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Loss of Organic Anion Transporting Polypeptide 1a1 Increases Deoxycholic Acid Absorption in Mice by Increasing Intestinal Permeability

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Deoxycholic acid (DCA) is a known hepatotoxicant, a tissue tumor promoter, and has been implicated in colorectal cancer. Male mice are more susceptible to DCA toxicity than female mice. Organic anion transporting polypeptide 1a1 (Oatp1a1), which is known to transport bile acids (BAs) *in vitro*, is predominantly expressed in livers of male mice. In addition, the concentrations of DCA and its taurine conjugate (TDCA) are increased in serum of Oatp1a1-null mice. To investigate whether Oatp1a1 contributes to the gender difference in DCA toxicity in mice, wild-type (WT) and Oatp1a1-null mice were fed a 0.3% DCA diet for 7 days. After feeding DCA, Oatp1a1-null mice had 30-fold higher concentrations of DCA in both serum and livers than WT mice. Feeding DCA caused more hepatotoxcity in Oatp1a1-null mice than WT mice. After feeding DCA, Oatp1a1-null mice expressed higher BA efflux-transporters (bile salt-export pump, organic solute transporter (Ost)α/β, and multidrug resistance-associated protein [Mrp]2) and lower BA-synthetic enzymes (cytochrome P450 [Cyp]7a1, 8b1, 27a1, and 7b1) in livers than WT mice. Intravenous administration of DCA and TDCA showed that lack of Oatp1a1 does not decrease the plasma elimination of DCA or TDCA. After feeding DCA, the concentrations of DCA in ileum and colon tissues are higher in Oatp1a1-null than in WT mice. In addition, Oatp1a1-null mice have enhanced intestinal permeability. Taken together, the current data suggest that Oatp1a1 does not mediate the hepatic uptake of DCA or TDCA, but lack of Oatp1a1 increases intestinal permeability and thus enhances the absorption of DCA in mice.

Key Words: **transporter; bile acid; liver proliferation; intestinal absorption; enterohepatic circulation.**

Organic anion transporting polypeptides (OATP/Oatps) mediate the Na^+-independent hepatocellular uptake of bile acids (BAs) and other organic compounds (Hagenbuch and Meier, 2003). In mice, Oatp1a1, 1a4, and 1b2 are predominantly expressed in liver and are all able to transport both conjugated and unconjugated BAs *in vitro* (Meier and Stieger, 2002). Recently, our lab showed that lack of Oatp1b2 transporter, which is orthologous to human OATP1B1 and OATP1B3, increases the concentrations of unconjugated but not conjugated BAs in serum of mice (Csanaky *et al*., 2011). In contrast, human OATP1B1 polymorphism considerably affects the disposition of both unconjugated and conjugated BAs, such as cholic acid (CA), ursodeoxycholic acid (UDCA), glycoursodeoxycholic acid (GUDCA), chenodeoxycholic acid (CDCA), and glycol-chenodeoxycholic acid (GCDCA) (Xiang *et al*., 2009). In addition, studies in Oatp1b2-null mice showed that Oatp1b2 transports only two out of total six model substrates of human OATP1B1 (Chen *et al*., 2008). Oatp1a1 and 1a4 have been shown to share a wide range of substrates of human OATP1B1 and 1B3 (Hagenbuch and Meier, 2003). Thus, the functions of human OATP1B1 and 1B3 are also possibly preserved in Oatp1a1 and 1a4 in livers of mice.

Oatp1a1 and 1a4 have different distributions in rodent liver. Rat Oatp1a1 has a homogeneous lobular distribution, whereas Oatp1a4 is predominantly expressed around the central vein in the liver (Kakyo *et al*., 1999; Reichel *et al*., 1999). Such differences are also observed for human OATP1B1 and 1B3, with OATP1B1 being expressed throughout the liver lobe, whereas OATP1B3 is highly expressed around the central vein (Konig *et al*., 2000a,b). Because the major uptake of BAs occurs in periportal hepatocytes (zone 1), Oatp1a1 is implicated in the uptake of BAs under normal conditions, whereas Oatp1a4 may assume a more important role in situations where Oatp1a1 is not the primary mediator for BA uptake (Aiso *et al*., 2000).

The secondary BA, deoxycholic acid (DCA), is known to be hepatotoxic. DCA is more hepatotoxic than CA and lithocholic acid and induces lipid peroxidation in livers of rats (Delzenne *et al*., 1992). DCA is also implicated in the pathogenesis of major diseases, with evidence for elevated DCA associated with increased risk of breast and colorectal cancer (Costarelli and Sanders, 2002; Rial *et al*., 2009). DCA induces cell

proliferation in a human colon cancer cell line (Zeng *et al.*, 2010). DCA can also induce the autophagic pathway in noncancer colon epithelial cells and contribute to cell survival (Payne *et al.*, 2009). In our preliminary studies, both male and female C57BL/6 mice were fed various concentrations of DCA to investigate DCA toxicity. When fed 1% DCA, all male mice died after 4 days, whereas there was no mortality in female mice after 1 week, revealing a sex difference in the susceptibility to DCA toxicity (Youcai Zhang, Peizhen Song, and Curtis D. Klaassen, unpublished data).

Sodium taurocholate cotransporting polypeptide (Ntcp), Oatp1a1, 1a4, and 1b2 are considered to be the major BA uptake transporters in livers of mice. Among these transporters, Oatp1b2 is expressed similarly in livers of male and female mice (Cheng *et al.*, 2005). Female mice have higher Ntcp and Oatp1a4 messenger RNA (mRNA) expression and possibly take up more BAs into livers than male mice (Cheng *et al.*, 2007). Thus, Ntcp and Oatp1a4 are not likely the reason why female mice are more resistant to DCA toxicity than male mice. Oatp1a1 is male predominant in livers of mice and has been shown to transport BAs, such as taurocholic acid (TCA) *in vitro* (Cheng *et al.*, 2006; Hagenbuch *et al.*, 2000). Therefore, it is likely that higher constitutive expression of Oatp1a1 may contribute to the hepatotoxicity of DCA in male mice. The purpose of the present study was to test the hypothesis that Oatp1a1 is a critical determinant of DCA disposition and toxicity in mice, and Oatp1a1-null mice were used as a model by which to test this hypothesis.

MATERIALS AND METHODS

Chemicals and reagents. DCA was purchased from Sigma-Aldrich Chemical Co. (St Louis, MO). BA standards were purchased from Steraloids, Inc. (Newport, RI) and Sigma-Aldrich.

Animals and breeding. Eight-week-old adult male and female C57BL/6 mice were purchased from Charles River Laboratories Inc. (Wilmington, MA). Oatp1a1-null mice (Deltagen, San Carlos, CA) were bred to homozygosity on the C57BL/6 background as previously described (Gong *et al.*, 2011). All mice were housed in an American Animal Associations Laboratory Animal Care accredited facility with a 12:12 h light:dark cycle and provided chow and water *ad libitum*.

BA feeding and sample preparation. Pelleted mouse feed (Teklad Rodent Diet #8604; Harlan Teklad, Madison, WI) was ground into a fine powder. The DCA-supplemented diets were made by mixing a certain percentage of DCA with control ground diet. Individually housed C57/BL6 and Oatp1a1-null mice (n = 5/gender) were fed a diet supplemented with 0.3% DCA. The DCA-supplemented diets (40 g) were added to a bowl in each mouse cage daily, and the remaining feed from the previous day was discarded. Cages were replaced daily to minimize contamination of feed with urine and feces. After 7 days, mice were anesthetized, blood was obtained by orbital bleeding, and centrifuged at 6000 g for 15 min to collect serum. Gallbladders were removed and stored at −80°C. Livers were harvested from the same animals, washed, frozen in liquid nitrogen, and stored at −80°C. The three small intestine segments, namely duodenum, jejunum, and ileum, as well as cecum and large intestine, were divided, the contents removed, and stored separately at −80°C.

BA extraction and analysis. BA analysis of serum and bile was according to a previously published method (Alnouti *et al.*, 2008). BA analysis of liver, ileum, and colon was according to a recent method (Zhang and Klaassen, 2010). To extract BAs from gallbladder, 1 ml of methanol was added to gallbladder premixed with 100 μl of internal standards (G-CDCA-d_4, 4 μg/ml and CDCA-d_4, 2 μg/ml). The mixture was vortexed vigorously, sonicated for 10 min, centrifuged at 16,000 g for 10 min, and the supernatant collected. The pellet was extracted with another 2 ml of methanol. The two supernatants were combined, evaporated under vacuum, and reconstituted in 1 ml of 50% methanol.

Intravenous administration of DCA and TDCA. Mice were anesthetized by ip injection of ketamine (100 mg/kg)-midazolam (5 mg/kg), and their body temperatures were maintained at 37°C by means of heating pads. Subsequently, the kidneys were ligated, the right carotid artery was cannulated with PE-10 tubing, and the common bile duct was cannulated with PE-10 tubing. DCA or TDCA was dissolved in saline and administered intravenously to wild-type (WT) and Oatp1a1-null mice (50 μmol/kg). This dose was chosen because it did not cause hemolysis or signs of cardiac or respiratory toxicity in preliminary experiments. After dosing, blood was collected at 0, 2, 5, 11, 21, 31, and 41 min. Pharmacokinetic parameters were calculated according to previously described work (Csanaky *et al.*, 2011).

Intestinal permeability test. Mice were acclimated to wire-bottomed metabolic cages for 1 day with free access to food and water. Mice were administrated 13.3 mg lactulose and 10.1 mg mannitol po (Sigma-Aldrich). Urine was collected the next 24 h in 10 μl 1% NaN_3. Urinary lactulose and mannitol were quantified by liquid chromatography-tandem mass spectrometry (LC-MS/MS) according to a previous method (Lostia *et al.*, 2008).

Total RNA isolation. Total RNA was isolated using RNA-Bee reagent (Tel-Test Inc., Friendswood, TX) according to the manufacturer's protocol. Total RNA concentrations were quantified spectrophotometrically at 260 nm. Integrity of RNA samples was determined by formaldehyde-agarose gel electrophoresis with visualization by ethidium bromide fluorescence under ultraviolet light.

Multiplex suspension array. Liver mRNA was quantified by multiplex suspension array (Panomics-Affymetrix, Inc., Fremont, CA). Individual gene accession numbers can be accessed at www.panomics.com (sets #21021 and #21151). Samples were analyzed using a Bio-Plex 200 System Array reader with Luminex 100 xMAP technology, and the data were acquired using Bio-Plex Data Manager version 5.0 (Bio-Rad, Hercules, CA). Assays were performed according to each manufactures' protocol. RNA data were normalized to Gapdh mRNA and are presented as relative light units.

Statistical analysis. Bars represent mean ± SEM (n = 5). Differences between mean values were tested for statistical significance ($p < 0.05$) by the two-tailed Student's t-test.

RESULTS

Hepatic mRNA Expression of Transporters

Oatp1a1-null mice show essentially no detectable hepatic Oatp1a1 mRNA or protein and no change in constitutive expression of Oatp1a4 or 1b2 (Gong *et al.*, 2011). However, in order to assess BA homeostasis in these mice, it is necessary to determine whether loss of Oatp1a1 function will alter all hepatic transporters involved in BA and cholesterol disposition. Ntcp, Oatp1a1, Oatp1a4, Oatp1b2, and Oatp2b1 are considered to be the BA-uptake transporters in mouse livers (Fig. 1a). Knockout of Oatp1a1 had little effect on the mRNA expression of Ntcp, Oatp1a4, Oatp1b2, and Oatp2b1 (Fig. 1a). Bile salt-export pump (Bsep) and multidrug resistance-associated

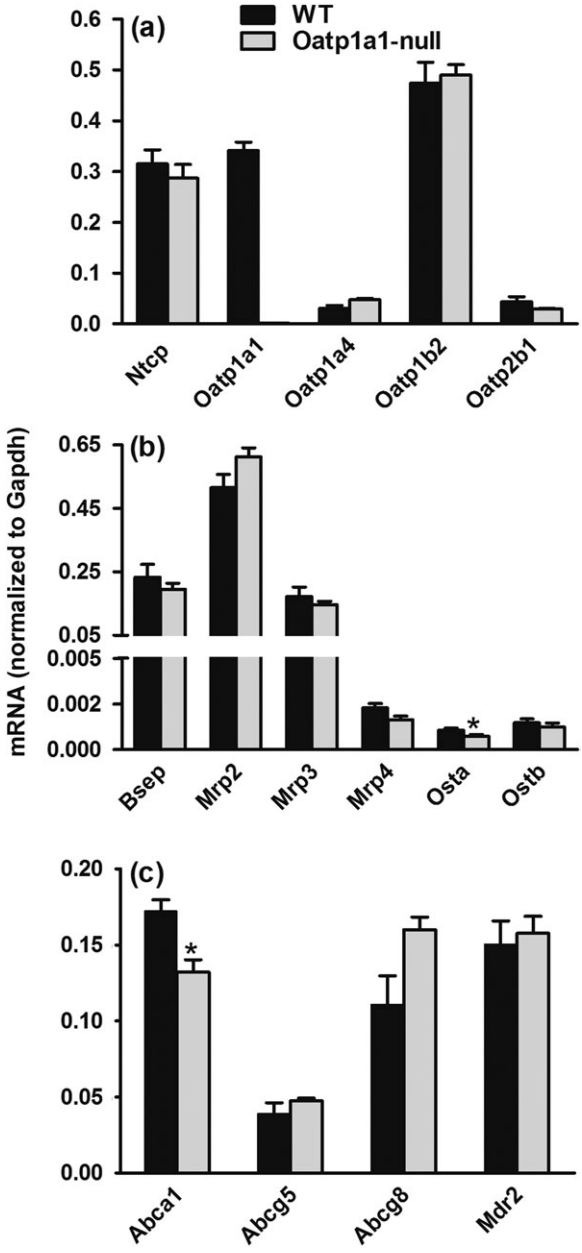

FIG. 1. mRNA expression of hepatic transporters. Total RNA from livers of male WT and Oatp1a1-null mice (n = 5/group) was analyzed by multiplex suspension array. The mRNA of each gene was normalized to GAPDH. All data are expressed as mean ± S.E. of five mice in each group. *, statistically significant difference between WT and Oatp1a1-null mice ($p < 0.05$).

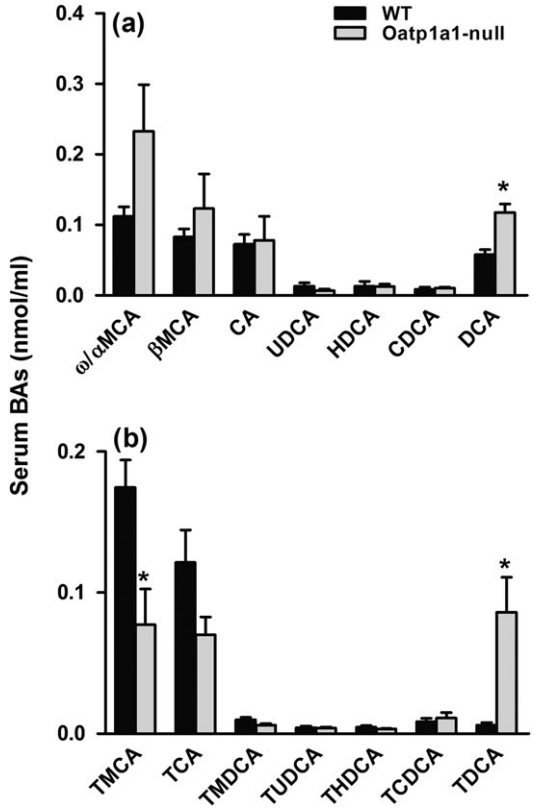

FIG. 2. BA concentrations in serum of WT and Oatp1a1-null male mice. The concentrations of conjugated (a) and unconjugated (b) BAs in serum of male WT and Oatp1a1-null mice (n = 5/group) were analyzed by ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). All data are expressed as mean ± S.E. of five mice in each group. *, statistically significant difference between WT and Oatp1a1-null mice ($p < 0.05$).

protein (Mrp)2 mediate BA efflux from hepatocytes into bile canaliculi, whereas Mrp3, Mrp4, and organic solute transporter (Ost)α/β are basolateral BA-efflux transporters that efflux BAs from liver back to the blood (Fig. 1b). Lack of Oatp1a1 had little effect on BA-efflux transporters, except that it decreased Ostα (32%). This decrease may not be biologically significant because Ostα is only minimally expressed in livers of mice. The mRNA expression of cholesterol transporters in livers of male WT and Oatp1a1-null mice are shown in Figure 1c. Lack of Oatp1a1 decreased the mRNA expression of the cholesterol-efflux transporter Abca1 about 23% but had no effect on Abcg5/8 or Mdr2. Taken together, lack of Oatp1a1 had little effect on BA or cholesterol transporters in livers of mice.

Concentrations of BAs in Serum of WT and Oatp1a1-Null Mice

Because Oatp1a1 is a hepatic uptake transporter, lack of Oatp1a1 may increase the serum concentrations of some BAs, which are possible endogenous substrates for Oatp1a1. Figure 2a illustrates the concentrations of unconjugated BAs in serum of male WT and male Oatp1a1-null mice. Lack of Oatp1a1 increased both ω/αMCA and DCA about 105% but had no effect on other unconjugated BAs, such as βMCA, CA, UDCA, hyodeoxycholic acid (HDCA), and CDCA. The concentrations of conjugated BAs are shown in Figure 2b. Compared with WT mice, serum concentration of TMCA (TαMCA + TβMCA) decreased about 60% in Oatp1a1-null mice. Lack of Oatp1a1 had little effect on the concentrations of TMDCA, TUDCA, THDCA, or TCDCA, whereas it markedly

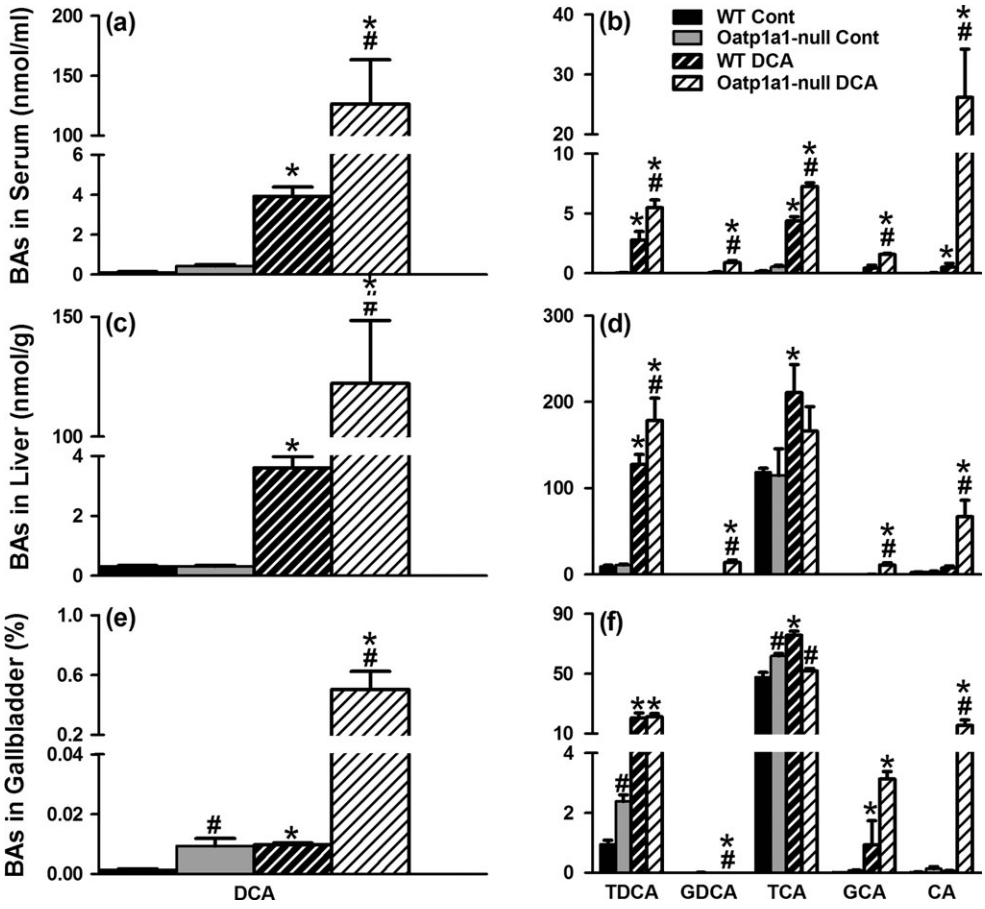

FIG. 3. Concentrations of DCA and DCA metabolites in serum, livers, and gallbladders of WT and Oatp1a1-null mice after feeding DCA for 7 days. The concentrations of DCA and DCA metabolites in serum (a and b), livers (c and d), and gallbladders (e and f) of male WT and Oatp1a1-null mice (n = 5/group) were analyzed by ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). All data are expressed as mean ± S.E. of five mice in each group. *, statistically significant difference between WT and Oatp1a1-null mice ($p < 0.05$). #, statistically significant difference between DCA-fed WT and DCA-fed Oatp1a1-null mice ($p < 0.05$).

increased TDCA (1050%) in serum of mice. Taken together, the alteration of BA concentrations in serum of Oatp1a1-null mice suggests that Oatp1a1 may play an important role in the homeostasis of DCA and muricholic acids (MCAs).

Concentrations of DCA Metabolites in Serum, Livers, and Gallbladders of WT and Oatp1a1-Null Mice Fed 0.3% DCA

The concentration of DCA and DCA metabolites were quantified from serum of mice after feeding DCA for 7 days. As shown in Figure 3a, the concentration of DCA in serum of control Oatp1a1-null mice was about 150% higher than that in control WT mice. After feeding DCA, serum DCA concentrations in Oatp1a1-null mice were about 32-fold higher than that in WT mice. The concentrations of TDCA, GDCA, TCA, glyco-cholic acid (GCA), and CA in serum of Oatp1a1-null mice were about 98, 1090, 65, 265, and 4900%, respectively, of that in WT mice (Fig. 3b).

As shown in Figure 3c, feeding DCA increased DCA concentrations in livers of both WT (1060%) and Oatp1a1-null (39,300%) mice (Fig. 3c). As a result, after feeding DCA, liver DCA concentrations in Oatp1a1-null mice were about 32-fold higher than that in WT mice. After feeding DCA, the concentrations of DCA metabolites, such as TDCA (40%), GCA (4340%), GDCA (9060%), and CA (780%) were also higher in livers of Oatp1a1-null mice than that in livers of WT mice (Fig. 3d). In contrast, after feeding DCA, the concentration of TCA was increased in livers of WT but not Oatp1a1-null mice.

Feeding DCA increased DCA in gallbladder bile about 600% in WT mice, whereas it increased about 5300% in Oatp1a1-null mice (Fig. 3e). Feeding DCA also increased GCA (240%), GDCA (660%), and CA (35,100%) in gallbladder bile of Oatp1a1-null more than in WT mice (Fig. 3f). Similar to liver, after feeding DCA, TCA in gallbladder bile of Oatp1a1-null mice was lower (40%) than WT mice. In contrast, feeding DCA increased TDCA in gallbladder bile similarly in WT and Oatp1a1-null mice.

Taken together, after feeding DCA, the concentrations of DCA in serum, livers, and gallbladders of Oatp1a1-null mice

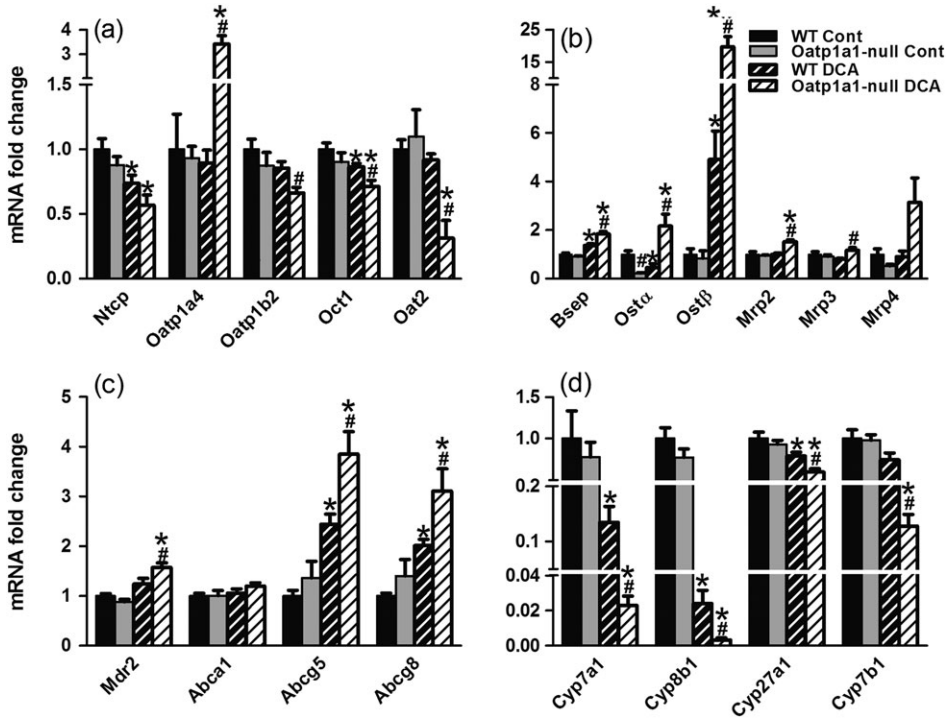

FIG. 4. mRNA of hepatic transporters and BA-synthetic enzymes from WT and Oatp1a1-null mice fed a 0.3% DCA diet. Total RNA from livers of male WT and Oatp1a1-null mice (n = 5/group) was analyzed by multiplex suspension array. The mRNA of each gene was normalized to GAPDH and presented as fold change related to the control group. All data are expressed as mean ± S.E. of five mice in each group. *, statistically significant difference between control and DCA-fed mice ($p < 0.05$). #, statistically significant difference between DCA-fed WT and DCA-fed Oatp1a1-null mice ($p < 0.05$).

were more than 30-fold higher than that in WT mice, suggesting that knockout of Oatp1a1 did not decrease hepatic uptake of DCA.

mRNA of Hepatic Transporters and BA-Synthetic Enzymes in WT and Oatp1a1-Null Mice After Feeding 0.3% DCA

The mRNA expression of hepatic uptake transporters are shown in Figure 4a. Feeding DCA decreased Ntcp mRNA in both WT (26%) and Oatp1a1-null (31%) mice. After feeding DCA, Oatp1a4 mRNA was not altered in WT mice but increased about 249% in Oatp1a1-null mice. In contrast, feeding DCA had little effect on Oatp1b2 in either WT or Oatp1a1-null mice. After feeding DCA, Oct1 mRNA was slightly decreased in both WT (13%) and Oatp1a1-null (19%) mice, whereas Oat2 mRNA was decreased only in Oatp1a1-null mice (79%).

The mRNA expression of hepatic BA-efflux transporters are shown in Figure 4b. After feeding DCA, Bsep mRNA was increased in both WT (36%) and Oatp1a1-null (93%) mice. Feeding DCA decreased Ostα mRNA about 55% in WT mice but increased Ostα mRNA about 195% in Oatp1a1-null mice. In contrast, feeding DCA increased Ostβ mRNA in both WT (390%) and Oatp1a1-null (1890%) mice. After feeding DCA, the mRNA expression of Mrp2 and Mdr2 were increased in Oatp1a1-null mice (57 and 70%) but not in WT mice.

Figure 4c shows the mRNA expression of hepatic cholesterol transporters. Feeding DCA increased Abcg5 and Abcg8 mRNA in both WT (144 and 101%, respectively) and Oatp1a1-null mice (249 and 171%, respectively).

The mRNA expression of BA-synthetic enzymes are shown in Figure 4d. Feeding DCA markedly suppressed cytochrome P450 (Cyp)7a1 mRNA in both WT (86%) and Oatp1a1-null (97%) mice. Feeding DCA also decreased Cyp8b1 and Cyp27a1 mRNA in both WT (97 and 21%, respectively) and Oatp1a1-null mice (99 and 36%, respectively). In contrast, feeding DCA decreased Cyp7b1 mRNA in Oatp1a1-null (-85%) but not WT mice.

Taken together, compared with WT mice fed DCA, Oatp1a1-null mice fed DCA expressed lower hepatic uptake transporters (Oatp1b2, Oct1, and Oat2), higher hepatic efflux transporters (Bsep, Ostα, Ostβ, Mrp2, Mrp3, Mdr2, Abcg5, and Abcg8), and lower BA-synthetic enzymes (Cyp7a1, Cyp8b1, Cyp27a1, and Cyp7b1), which together may prevent the hepatic accumulation of BAs.

Plasma Elimination of DCA and TDCA in WT and Oatp1a1-Null Mice

Because knockout of Oatp1a1 increased the concentrations of DCA and TDCA in serum of mice and the data from feeding DCA suggests that knockout of Oatp1a1 does not prevent the

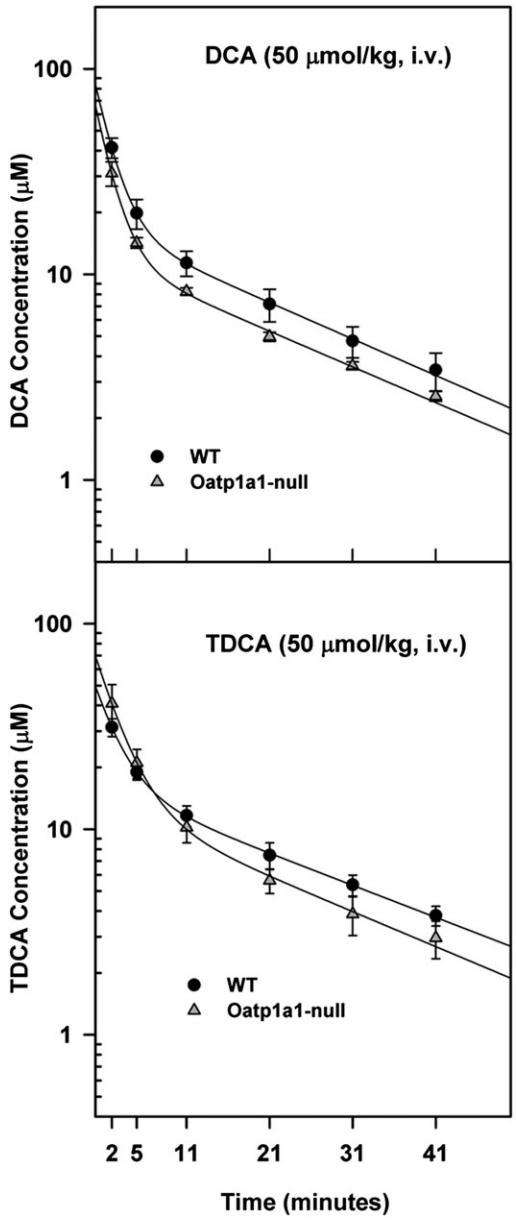

FIG. 5. Plasma distribution of DCA and TDCA in WT and Oatp1a1-null mice. DCA or TDCA was administered intravenously to WT and Oatp1a1-null mice (50 μmol/kg, $n = 6$/group). Blood was collected at 0, 2, 5, 11, 21, 31, and 41 minutes after dosing. DCA and TDCA concentrations in serum were quantified by ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS).

hepatic uptake of DCA, kinetic studies of iv injected DCA or TDCA in WT and Oatp1a1-null mice were performed to further determine the role of Oatp1a1 in the disposition of DCA and TDCA. Figure 5 illustrates the plasma elimination of an intravenous dose of DCA or TDCA (50 μmol/kg). The plasma disappearance curves indicate that the elimination of DCA or TDCA can be described by a two-compartment open model of elimination. After DCA administration, plasma DCA concentration tended to, but not significantly, decrease faster in Oatp1a1-null than WT mice. After TDCA administration, the plasma elimination of TDCA showed no significant difference between WT and Oatp1a1-null mice.

Concentrations of DCA and DCA Metabolites in Ileum and Colon Tissues of WT and Oatp1a1-Null Mice Fed 0.3% DCA

In an attempt to determine whether knockout of Oatp1a1 might increase the intestinal absorption of DCA, the concentrations of DCA and its metabolites were quantified in ileum and colon tissues of WT and Oatp1a1-null mice. Feeding DCA increased the concentration of DCA about 33 nmol/g in ileum tissue of WT mice and about 84 nmol/g in Oatp1a1-null mice (Fig. 6a). Feeding DCA increased TDCA concentration about 170 nmol/g in ileum tissue of WT mice and about 450 nmol/g in Oatp1a1-null mice (Fig. 6b). Feeding DCA had little effect on CA concentration in ileum tissue of WT and Oatp1a1-null mice. Feeding DCA increased TCA concentration about 450 nmol/g in ileum tissue of WT mice but not significantly in Oatp1a1-null mice. In contrast, feeding DCA increased GCA and GDCA concentrations about 74 and 31 nmol/g, respectively, in ileum tissue of Oatp1a1-null mice but not in WT mice.

As shown in Figure 6c, feeding DCA increased DCA concentration about 80 nmol/g in colon tissue of WT mice and about 180 nmol/g in Oatp1a1-null mice. Feeding DCA had little effect on TDCA concentration in colon tissue of WT and Oatp1a1-null mice, whereas it increased GDCA concentration about 0.6 nmol/g in colon tissue of Oatp1a1-null but not WT mice (Fig. 6d). Similar to ileum tissue, feeding DCA increased TCA concentrations about 20 nmol/g in colon tissue of WT but not Oatp1a1-null mice. Feeding DCA increased GCA concentration about 0.8 nmol/g in colon tissue of Oatp1a1-null but not WT mice. In contrast, feeding DCA increased CA concentration about 20 nmol/g in colon tissue of both WT and Oatp1a1-null mice.

Taken together, after feeding DCA, Oatp1a1-null mice had higher concentrations of DCA in both ileum and colon tissues than WT mice, suggesting that knockout of Oatp1a1 increases the intestinal absorption of DCA in mice.

mRNA of BA and Cholesterol Transporters in Ilea of WT and Oatp1a1-Null Mice

To determine whether the enhanced intestinal absorption of DCA in Oatp1a1-null mice is via the intestinal transporters, the mRNA expression of major BA and cholesterol transporters in ilea of WT and Oatp1a1-null male mice were quantified. Knockout of Oatp1a1 did not affect the major BA transporters, namely Asbt, Ostα, and Ostβ in ilea of mice (Fig. 7a). Compared with WT mice, Mrp3 mRNA was increased about 33%, and Mrp2 mRNA was decreased about 34% in ilea of Oatp1a1-null mice. Figure 7b shows the mRNA expression of cholesterol transporters. Knockout of Oatp1a1 increased Abca1 mRNA about 30% in ilea of mice. Taken together, knockout of Oatp1a1 had little effect on ileal BA-transporters.

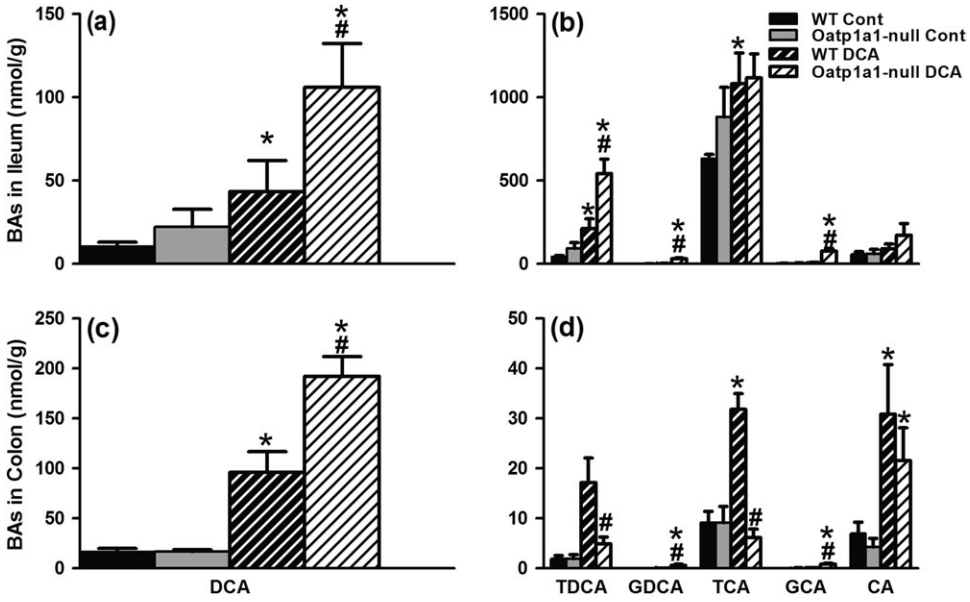

FIG. 6. Concentrations of DCA and DCA metabolites in ileum and colon tissue of WT and Oatp1a1-null mice after feeding DCA for 7 days. The concentrations of DCA and DCA metabolites in ilea (a and b) and colons (c and d) of male WT and Oatp1a1-null mice (n = 5/group) were analyzed by ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). All data are expressed as mean ± S.E. of five mice in each group. *, statistically significant difference between control and DCA-fed mice ($p < 0.05$). #, statistically significant difference between DCA-fed WT and DCA-fed Oatp1a1-null mice ($p < 0.05$).

Intestinal Permeability in WT and Oatp1a1-Null Mice

As a secondary BA, DCA is thought to be reabsorbed from the intestine by passive diffusion (Hofmann and Hagey, 2008). To determine whether knockout of Oatp1a1 alters intestinal permeability, the urinary excretion of lactulose and mannitol was determined in both WT and Oatp1a1-null mice gavaged with lactulose and mannitol. The lactulose/mannitol ratio in urine of Oatp1a1-null mice was about 2.5-fold higher than that in urine of WT mice (Fig. 8).

DISCUSSION

Oatp1a1 is highly expressed in liver and kidney but almost undetectable in the intestinal tract of mice (Cheng *et al*., 2005). Oatp1a1 has been shown *in vitro* to transport BAs, such as TCA (Hagenbuch *et al*., 2000). Therefore, it was expected that concentrations of some BAs would be higher in serum of Oatp1a1-null mice. Lack of Oatp1a1 increased the concentrations of DCA and TDCA in serum of mice, suggesting that Oatp1a1 likely mediates the hepatic uptake of DCA and TDCA. However, the total BA concentration in serum was decreased in Oatp1a1-null mice, mainly due to the decreased concentrations of TMCA and TCA. This indicates that Oatp1a1 may play a role other than hepatic uptake in the enterohepatic circulation of BAs. Because the hepatic expression of other BA transporters remain unchanged in Oatp1a1-null mice, the alterations of BA concentrations in serum of Oatp1a1-null mice are unlikely due to secondary changes in other BA transporters.

We noticed in a preliminary study that DCA caused more toxicity in male than female mice. Oatp1a1 is predominantly expressed in livers of male mice, and knockout of Oatp1a1 increases the concentrations of DCA and TDCA in serum of mice. Therefore, it is likely that the higher expression of Oatp1a1 in male mice might cause increased transport of DCA into liver, and thus result in more DCA-induced hepatotoxicity in male mice than in female mice. As expected, after feeding DCA, the concentration of DCA in serum of Oatp1a1-null mice was more than 30-fold higher than in WT mice. However, this is not due to decreased hepatic uptake of DCA by lack of Oatp1a1 because the concentrations of DCA in livers of Oatp1a1-null mice were also 30-fold higher than in WT mice after feeding DCA. In addition, after feeding DCA, Oatp1a1-null mice had about 50-fold more DCA in their gallbladders than WT mice, indicating that the abnormal increase of DCA in serum of Oatp1a1-null mice fed DCA is not due to a block in liver efflux. Furthermore, after feeding DCA, the concentrations of DCA metabolites, such as TDCA, GCA, GDCA, and CA in serum, livers, and gallbladders were all higher in Oatp1a1-null mice than in WT mice. Intravenous injection of a high dose of DCA or TDCA showed that Oatp1a1-null mice had a similar volume of distribution and clearance as WT mice (Supplementary table 1). Taken together, lack of Oatp1a1 does not prevent hepatic uptake of DCA or TDCA in mice.

Feeding 0.3% DCA increased the liver/body weight ratio about 50% in Oatp1a1-null but not in WT mice (Supplementary fig. 1F). In addition, feeding DCA increased serum TG and serum conjugated bilirubin in Oatp1a1-null mice but not in WT mice (Supplementary fig. 2). Although feeding DCA increased

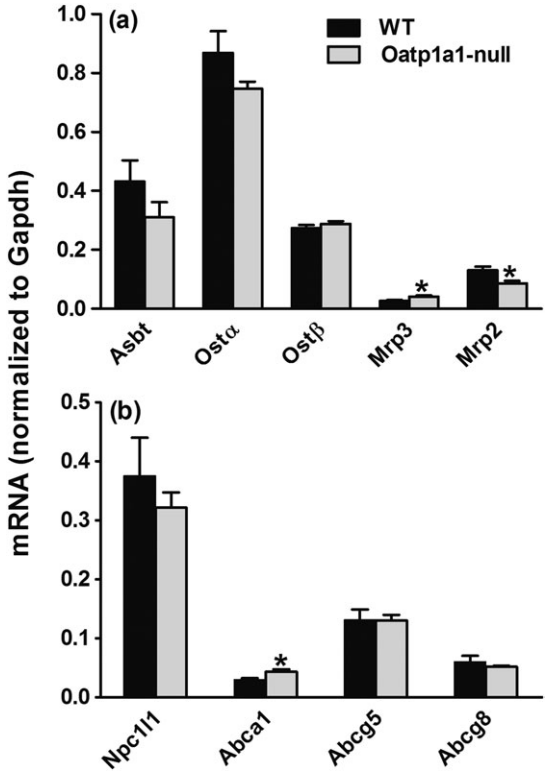

FIG. 7. mRNA of BA- and cholesterol-transporters in ilea of WT and Oatp1a1-null mice. Total RNA from ileums of WT and Oatp1a1-null mice (n = 5/group) was analyzed by multiplex suspension array. The mRNA of each gene was normalized to GAPDH. All data are expressed as mean ± S.E. of five mice in each group. #, statistically significant difference between WT and Oatp1a1-null mice ($p < 0.05$).

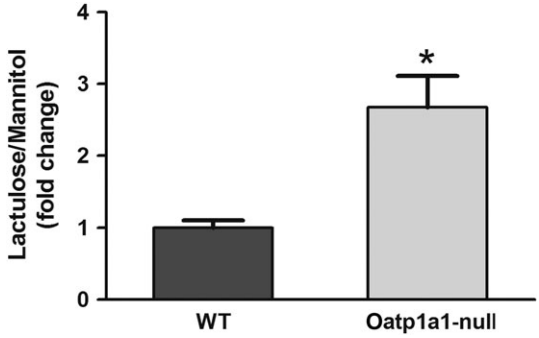

FIG. 8. Intestinal permeability in WT and Oatp1a1-null mice. Mice ($n = 4$/group) were gavaged with 13.3 mg lactulose and 10.1 mg mannitol, and urine was collected for 20 hr. The concentration of mannitol and lactulose were quantified by ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) and presented as fold change related to WT mice. *, statistically significant difference between WT and Oatp1a1-null mice ($p < 0.05$).

serum alanine aminotransferase (ALT) more in WT than Oatp1a1-null mice, the low ALT values (<300 U/l) indicate that feeding DCA did not cause significant liver damage in either WT or Oatp1a1-null mice (Supplementary fig. 2). Histological analysis showed that feeding DCA increased mitotic and apoptotic activities in livers of Oatp1a1-null mice but not in WT mice (data not shown). Consistently, after feeding DCA, Oatp1a1-null mice had higher mRNA expression of proliferation markers, such as antigen identified by monoclonal Ki-67 (Ki67), topoisomerase II alpha (Top2α), cyclin D1 (Ccnd1), collagen type 1 alpha 1 (Col1a1), and growth arrest and DNA-damage-inducible beta (Gadd45β) than did WT mice (Supplementary fig. 3). Taken together, feeding DCA enhances hepatocyte proliferation in livers of mice, which is more prominent in Oatp1a1-null than WT mice.

The present study indicates that mice have a tremendous ability to maintain total BA concentrations in liver. Although feeding DCA increased the concentrations of DCA and its metabolites in livers of WT mice, concentrations of other BAs were decreased. After feeding DCA, Oatp1a1-null mice had higher concentrations of TαMCA, αMCA, βMCA, and ωMCA but lower TβMCA, TCDCA, and TUDCA in livers than did WT mice (Supplementary fig. 4). Therefore, after feeding DCA, WT mice only had slightly increased total BA concentrations in livers (Supplementary fig. 5). In contrast, feeding DCA to Oatp1a1-null mice markedly increased the concentrations of unconjugated BAs in their livers, especially unconjugated DCA. The homeostasis of hepatic BAs is regulated not only by transporters but also by the *de novo* biosynthesis of BAs from cholesterol. Due to the higher concentrations of BAs in liver, Oatp1a1-null mice fed DCA had higher mRNA expression of BA-efflux transporters (Bsep, Ostα/β, and Mrp2) and lower mRNA expression of BA-synthetic enzymes (Cyp7a1, 8b1, 27a1, and 7b1) in liver than did WT mice fed DCA.

During DCA feeding, livers of mice can convert DCA to more hydrophilic and possibly less toxic metabolites. DCA can be conjugated with taurine and glycine to form less toxic TDCA and GDCA. DCA can also be "repaired" in the liver by being hydroxylated back to CA, which is further conjugated with taurine or glycine. After feeding DCA, Oatp1a1-null mice had higher concentrations of DCA and its metabolites (TDCA, GDCA, CA, TCA, and GCA) in livers than did WT mice. Interestingly, after feeding DCA, concentrations of almost all MCAs were decreased in livers of WT mice, whereas concentrations of TαMCA and ωMCA were markedly increased in livers of Oatp1a1-null mice. This suggests that TαMCA and ωMCA might also be metabolites of DCA in Oatp1a1-null mice.

Feeding DCA has more prominent effects in male than female Oatp1a1-null mice, probably because Oatp1a1 is a male-predominant transporter in mice. After feeding DCA, male Oatp1a1-null mice had 30-fold more DCA in serum and livers than male WT mice, whereas female Oatp1a1-null mice had similar levels of DCA as female WT mice (Supplementary fig. 6). Therefore, knockout of Oatp1a1 has more dramatic effects on BA metabolism of male than female mice. Consistently, after feeding DCA, the mRNA expression of Ostα/β, Bsep, Ki67, Gadd45β, and Top2α were increased more in livers of male than female Oatp1a1-null mice (Supplementary fig. 7). The mRNA expression of some other

genes, such as Oatp1a4, Ccnd1, and proliferating cell nuclear antigen (Pcna), were increased in livers of male Oatp1a1-null mice but not in female Oatp1a1-null mice. Taken together, there is a strong relationship between the expression of Oatp1a1 and the disposition of DCA in mice.

Oatp1a1-null mice appear to have increased intestinal absorption of DCA. DCA is mainly formed by intestinal bacteria in the cecum and colon and is thought to be reabsorbed into enterocytes by passive diffusion (Hofmann and Hagey, 2008). Lack of Oatp1a1 has little effect on the BA transporters in intestine, namely Asbt and Ostα/β. However, lack of Oatp1a1 increases the intestinal permeability of mice. This suggests that the abnormal increase of DCA in serum, livers, and gallbladders of Oatp1a1-null mice after feeding DCA is due to passive rather than active intestinal absorption of DCA. Compared with the 30-fold higher concentration of DCA in serum and livers of Oatp1a1-null male mice, they only had a twofold higher DCA concentration in ileum and colon tissues than WT mice, indicating that the majority of DCA is probably reabsorbed in the cecum, which contains the majority of intestinal contents and bacteria.

The increased intestinal permeability in Oatp1a1-null mice may be due to alterations in intestinal bacteria. The gut microbiota interact with the host through metabolic exchange and cometabolism of substrates. Gong *et al*. (2011) reported that Oatp1a1-null mice have increased urinary excretion of isethionic acid, a taurine metabolite produced by intestinal bacteria, suggesting that intestinal bacteria are altered in Oatp1a1-null mice. In the present study, Oatp1a1-null mice have higher concentrations of DCA and TDCA in serum. Oatp1a1-null mice also have higher concentrations of DCA and TDCA in feces (data not shown). Because DCA is formed from 7-dehydroxylation of CA by intestinal bacteria, this suggests that lack of Oatp1a1 may increase the bacteria that produce DCA in the intestine. Consistently, Oatp1a1-null mice have higher density of bacteria in the large intestine (Youcai Zhang, Peizhen Song, and Curtis D. Klaassen, unpublished data). Further research will be required to determine how the lack of Oatp1a1 alters the intestinal bacteria.

To conclude, the present study shows a critical role of Oatp1a1 in DCA disposition of mice. Although lack of Oatp1a1 increases the concentrations of DCA and TDCA in serum, Oatp1a1 does not appear to mediate the hepatic uptake of DCA or TDCA. Instead, lack of Oatp1a1 increases the intestinal permeability and thus increases intestinal absorption of DCA. As a result, Oatp1a1-null mice fed DCA have more than 30-fold higher concentrations of DCA in serum, livers, and gallbladders than do WT mice fed DCA.

SUPPLEMENTARY MATERIALS

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

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