Fibroblast Growth Factor 19 Treatment Ameliorates Disruption of Hepatic Lipid Metabolism in Farnesoid X Receptor (Fxr)-Null Mice

Masaaki Miyata,* Yumi Sakaida, Hitomi Matsuzawa, Kouichi Yoshinari, and Yasushi Yamazoe

Division of Drug Metabolism and Molecular Toxicology, Graduate School of Pharmaceutical Sciences, Tohoku University; 6–3 Aoba, Aramaki, Aoba-ku, Sendai 980–8578, Japan.

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Human fibroblast growth factor 19 (FGF19) is an enterohepatic hormone that is involved in the regulation of hepatic metabolism of bile acids, lipids, and glucose. Farnesoid X receptor (Fxr)-null mice exhibit steatosis-like symptoms, showing higher hepatic lipid levels than with the wild-type mice. We investigated the influence of FGF19 treatment on hepatic lipogenesis in Fxr-null mice. Recombinant FGF19 treatment (400 μg/kg/d) for 3 d prevented the accumulation of lipid droplets and decreased serum alanine aminotransferase activity and hepatic lipid levels, including those of triglycerides and free fatty acids. The treatment significantly decreased the hepatic mRNA levels of acetyl-CoA carboxylase 1 (Acc1), Cyp7a1, and sterol regulatory element-binding protein-1c (Srebp-1c) as well as those of acetyl-CoA carboxylase 2 (Acc2), stearoyl CoA desaturase 1 (Scd1), and Cyp7a1. FGF19 treatment (4 μg/kg/d) for 3 d also decreased the hepatic free fatty acid levels and mRNA levels of Acc1, Cyp7a1, and Srebp-1c. These results indicate that FGF19-mediated signaling ameliorates disrupted hepatic lipogenesis in Fxr-null mice.

Key words: fibroblast growth factor 19; farnesoid X receptor; lipogenesis; Cyp7a1; acetyl-CoA carboxylase 1

Farnesoid X receptor (FXR) is a bile acid sensor that protects the liver from bile acid toxicity. Recently, the physiological role of FXR has been expanded to include the regulation of the homeostasis of lipids and glucose as well as of bile acid.1–4 Over the past decade, many studies have indicated the presence of several FXR signaling pathways that regulate hepatic lipid metabolisms (lipogenesis). FXR directly regulates the expression of the genes involved in hepatic lipogenesis, such as acetyl-CoA carboxylase 1 (Acc1) and fatty acid synthase (FAS).5 On the other hand, FXR down-regulates hepatic lipogenesis by the induction of small heterodimer partner (SHP), which is a negative regulator of sterol regulatory element-binding protein-1c (SREBP-1c)-mediated lipogenic signaling.6 Moreover, recent research has highlighted the role of FXR-dependent fibroblast growth factor 19 (FGF19) signaling in the regulation of hepatic lipid metabolism.7–10

FGF19 and FGF15 (the mouse ortholog of FGF19) are members of the FGF family.11,12 They are specifically expressed in the intestine and function as endocrine hormones, regulating various metabolic processes.13,14 FGF15/19 acts on the liver through the activation of FGF receptor 4 (FGFR4).15,16 FGF15/19 has been reported to down-regulate the hepatic expression of CYP7A1 to reduce bile acid synthesis.8,16,17 Studies using FGF19-transgenic mice and FGF19-treated hyperlipidemic mice have indicated that FGF19 also regulates the hepatic levels of lipids, such as triglyceride (TG) and cholesterol.7,9 However, how the FGF15/19-FGFR4 signal controls the hepatic lipid levels remains unclear. Fxr-null mice have disrupted lipogenesis and their livers show elevated levels of not only hepatic bile acids but also TGs and free fatty acids (FFAs).18 Furthermore, insulin resistance has been observed in these mice.19–21 Fxr-null mice are now considered to be models of metabolic syndrome with hepatic steatosis. Fxr-null mice, lacking FXR signaling, show lower expression levels of intestinal Fgf15 and hepatic Shp mRNA.17,18 Therefore, not only the lack of direct FXR signaling but also the attenuation of FGF15- and SHP-mediated signaling contributes to the elevation of the hepatic lipid levels in Fxr-null mice. However, whether FGF15/19 signaling compensates for the lack of FXR signaling that regulates hepatic lipogenesis remains unknown.

We investigated the influence of human FGF19 treatment on the hepatic lipid levels in Fxr-null mice to verify the role of FGF15/19 signaling in the regulation of hepatic lipogenesis. The results showed that FGF19 treatment significantly decreased the elevated hepatic lipid levels in Fxr-null mice.

MATERIALS AND METHODS

Animal Treatment, Sample Collection, and Histological Analysis Fxr-null mice20 and wild-type mice were housed under a standard 12-h light–dark cycle (9 a.m.—9 p.m.). Before beginning the experiments, the mice were fed standard rodent chow (CE2; CLEA Japan, Tokyo, Japan) and water when needed. Age-matched groups of 8—10-week-old female mice were intravenously injected with recombinant FGF19 (400 μg or 4 μg/kg/d) or vehicle (saline) through the tail vein once a day for 3 d. They were killed 6 h after the final injection. Liver tissues were frozen in liquid nitrogen and stored at −80°C until use. For histological analysis, some of the frozen liver sections were stained with Oil Red O using standard procedures. All experiments were performed in accordance with the Guidelines for Animal Experiments of Tohoku University (Sendai, Japan). The protocol was approved by the Institutional Animal Care and Use Committee at Tohoku University (Permission No. 22-Pharm-Animal-6).

Expression and Purification of Recombinant Human FGF19 Human FGF19 DNA fragment containing the amino acid coding region (3—215) was amplified by polymerase chain reaction (PCR) with the primers, 5'-GGCGCAATATGCTGCTTCTTCTCGGAGCGCGG-3' containing an NdeI site and 5'-GGCGAAGCTTACTTCTCAAAAGCTGGG-3' containing a HindIII site and cDNA derived from...
Caco-2 cells (Riken BioResource Center, Ibaraki, Japan) as the template by using KOD-FX (Toyobo, Osaka, Japan). After the initial denaturation at 94°C for 2 min, DNA was amplified for 30 cycles at 98°C for 10 s and 68°C for 1 min. The PCR product was digested with Ndel and HindIII and ligated into Ndel and HindIII sites of the pET-28a(+) vector (Merck, Rahway, NJ, U.S.A.). The recombinant plasmid was transformed into Origami 2(DE3) (Merck). Recombinant FGF19 protein was purified on a Ni-NTA column (Qiagen, Valencia, CA, U.S.A.) as per the manufacturer's instructions. The purity of the isolated recombinant FGF19 protein was estimated to be >98% by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Protein concentration was determined using the Bradford assay.

**Determination of Hepatic Diagnostic Marker Levels and Bile Acid Levels** Serum alanine amino transferase (ALT) activity was determined using a commercial kit, Transaminase CII-B-test Wako (Wako Pure Chemicals, Osaka, Japan). Serum alkaline phosphatase (ALP) activity was determined using a commercial kit, Transaminase CII-B-test Wako (Wako Pure Chemicals, Osaka, Japan). Serum bile acid levels were measured by high-performance liquid chromatography (HPLC) as previously described.

**Determination of Hepatic Lipid Levels** A liver section of was homogenized in 2 volumes of 75 mM KCl and 75 mM KH2PO4-K2HPO4 buffer (pH 7.4). The homogenate was diluted with four times the amount of buffer containing 2% Triton X-100 and was incubated at 65°C for 30 min. After centrifugation, the supernatants were assessed for the hepatic TG and FFA levels compared with wild-type mice. FGF19 treatment (400 μg/kg/d) significantly reduced the FFA levels to those in vehicle-treated mice (Fig. 1). FGF19 treatment (400 μg/kg/d) for 3 consecutive days through tail vein injection. Livers were dissected 6 h after the last injection. Data are shown as the mean ± S.D. (n=5—8). The significant differences were assessed by Dunnett's test (##p<0.01 vs. corresponding control mice) or Student's t-test (##p<0.001 vs. vehicle-treated Fxr-null mice).

### Table 1. Influence of FGF19 Treatment on Relative Liver Weight and Liver Diagnostic Markers

<table>
<thead>
<tr>
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<th>Fxr-null</th>
<th>Wild-type</th>
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</thead>
<tbody>
<tr>
<td>Liver weight (g)</td>
<td>1.27±0.24</td>
<td>1.05±0.06</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>19.7±0.86</td>
<td>20.6±0.66</td>
</tr>
<tr>
<td>Liver/body weight ratio (%)</td>
<td>6.46±1.19</td>
<td>5.12±0.21</td>
</tr>
<tr>
<td>ALT (IU/l)</td>
<td>82.7±48.7</td>
<td>19.1±0.3***</td>
</tr>
<tr>
<td>ALP (IU/l)</td>
<td>151.4±67.1</td>
<td>68.7±7.7***</td>
</tr>
<tr>
<td>Hepatic bile acid (μmol/g liver)</td>
<td>1.43±0.91</td>
<td>0.51±0.11***</td>
</tr>
</tbody>
</table>

Mice received vehicle or FGF19 (4, 400, 4000 μg/kg/d) for 3 consecutive days through tail vein injection. Livers were dissected 6 h after the last injection. Data are shown as the mean±S.D. (n=5—8). The significant differences were assessed by Dunnett's test (*p<0.05 vs. corresponding control mice) or Student's t-test (##p<0.01, ***p<0.001 vs. vehicle-treated Fxr-null mice).
wild-type mice. However, FGF19 treatments (4, 400 μg/kg/d) had no effect on the hepatic TC levels. To confirm that the reduction of the hepatic TG levels was FGF19-mediated, we performed Oil Red O staining of liver sections. Histological analysis revealed many Oil Red O-positive lipid droplets in the livers of vehicle-treated Fxr-null mice (Fig. 2). The number of lipid droplets was markedly decreased in the livers of Fxr-null mice treated with FGF19 (400 μg/kg/d). These results indicate that FGF19 has a preventive effect on the hepatic accumulation of lipid droplets in Fxr-null mice.

**Influence of FGF19 Treatment on Hepatic mRNA Levels of Genes Involved in Lipogenesis** We determined the hepatic mRNA levels of genes involved in lipogenesis in order to investigate the mechanisms of the FGF19-mediated changes in the lipid levels in Fxr-null mice (Fig. 3). The hepatic mRNA levels of Cyp7a1, the target gene of FGF15 signaling, were considerably decreased in mice by FGF19 treatment (4 μg/kg/d or 400 μg/kg/d). Thus, intravenously injected FGF19 probably acted on the livers of mice. The hepatic mRNA levels of Shp, a negative regulator of Srebp-1c expression, were significantly increased in the mice treated with FGF19 (400 μg/kg/d), whereas the mRNA levels of Srebp-1c and Acc1, but not Fas, were decreased in the mice treated with FGF19 (4, 400 μg/kg/d). Consistent with previous reports using mice treated with FGF19 (1 mg/kg/d), the hepatic mRNA levels of Acc2, a negative regulator of fatty acid β-oxidation, and Scd1 were significantly decreased in Fxr-null mice by treatment with FGF19 (400 μg/kg/d). The hepatic mRNA levels of fatty acid β-oxidation related genes, carnitine palmitoyltransferase 1a (Cpt1a), carnitine palmitoyltransferase 2 (Cpt2), long chain acyl-CoA dehydrogenase (Acadl), medium chain acyl-CoA dehydrogenase (Acadm), and short chain acyl-CoA dehydrogenase (Acads), were significantly decreased in Fxr-null mice by treatment with FGF19 (400 μg/kg/d). The ratios of Cpt1a, Cpt2, Acadl, Acadm and Acads mRNA levels in mice treated with FGF19 (400 μg/kg/d) to those in vehicle-treated mice were 0.45, 0.65, 0.70, 0.58 and 0.75 respectively. We also determined the hepatic mRNA levels of genes involved in lipid transport. FGF19 treatments (4, 400 μg/kg/d) significantly decreased the hepatic mRNA levels of Cd36, which encodes a fatty acid uptake transporter in Fxr-null mice.
DISCUSSION

We demonstrated that the FGF19 treatment of Fxr-null mice significantly decreased their hepatic levels of TGs and FFAs to those observed in wild-type vehicle-treated mice. This suggests that FGF19 signaling compensates for the disruption of hepatic lipid metabolism due to the absence of FXR signaling. Furthermore, our data suggest that the attenuation of enterohepatic FGF15 signaling contributes, at least in part, to the elevation of the hepatic lipid levels in Fxr-null mice.

Significant decreases were observed in the hepatic FFA levels, but not in the TG levels in Fxr-null mice treated with a low dose of FGF19 (4 μg/kg/d). Regulation of the hepatic FFA levels might be more sensitive to FGF15/19 signaling than that of the hepatic TG levels. Significant decreases in the hepatic mRNA levels of Cd36, a fatty acid uptake transporter, and Acc1, a lipogenic enzyme, were also observed in Fxr-null mice treated with FGF19 (4 μg/kg/d). These results suggest that FGF19-mediated reduction of hepatic Cd36 and Acc1 expression is possibly involved in the reduction of the hepatic FFA levels in Fxr-null mice treated with a low dose of FGF19 (4 μg/kg/d).

FGF19 treatment increased the hepatic Shp mRNA levels in Fxr-null mice treated with FGF19 (400 μg/kg/d), whereas it decreased the Srebp-1c, Acc1, and Scd1 mRNA levels. We propose that decreases in SREBP-1c-mediated lipogenesis are possibly, at least in part, involved in the reduction of the hepatic TG and FFA levels in Fxr-null mice treated with FGF19 (400 μg/kg/d). Decreased expression of many fatty acid β-oxidation-related genes was observed in Fxr-null mice by treatment with FGF19 (400 μg/kg/d). It is unlikely that fatty acid β-oxidation contributes to the decrease in the hepatic TG and FFA levels in Fxr-null mice treated with FGF19. Recently, it has been reported that fatty acid β-oxidation was decreased in perfused livers from mice infected with FGF15-expressing adenovirus for 3 d. FGF19 may act on the down-regulation of hepatic lipogenesis and hepatic fatty acid uptake, thereby reducing the hepatic TG and FFA levels in Fxr-null mice.

Although recent reports suggest an important role of FGF15/19 signaling in the regulation of hepatic lipogenesis, the underlying mechanism remains largely unknown. In primary hepatocytes, FGF19 suppresses the insulin-mediated elevation of hepatic lipid synthesis. Thus, FGF15/19 signaling may be involved in the regulation of hepatic lipogenesis through an insulin signaling pathway. Conversely, it has been reported that FGF19 activates the insulin-independent endocrine pathway that regulates hepatic protein and glycogen metabolism. FGF19 treatment has been clearly shown to decrease the hepatic TG and FFA levels in Fxr-null mice in which the hepatic insulin signaling pathway was markedly blunted. The relationship between FGF19 and insulin signaling in hepatic lipogenesis remains unclear.

Fxr-null mice show increased hepatic oxidative stress as well as increased the TG and FFA levels. The FGF19 treatment in Fxr-null mice decreased the serum ALT and ALP activities as well as the hepatic TG and FFA levels. Recent studies have indicated the protective role of FGF19 signaling in chemical-induced liver injury. Because FGF15/19 signaling is up-regulated by FXR activation, it probably participates in the protection of hepatic function. The present study raises the possibility of application of the FGF19 signaling pathway to liver disorders, such as non-alcoholic fatty liver...
disease with insulin resistance, since Fxr-null mice seem to be models of the metabolic syndrome with hepatic steatosis. Conversely, Fgf4-null mice exhibited features of the metabolic syndrome that include hyperlipidemia, glucose intolerance, and insulin resistance, whereas Fgfr4-null mice were resistant to hepatic steatosis induced by a high-fat diet.32) In a tetracycline-induced fatty liver model, FGFR4 inhibition reduced the accumulation of hepatic lipids.33) Further studies are necessary to understand the relationship between FGF19/FGFR4 signaling and protection against hepatic steatosis.

In summary, we demonstrated that FGF19 treatment decreased the hepatic TG and FFA levels and the serum ALT activities in Fxr-null mice. We thereby conclude that FGF19 signaling ameliorates disrupted hepatic lipogenesis caused due to the absence of FXR signaling.

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REFERENCES