, Q is the urine flow, and CP is the plasma bile acid concentration, [*H]inulin clearance was determined as described in MATERIALS AND METHODS. *Significant difference between knockout cholic acid (CA) and wild-type CA (P < 0.001; n = 6–7).

Fig. 1. Urinary bile acid, but not [*H]inulin, clearance is higher in Ostα-deficient mice than in wild-type controls on a cholic acid diet. Endogenous bile acid clearance (K) was determined by the equation K = (CU × Q)/CP, where CU is the urinary bile acid concentration, Q is the urine flow, and CP is the plasma bile acid concentration. [*H]inulin clearance was determined as described in MATERIALS AND METHODS. *Significant difference between knockout cholic acid (CA) and wild-type CA (P < 0.001; n = 6–7).

Fig. 2. Ostα-deficient mice are partially protected from liver injury after 1% CA feeding. Serum alanine aminotransferase (ALT) and alkaline phosphatase (ALP) levels are lower in the Ostα-deficient mice. *Significant difference between basal and CA feeding (P < 0.05; n = 5–6).

Fig. 3. Fecal bile acid concentrations are higher in Ostα-deficient mice than in wild-type controls on days 1 and 2. Bile acid concentration was determined in feces collected each day over the 5-day 1% cholic acid feeding. Ostα-deficient mice had significantly higher concentrations over the first 2 days, but then the wild-type mice increased to similar levels. *Significant difference between wild-type and knockout mice (P < 0.05; n = 8).
acid output over the entire 5 days was calculated ($n = 2$ in each group), the Ostα-deficient mice had approximately twofold more fecal bile acid output over the 5 days of cholic acid feeding (Table 1). These data are similar to previous reports (13), where the authors hypothesize that intestinal Asbt would be downregulated over the 5-day period of cholic acid feeding in wild-type mice, resulting in increased bile acid output into the feces. Quantitative RT-PCR and Western blotting of ileal tissue in the present study confirmed that Asbt mRNA and protein levels were indeed reduced significantly in the wild-type mice after 1% cholic acid feeding (Fig. 4). Furthermore, the low basal levels in the Ostα-deficient mice (Fig. 4) presumably explains the greater loss of bile acids in the feces after only 1 and 2 days of bile acid feeding. Thus it appears that the Ostα-deficient mice are protected from the increased plasma bile acids after cholic acid feeding by eliminating the excess bile acids via the intestine into the feces. Under these conditions, bile acid clearance via the intestine is $\sim 4,500$ times higher than the urinary bile acid clearance in the Ostα-deficient mice.

**Adaptive changes in bile acid transporters in the kidney and intestine.** In the kidney, cholic acid feeding of wild-type mice did not alter the mRNA expression of Asbt or Mrp2, whereas Mrp4 and Oat3 mRNA were both significantly increased (Fig. 5A). In contrast, in the Ostα-deficient mice, mRNA for Mrp4 and Oat3 were unchanged after cholic acid feeding (Fig. 5A). This may be the result of lower levels of plasma bile acids due to the increased excretion in the feces.

In the ileum, the Ostα-deficient mice had significantly lower expression of Fxr mRNA under basal feeding conditions compared with the wild-type controls (Fig. 5B). After cholic acid feeding, the Fxr mRNA expression was unchanged in both Ostα-deficient and wild-type mice (Fig. 5B). However, mRNA
for both ileal Shp and Fgf15 were significantly elevated in both groups of mice after cholic acid feeding (Fig. 5B).

DISCUSSION

We have previously demonstrated that Osta-deficient mice are partially protected from liver injury in a model of obstructive cholestasis due to increased excretion of bile acids into the urine (17). To assess the role of Osta-Ostβ in bile acid overload when the biliary pathway is still intact, we examined renal and intestinal function and clearance of bile acids in wild-type and Osta-deficient mice in basal and 1% cholic acid feeding conditions. Our findings indicate that there is no difference in the GFR between wild-type and Osta-deficient mice. However, after cholic acid feeding, renal clearance of bile acids is significantly higher in the Osta-deficient mice than in the wild-type controls. These mice also have lower levels of plasma ALT and lower levels of plasma and hepatic bile acids than wild-type, cholic acid-fed mice. However, the magnitude of bile acid loss in the urine is minimal. Instead, both wild-type and Osta-deficient mice excrete significant amounts of bile acids in the feces in response to cholic acid feeding. Osta-deficient mice excrete approximately twofold more bile acids into the feces after cholic acid feeding, which may account for the partial protection from liver injury in this model. Indeed, estimates based on an average urine output of 1 ml urine/day suggest that, when a 1% cholic acid diet was fed, wild-type mice excreted ~2,000 times more bile acids in the feces than the urine, whereas the Osta-deficient animals excreted ~4,500 times more bile acids in the feces than in the urine.

In humans, 10–30% of circulating bile acids are not bound to albumin (15) and can be filtered at the glomerulus where they are efficiently reabsorbed in the proximal tubule by Asbt (5, 6). However, to return to the circulation, bile acids must be transported from the proximal tubule into the renal vasculature across its basolateral membrane. This membrane contains two bile acid efflux transporters, Ostα-Ostβ and Mrp3. The relative importance of Ostα-Ostβ in the kidney in cholestasis was recently demonstrated in Osta-deficient mice subjected to a bile acid stress by ligation of the common bile duct. Protein expression of renal Mrp3 did not change, but the urinary excretion of bile acids was elevated by approximately threefold in the Osta-deficient mice compared with wild-type mice (17). Although additional alterations were found in other renal transporters (downregulation of Asbt and upregulation of Mrp2 and Mrp4) after BDL, a detailed study of renal function, including GFR and renal clearances of bile acids, has not been done in the Osta-deficient mouse. We show that these mice do not have significantly different GFR, urine flow, or renal bile acid clearance under basal feeding conditions. In obstructive cholestasis in rats, it has been reported that GFR is not altered, but tubular reabsorption rates of [3H]taurocholate and [3H]cholate are decreased due to a functional downregulation of Asbt (14, 16). In this study, we confirm that GFR was not significantly different under basal conditions or after cholic acid feeding in the Osta-deficient mice but demonstrate that renal clearance of bile acids was higher in Osta-deficient mice than in controls when they were placed under a bile acid load. However, plasma and hepatic levels of bile acids did not reach levels as high as seen after BDL (Table 1 and Ref. 17); therefore, the kidney was not exposed to the same level of bile acid stress as in the previous model. The levels in the wild-type, cholic acid-fed mice were high enough to induce upregulation of renal Mrp4 and Oat3 (4); however, these were not altered during cholic acid feeding in Osta-deficient mice. The increase in renal clearance of bile acids in the knockout mice is likely explained entirely to the deficiency of Osta-Ostβ.

Although the cholic acid feeding model was not as severe as the BDL model, the mice displayed hepatic injury, as demonstrated by liver function tests, as well as upregulation of intestinal Shp and Fgf15 and downregulation of hepatic Cyp7a1. Although liver histology revealed no obvious fibrosis, necrosis, or bile duct proliferation (data not shown), lower levels of serum ALT were seen in the bile acid-fed Osta-deficient mice, suggesting less liver injury than the wild-type controls. This partial protection from liver injury correlated with a greater increase in fecal bile acid excretion in the Osta-deficient mice. Ileal Asbt expression is downregulated in these mice (Fig. 4 and Refs. 1, 13), and thus bile acids are not reabsorbed efficiently from the intestine. Within 1 day of cholic acid feeding, these mice already have approximately fourfold higher concentration of bile acids in the feces compared with wild-type controls. The wild-type mice begin to show an increase after 2 days, and by 5 days the fecal bile acid concentration was similar to the Osta-deficient mice. We show that this is due to the downregulation of ileal Asbt mRNA and protein in the wild-type mice after 5 days of elevated cholic acid, as has been suggested by Rao et al. (13).

In conclusion, the present studies demonstrate that Osta-deficient mice are partially protected from liver injury induced by cholic acid feeding by excreting excess bile acids into the feces. Osta deficiency also results in an increase in renal bile acid clearance despite maintenance of a normal GFR. Although liver injury in this model was less severe than following bile duct obstruction, the findings suggest that strategies directed at inhibition of ileal bile acid transporters might be more effective in early stages of cholestatic liver injury where bile acid excretion into bile still exists.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


