Bile acid flux through portal but not peripheral veins inhibits CYP7A1 expression without involvement of ileal FGF19 in rabbits

Quan Shang,1,2 Grace L. Guo,3 Akira Honda,4 Daniel Shi,2 Monica Saumoy,2 Gerald Salen,1 and Guorong Xu1,2

1Department of Medicine, Rutgers, New Jersey Medical School, Newark, New Jersey; 2Medical Research Service, Veterans Affairs Medical Center, East Orange, New Jersey; 3Department of Pharmacology and Toxicology, Ernest Mario School of Pharmacy, Rutgers University, Piscataway, New Jersey; and 4Department of Gastroenterology, Ibaraki Medical Center, Tokyo Medical University, Ibaraki, Japan

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Shang Q, Guo GL, Honda A, Shi D, Saumoy M, Salen G, Xu G. Bile acid flux through portal but not peripheral veins inhibits CYP7A1 expression without involvement of ileal FGF19 in rabbits. Am J Physiol Gastrointest Liver Physiol 307: G479–G486, 2014. First published July 3, 2014; doi:10.1152/ajpgi.00062.2014.—It was proposed that CYP7A1 expression is suppressed through the gut-hepatic signaling pathway fibroblast growth factor (FGF) 15/19-fibroblast growth factor receptor 4, which is initiated by activation of farnesoid X receptor in the intestine rather than in the liver. The present study tested whether portal bile acid flux alone without ileal FGF19 could downregulate CYP7A1 expression in rabbits. A rabbit model was developed by infusing glycodeoxycholic acid (GDCA) through the splenic vein to bypass ileal FGF19. Study was conducted in four groups of rabbits: control; bile fistula + bovine serum albumin solution perfusion (BF); BF + GDCA (by portal perfusion); and BF + GDCA-F (by femoral perfusion). Compared with only BF, BF + GDCA (6 h portal perfusion) suppressed CYP7A1 mRNA, whereas BF + GDCA-F (via femoral vein) with the same perfusion rate of GDCA did not show inhibitory effects. Meanwhile, there was a decrease in ileal FGF19 expression and portal FGF19 protein levels, but an equivalent increase in biliary bile acid outputs in both GDCA perfusion groups. This study demonstrated that portal bile acid flux alone downregulated CYP7A1 expression with diminished FGF19 expression and protein levels, whereas the same bile acid flux reaching the liver through the hepatic artery via femoral vein had no inhibitory effect on CYP7A1. We propose that bile acid flux through the portal venous system may be a kind of “intestinal factor” that suppresses CYP7A1 expression.

cholesterol 7α-hydroxylase; fibroblast growth factor 19; bile acid synthesis

IT HAS BEEN PROPOSED THAT activated farnesoid X receptor (FXR) stimulates short heterodimer partner (SHP) expression in the liver, which in turn inhibits liver receptor homolog-1 (LRH-1)-mediated transcriptional activation of the CYP7A1 gene (8, 19). This hypothesis involving a cascade reaction in the liver provides an explanation at the molecular level for the indirect inhibition of CYP7A1 by bile acid-activated hepatic FXR. FTF (LRH-1) and hepatocyte nuclear factor 4 (HNF4) binding sites overlap in the bile acid response element II (2, 5). Recently, it was suggested that, in the liver, SHP inhibited CYP7A1 transcription by interacting with HNF4/LRH-1 at their overlapping binding sites in the CYP7A1 promoter region (16). However, observations from several other studies suggested that the intestine might be responsible for downregulation of CYP7A1. When the bile duct was ligated in rats (6) or mice (14) where bile acids accumulated in the liver and was blocked from reaching the intestine, CYP7A1 expression unexpectedly was increased, indicating a role of intestine in suppression of CYP7A1. Furthermore, it was observed that bile acids infused through peripheral (4, 21, 25) and portal venous systems (20) did not downregulate CYP7A1 and bile acid synthesis. Pandak et al. (21) proposed that an intestinal factor might be necessary for the feedback regulation of CYP7A1.

FGF15 in mice (14) or its human ortholog FGF19 (11) are target genes of FXR. Recently, it was proposed that FGF15 is selectively induced by bile acids-FXR in the ileum and acts as an intestinal-hepatic signal through FGF receptor 4 (FGFR4) integrating with SHP to repress CYP7A1 expression in the liver (14). Furthermore, it was hypothesized that activation of FXR- FGF15 in the intestine but not activation of FXR-SHP in the liver regulates CYP7A1 gene expression (15). This hypothesis challenged the idea that the expression of CYP7A1 is regulated by the bile acid flux through the liver by activation of a hepatic FXR-SHP cascade. More recently, Kong et al. reported (17) that both FGFR4 and SHP are responsible for suppressing Cyp7a1 expression in the liver of mice after activation of FXR but FGFR4 seems more important.

It has been reported that many pathways and factors are involved in regulation of hepatic CYP7A1 expression (3, 13). In the present study, we focused on whether an increase of bile acid flux alone without effects from ileal FGF19 could downregulate expression of CYP7A1 in rabbits. As a target gene of FXR, FGF19 expression in the ileum reflects the changes in the bile acid flux passing through the ileum. Thus, it is difficult to differentiate whether the induced FGF19 expression in the ileum or the increased bile acid flux or both are responsible for inhibition of CYP7A1 when bile acid is administered orally. In this study, we developed a model in rabbits that allows increased bile acid flux administered directly through the portal (portal) vein to bypass the ileum. Our data demonstrated that increasing bile acid flux through the portal venous system but not the peripheral venous system suppressed CYP7A1 expression without involvement of FGF19 in the ileum.

MATERIALS AND METHODS

Animal Experiments

The experiment included two parts: part 1, preliminary study, where 75 mg of glycodeoxycholic acid sodium salt (GDCA)/h were infused and part 2 where 100 mg of GDCA/h were infused. New
Zealand White rabbits used in either study parts 1 or 2 were divided into four study groups: rabbits fed only regular rabbit chow (control); bile fistula (BF) + portal venous perfusion of 10% bovine serum albumin (BSA) solution (BF); bile fistula + portal venous perfusion of GDCA solution (BF + GDCA); and bile fistula + femoral venous perfusion of GDCA (BF + GDCA-f). There were six rabbits in each study group of part 1 and eight rabbits in each group of part 2.

**Bile fistula.** The operation was performed under anesthesia (by ketamine 40 mg/kg and xylazine 4 mg/kg iv) in aseptic conditions. After ligation of the cystic duct, the common bile duct was cannulated using a custom-made tubing system composed of a Teflon tip and silicone tube. This biliary tubing was exteriorized subcutaneously through a skin incision on the rear back of the animal. Bile was collected continuously for 6 h. The bottle for bile collection was usually changed every hour for calculation of biliary bile acid outputs. Bile collected during the first 15 min in the rabbits fed regular chow was used for calculation of biliary bile acid outputs in controls (baseline value). The biliary bile acid outputs in controls (μmol/h) = total bile acid concentration (μmol/l) × amount of collected bile within 15 min (l) ÷ 15 × 60. The biliary bile acid outputs in the BF group (μmol/h) = total bile acid concentration (μmol/l) in the bile collected (in the rabbits only fed regular chow) during the 6th h × amount of collected bile (l) within 60 min ÷ 60 × 60. The biliary bile acid outputs of the BF + GDCA or BF + GDCA-f group = the mean value of the biliary bile acid outputs at the 5th and 6th h during the GDCA perfusion. During that time, biliary bile acid outputs almost reached steady state. In addition, because liver and ileal tissues were collected immediately after the 6th h, changes in CYP7A1 mRNA expression reflected the effect of the bile acid flux at that moment.

**Portal venous perfusion.** After the bile fistula had been constructed, a primary branch of the portal venous system located within the gastrosplenic ligament was cannulated toward the splenic vein. Immediately after the cannulation, portal perfusion was started. GDCA solution infused at the BF + GDCA group was 6 mg (in part 1 study) or 8 mg (in part 2) GDCA in each milliliter of 10% BSA solution. The perfusion was continued for 6 h at a rate of 12.5 ml/h such that GDCA was infused at 75 mg/h (159 μmol/h) in the part 1 study and 100 mg/h (212 μmol/h) in part 2. In the BF alone group, only 10% BSA solution was infused at 12.5 ml/h for 6 h through the splenic (portal) vein.

**Femoral (peripheral) venous infusion.** After the bile fistula was established, an incision was made at the inguinal area, and the femoral vein was dissected and cannulated. The GDCA solution was continuously infused through the femoral vein into the peripheral venous system at a rate of 12.5 ml/h for 6 h. The infused GDCA solution was exactly the same as used for portal perfusion: 6 mg (159 μmol/h in part 1 study) or 8 mg (212 μmol/h in part 2) GDCA in each milliliter of 10% BSA solution. Increase of the infused GDCA in the part 2 study was to further confirm that bile acid flux returning to the liver via the femoral vein-hepatic artery would not downregulate CYP7A1 expression.

To standardize effects of circadian rhythm of CYP7A1, all of the portal and femoral perfusions with GDCA in BSA or BSA alone were started between 9:00 and 10:00 A.M. After the surgery to construct bile fistula and cannulation, the animals did not remain anesthetized until the 6 h of perfusion were completed. All studied rabbits including controls were fasted from 5:00 P.M. the day before the experiment, and animals were killed between 3:00 and 4:00 P.M. following the 6 h of intravenous perfusion. Blood (from the portal vein and vena cava), liver tissue, and ileal mucosa were collected under general anesthesia immediately before death.

The animal protocol was approved by the Institutional Animal Care and Use Committee at the Veterans Affairs Medical Center (East Orange, NJ).

**Measurement of FGF19 Protein Concentrations in Plasma Samples**

FGF19 protein levels in plasma samples were measured using an “in-house” designed indirect ELISA method as previously described (22). One hundred microliters of the standards and rabbit plasma samples were added to each well. FGF19 goat polyclonal IgG antibody (Everest Biotech Ltd, Upper Heyford, UK) was used to coat the plates.

The minimum detectable plasma FGF19 protein concentration was 15.6 pg/ml. This method showed a strong linear relationship between the standard FGF19 protein concentrations (from 15.6 to 1,000 pg/ml) and the corresponding absorbance (optical density).

The coefficient of variation (CV) of this method was determined at low (L, 32 pg/ml), medium (M, 75 pg/ml), and high (H, 200 pg/ml) standard FGF19 protein concentrations (n = 20 for each concentration). Intra-assay CV was 11% (L), 4% (M) and 3.6% (H) and interassay CV was 7.8% (L), 4.3% (M), and 4.8%.

**Assays for Plasma 7α-Hydroxy-4-Cholesten-3-One Concentrations**

Plasma 7α-hydroxy-4-cholesten-3-one (C4) concentrations were quantified by LC-MS/MS as described previously (12). Briefly, deuterium-labeled C4 was added to 20 μl of plasma as internal standard, and C4 was extracted with acetonitrile. After derivatization into the picolinyl ester, the sample was analyzed by the LC-ESI-MS/MS system.

**Assays for mRNA Expression**

The mRNA expression was determined quantitatively by the standard real-time PCR method. Total RNA was isolated using TRIzol reagent (Sigma, St. Louis, MO) according to the manufacturer’s instructions. For quantitative RT-PCR analysis, 1 μg total RNA was transcribed to cDNA using the high cDNA capacity reverse transcription kit (Applied Biosystems, Foster City, CA). The qRT-PCR was performed using TaqMan Expression Assays (containing primer and probe) designed by Applied Biosystems for rabbit CYP7A1, SHP, Na+-taurocholate cotransporting polypeptide (NTCP), and FGF19. Each gene expression was expressed as relative units of the ratio between the controls (regular chow-fed) and treated rabbits.

**Measurements of Portal Blood Bile Acid Concentrations**

The bile acid concentrations were measured by capillary gas liquid chromatography as described previously (22). Ten micrograms of glycocholic acid were added as an internal standard to each 1 ml of plasma sample. The bile acid methyl esters were applied to a capillary gas chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with a 25-m fused silica CP-Sil 5-CB capillary column.

**Statistical Analysis**

Data of bile acid (concentration and biliary outputs) and FGF19 protein in the text and Figs. 1–4 are shown as means ± SD. Statistical difference among multistudy groups was tested using ANOVA followed by the Bonferroni multiple-comparisons test. GraphPad InStat, Instant Biostatistics Version 3.0 (GraphPad Software, San Diego, CA) was used for these statistical evaluations.

Because the data in some genes’ expression were not distributed normally, the Kruskal-Wallis test (nonparametric test) was employed to evaluate significant difference and was followed by Bunn’s multiple-comparisons test to identify statistically significant difference in all the gene expressions between selected study groups. Data of gene expression in the text are presented as medians and in Figs. 1–4 as median with range. GraphPad Prism, version 5.0 (GraphPad Software) was used for these nonparametric statistical evaluations and to make relevant graphs.
RESULTS

Part 1 Study (GDCA was infused at 159 μmol/h)

The portal bile acid concentrations that represent the bile acid flux in the BF + GDCA group (83 ± 21 μmol/l) increased 2.3-fold (P < 0.001) and 46% (P < 0.01), respectively, compared with the BF group (36 ± 10 μmol/l) and the controls (57 ± 14 μmol/l). In contrast, the portal bile acid concentrations in the BF + GDCA-f group (37 ± 13 μmol/l) did not change (Fig. 1A). Perfusion of GDCA either through the portal or peripheral venous system increased bile acid concentrations in the peripheral blood both (P < 0.001) in the BF + GDCA (13 ± 3.4 μmol/l) and BF + GDCA-f (11.6 ± 3 μmol/l) groups compared with the BF (4.1 ± 1.5 μmol/l) or the control (4.8 ± 1.1 μmol/l) group. After 6 h of the GDCA perfusion, the biliary bile acid outputs in the BF + GDCA (159 ± 32 μmol/h) and BF + GDCA-f (171 ± 30 μmol/h) groups were similar and increased significantly compared with the BF group (47 ± 21 μmol/h, P < 0.001) and the control group (94 ± 39 μmol/h, P < 0.01). Figure 1D shows the kinetic changes of the biliary bile acid outputs (means ± SE) during the 6 h of treatment. It suggests that the bile acid flux passing through the liver was similar in the portal and femoral perfusion groups during the whole perfusion time.

FGF19 protein levels in the portal blood (Fig. 2) did not increase but rather decreased both in the BF + GDCA (85 ± 31 pg/ml, P < 0.01) and the BF + GDCA-f (87 ± 33 pg/ml, P < 0.01) groups compared with the BF group (148 pg/ml, P < 0.05) or the control group (166 ± 56 pg/ml, P < 0.01).

CYP7A1 mRNA levels (Fig. 2) were suppressed after 6 h of GDCA perfusion through the portal vein in the BF + GDCA group (median: 0.33 relative units) but not via the femoral vein in the BF + GDCA-f group (1.09 relative units) compared with the BF (1.00 relative units) (P < 0.01) or the control (0.96 relative units) (P < 0.05) groups.

Ileal FGF19 mRNA levels (Fig. 2) in the BF + GDCA group (median: 0.02 relative units) were not decreased significantly compared with the BF group (0.13 relative units) but were lower (P < 0.001) than the control group (0.58 relative units). There was no significant difference in FGF19 mRNA between the BF + GDCA and BF + GDCA-f (0.14 relative units) groups.

Hepatic FGF19 mRNA levels in the BF + GDCA (median: 0.56 relative units) and BF + GDCA-f (0.77 relative units) groups did not increase compared with either the BF (1.01 relative units) or the control (0.65 relative units) group.

Hepatic SHP mRNA in the BF + GDCA group (median: 2.19 relative unit) increased (P < 0.01) compared with the BF group (0.88 relative units). Hepatic SHP mRNA in the BF + GDCA-f group (1.58 relative units) did not increase significantly compared with either the BF or control groups. The expression of SHP mRNA in the ileum was diminished (P < 0.05) in the BF + GDCA group (median: 0.32 relative units) and the BF + GDCA-f group (0.24 relative units) compared with the control (1.11 relative units). The ileal SHP mRNA levels in the BF group (0.72 relative units) were not significantly lower than the control and not significantly higher than the BF + GDCA and BF + GDCA-f groups either (Fig. 2).

Part 2 Study (GDCA was infused at 212 μmol/h)

The portal bile acid concentrations (Fig. 3A) in the BF + GDCA group (118 ± 29 μmol/l) increased threefold (P < 0.001) compared with the BF group (36 ± 14 μmol/l) and 93% (P < 0.001) compared with the control group (61 ± 21 μmol/l). The portal bile acid concentrations did not rise in the BF + GDCA-f group where GDCA was infused through the femoral vein (34 ± 10 μmol/l) compared with either the BF or the controls. Perfusion of GDCA either through the portal or peripheral venous system increased bile acid concentrations in the peripheral blood in the BF + GDCA group 3.8-fold (P < 0.001) and BF + GDCA-f group 4-fold (P < 0.001) compared with the BF group (4 ± 2.3 μmol/l). During the GDCA perfusion, the biliary bile acid outputs in the BF + GDCA

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(223 ± 36 μmol/h) and BF + GDCA-f (245 ± 47 μmol/h) groups increased similarly and were two times larger (P < 0.001) than the baseline value in the controls (80 ± 30 μmol/h) and six times larger (P < 0.001) than the BF group (34 ± 13 μmol/h). Figure 3D also suggests that the transhepatic bile acid flux was similar in the portal and femoral GDCA perfusion groups during the 6 h of treatment.

FGF19 protein levels in the portal blood did not increase (Fig. 4) after GDCA perfusion either through the portal (BF + GDCA) or the femoral vein (BF + GDCA-f). Portal FGF19 protein levels were decreased (P < 0.05) in the BF + GDCA group (0.06 relative units) compared with either the BF (0.22 relative units) or the control (1.00 relative units) group. However, plasma C4 levels in the BF + GDCA-f group (1.92 ± 1.08 ng/ml, n = 8) were not decreased compared with the BF or control group.

CYP7A1 mRNA levels (Fig. 4) were suppressed after 6 h of GDCA perfusion through the portal vein in the BF + GDCA group (median: 0.06 relative units) compared with either the BF (1.13 relative units, P < 0.01) or the control (0.78 relative units, P < 0.05) group. However, in the BF + GDCA-f group (1.02 relative units) where GDCA was infused via the femoral vein, CYP7A1 mRNA levels were not suppressed, although the rate of infused GDCA was increased up to 212 μmol/h.

Ileal FGF19 mRNA levels in the BF + GDCA group (median: 0.04 relative units) were lowest among the studied groups (Fig. 4). Ileal FGF19 mRNA levels were not significantly lower than the BF group (0.22 relative units) but lower than the control group (1.00 relative units, P < 0.01) and the BF + GDCA-f group (0.35 relative units, P < 0.05).

Hepatic FGF19 mRNA levels (Fig. 4) in the BF + GDCA group (median 0.66 relative units) were lower (P < 0.05) than the BF group (1.37 relative units) and the BF + GDCA-f group (1.66 relative units).
The expression of hepatic SHP mRNA was identical to the increase of bile acid flux through the liver regardless whether GDCA was infused via the portal or the femoral vein. Hepatic SHP mRNA increased \((P < 0.05)\) in the BF + GDCA group (median: 1.81 relative units) and the BF + GDCA-f group (2.02 relative units) compared with the BF group (0.76 relative units). In contrast, the expression of SHP in the ileum was diminished in the GDCA perfusion group (Fig. 4). Ileal SHP mRNA levels in the BF + GDCA group (median: 0.20 relative units) were significantly \((P < 0.01)\) lower than the control group (1.11 relative units). However, the ileal SHP mRNA levels in the BF group (0.49 relative units) were not significantly lower than the control and not significantly higher than the BF + GDCA and BF + GDCA-f (0.31 relative units) groups either.

The expression of NTCP mRNA, the bile acid transporter that transfers bile acids from the portal blood into the liver in the BF + GDCA group (median: 0.33 relative units), was decreased significantly \((P < 0.01)\) compared with the control group (1.06 relative units) but not the BF group (0.73 relative units). NTCP mRNA levels in the BF + GDCA-f group (0.60 relative units) and the BF group did not reduce significantly compared with the controls (Fig. 4).

**DISCUSSION**

This study demonstrated that bile acid flux alone downregulated CYP7A1 mRNA expression without involvement of ileal FGF19. In this rabbit model with bile fistula, GDCA was infused through the splenic vein (portal venous system). The infused bile acid (GDCA) to the liver did not pass through the ileum to activate ileal FXR to induce ileal FGF19. Thus, the infused GDCA and the initially existed bile acid flux were not able to reach the intestine after secreted from the liver because of total bile drainage through the bile fistula. In the rabbits where GDCA was infused through the portal vein (the BF + GDCA group), the expression of FGF19 mRNA in the ileum decreased and FGF19 protein levels in the portal blood, which presumably is the path for ileal FGF19 to reach the liver, did not increase (Fig. 2). Thus, the suppression of CYP7A1 mRNA expression after portal GDCA perfusion (BF + GDCA) was solely due to the passage of the increased bile acid flux, whereas in the BF group that was infused the same amount of BSA solution without GDCA CYP7A1 expression was not suppressed. It must be emphasized that CYP7A1 expression was inhibited despite the diminished FGF19 expression in the ileum that should have induced the expression of CYP7A1 according to the gut-hepatic signaling theory.

In the present study, we examined genes related to the FXR-SHP pathway. Hepatic SHP mRNA levels were increased in the BF + GDCA groups associated with inhibited CYP7A1 mRNA in the liver and diminished FGF19 mRNA in the ileum. The results suggested that, under this experimental condition, the FXR-SHP pathway but not the ileal FGF19 signaling pathway was involved in the downregulation of CYP7A1 expression. However, it should be emphasized that there are other pathways through which GDCA could downregulate CYP7A1 (3, 13). Particularly, in the present study, GDCA perfusion could also activate the c-jun NH2-terminal kinase signaling cascade (9, 10, 18) to downregulate CYP7A1 transcription. Although GDCA might suppress CYP7A1 through activating the FGF19/FGFR4 signaling pathway in the liver (24), in the present study, hepatic FGF19 mRNA levels were not increased in the BF + GDCA group. In the bile acid perfusion studies, only GDCA was tested because in rabbits DCA constituted >90% of the bile acids in the pool such that increasing GDCA flux would more likely reflect the regulatory effects of portal bile acid flux. Although chenodeoxycholic acid (CDCA) is a more potent FXR activator than DCA, it is very toxic in rabbits. Rabbits are coprophagic, and feeding CDCA would produce more lithocholic acid to cause severe liver damage (7).

In contrast to the suppression of CYP7A1 mRNA expression in the rabbits with portal GDCA perfusion, CYP7A1 mRNA levels in the GDCA-f group were not reduced compared with the BF or control group even after the infused GDCA was...
increased to 212 μmol/h. The different response of CYP7A1 expression to the bile acid perfusion through the portal and femoral veins was not due to the effects of FGF19 from the ileum. FGF19 mRNA levels in the ileum were suppressed both in the BF/GDCA and BF/GDCA-f groups (Figs. 2 and 4), and FGF19 protein levels in the portal blood were not different between these two groups (Figs. 2 and 4). It must be pointed out that biliary bile acid output (97% was GDCA) during the femoral GDCA perfusion was almost the same as portal GDCA perfusion, suggesting that the bile acid flux passing through the liver every hour was similar in the rabbits infused either GDCA through the portal or the femoral (peripheral) veins. In addition, total bile acid concentrations in the peripheral blood were almost identical between the rabbits with the portal (BF + GDCA) and peripheral (BF + GDCA-f) GDCA perfusion (Figs. 1 and 3). The key difference was that, in the BF + GDCA group, the additional bile acid (GDCA) flux was infused through the portal vein, whereas, in the BF + GDCA-f group, infusion was through the femoral vein. As a result, bile acid concentrations in the portal blood in the rabbits infused GDCA through the portal vein (BF + GDCA) were three times (P < 0.001) higher than through the peripheral vein (BF + GDCA-f). When compared with baseline values in the controls, bile acid concentrations in the portal blood increased 46 and 93%, respectively, in the portal GDCA perfusion at 159 and 212 μmol/h. The result of CYP7A1 mRNA expression from the BF + GDCA-f group was consistent with the finding of Pandak et al. (21). However, when the BF + GDCA-f was compared with the study of BF + GDCA (portal GDCA perfusion), it demonstrates that, in fact, bile acid flux alone without passing through the intestine could downregulate CYP7A1 expression and must be returned to the liver via the portal vein but not the hepatic artery (through the femoral vein). This finding suggests that the sensor activated by the bile acid flux that triggers the process to downregulate CYP7A1 expression might be located along the hepatic portal venous system. Thus, we postulate that the absorbed bile acid passing through the intestine physiologically may be a kind of “intes-
tinal factor,” since all of the bile acids absorbed through the intestine should return to the liver only via the portal venous system. The above findings obtained in rabbits may help to explain the observations in rats (6) and mice (14) with ligated common bile ducts. In those animals, bile acids accumulated in the liver, whereas CYP7A1 was not suppressed but rather upregulated. This was because the sensor in the portal venous area received a message of sharply reduced bile acid flux from the portal blood due to the ligation of the common bile duct that blocks the enterohepatic bile acid circulation. This “false” message resulted in stimulation of CYP7A1 expression. Moreover, the toxic effects of the accumulated bile acids in the liver may also increase CYP7A1 mRNA as previously observed in rats fed deoxycholic acid (23). In addition, the findings in the present study also could explain the observations in cholic acid-fed rats and rabbits where CYP7A1 mRNA levels were repressed with increased bile acid flux but without enhanced FGFI5/19 protein levels in the portal blood (22).

We should also be aware that, if the hepatic bile acid sensor would be sensitive only to the bile acid concentration but not the flux, the results observed in the femoral perfusion group might be possibly also due to dilution of the infused bile acid concentrations in the peripheral blood.

It has been reported that plasma C4 levels could be used as the marker reflecting CYP7A1 activity and bile acid synthesis (1). The inhibition of CYP7A1 mRNA expression in the BF + GDCA group was further supported by the evidence that plasma C4 levels decreased significantly in the portal but not the femoral GDCA perfusion group (Fig. 4). We noted that Nagano et al. (20) did not see repression of CYP7A1 mRNA by portal cholic acid perfusion in rats. This result might be due to different species and difference in the infused bile acids, since CA is considered a weaker FXR ligand than DCA. In our study, GDCA was infused via a primary branch of the portal venous system located within the gastrosplenic ligament. Rabbit study, GDCA was infused via a primary branch of the portal venous system located within the gastrosplenic ligament. Since CA is considered a weaker FXR ligand than DCA. In our study, GDCA was infused via a primary branch of the portal venous system located within the gastrosplenic ligament. Rabbit study, GDCA was infused via a primary branch of the portal venous system located within the gastrosplenic ligament. Since CA is considered a weaker FXR ligand than DCA. In our study, GDCA was infused via a primary branch of the portal venous system located within the gastrosplenic ligament. Rabbit study, GDCA was infused via a primary branch of the portal venous system located within the gastrosplenic ligament. Since CA is considered a weaker FXR ligand than DCA.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: Q.S., A.H., and G.X. performed experiments; Q.S., A.H., and G.X. analyzed data; Q.S., A.H., and G.X. interpreted results of experiments; Q.S., G.L.G., A.H., D.S., M.S., G.S., and G.X. approved final version of manuscript; G.L.G., M.S., G.X. edited and revised manuscript; D.S. and G.X. drafted manuscript; G.X. conception and design of research; G.X. prepared figures.

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