Hepatoprotective Effects of the Dual Peroxisome Proliferator-Activated Receptor Alpha/Delta Agonist, GFT505, in Rodent Models of Nonalcoholic Fatty Liver Disease/Nonalcoholic Steatohepatitis

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Nonalcoholic fatty liver disease (NAFLD) covers a spectrum of liver damage ranging from simple steatosis to nonalcoholic steatohepatitis (NASH), fibrosis, and cirrhosis. To date, no pharmacological treatment is approved for NAFLD/NASH. Here, we report on preclinical and clinical data with GFT505, a novel dual peroxisome proliferator-activated receptor alpha/delta (PPAR-α/δ) agonist. In the rat, GFT505 concentrated in the liver with limited extrahepatic exposure and underwent extensive enterohepatic cycling. The efficacy of GFT505 was assessed in animal models of NAFLD/NASH and liver fibrosis (Western diet [WD]-fed human apolipoprotein E2 [hApoE2] transgenic mice, methionine- and choline-deficient diet-fed db/db mice, and CCl4-induced fibrosis in rats). GFT505 demonstrated liver-protective effects on steatosis, inflammation, and fibrosis. In addition, GFT505 improved liver dysfunction markers, decreased hepatic lipid accumulation, and inhibited proinflammatory (interleukin-1 beta, tumor necrosis factor alpha, and F4/80) and profibrotic (transforming growth factor beta, tissue inhibitor of metalloproteinase 2, collagen type I, alpha 1, and collagen type I, alpha 2) gene expression. To determine the role of PPAR-α-independent mechanisms, the effect of GFT505 was assessed in hApoE2 knock-in/PPAR-α knockout mice. In these mice, GFT505 also prevented WD-induced liver steatosis and inflammation, indicating a contribution of PPAR-α-independent mechanisms. Finally, the effect of GFT505 on liver dysfunction markers was assessed in a combined analysis of four phase II clinical studies in metabolic syndrome patients. GFT505 treatment decreased plasma concentrations of alanine aminotransferase, gamma-glutamyl transpeptidase, and alkaline phosphatase. Conclusion: The dual PPAR-α/δ agonist, GFT505, is a promising liver-targeted drug for treatment of NAFLD/NASH. In animals, its protective effects are mediated by both PPAR-α-dependent and -independent mechanisms. (Hepatology 2013;58:1941-1952)

Abbreviations: ALT, alanine amino transferase; ALP, alkaline phosphatase; αSMA, alpha smooth muscle actin; AST, aspartate amino transferase; CCL5, chemokine (C-C motif) ligand 5; CoA, coenzyme A; Col1α1, collagen type I, alpha 1; Col1α2, collagen type I, alpha 2; FFAs, free fatty acids; GGT, gamma glutamyl transpeptidase; hApoE2, human apolipoprotein E2; HDL, high-density lipoprotein; HSCs, hepatic stellate cells; IL-1β, interleukin-1 beta; IP, intraperitoneal; IR, insulin resistance; KCs, Kupffer cells; KI, knock-in; KO, knockout; MCD, methionine and choline deficient; MetS, metabolic syndrome; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PPAR, peroxisome proliferator-activated receptor; SD, Sprague-Dawley; TGs, triglycerides; TGF-β, transforming growth factor beta; TIMP, tissue inhibitor of metalloproteinase; TNF-α, tumor necrosis factor alpha; WD, Western diet.
nonalcoholic steatohepatitis (NASH) to fibrosis, and irreversible cirrhosis. NAFLD is frequently observed in patients with central obesity or diabetes and its prevalence is increasing with the epidemics of type 2 diabetes and obesity, such that NAFLD is now the most common liver disease in Western countries. NASH is defined by the presence of steatosis coexisting with hepatic inflammation and hepatocellular injury. Although simple steatosis is generally a benign condition, NASH can have a dire prognosis resulting from concomitant evolving fibrosis and progression to cirrhosis. Patients with NASH have increased liver-related mortality, and NASH-induced cirrhosis can result in end-stage liver disease, including the development of hepatocellular carcinoma.

Efficacious therapeutic agents for the treatment of NASH are lacking. Several pharmacological agents have been studied with the aim of improving insulin sensitivity and reducing the proinflammatory mediators potentially involved in the development and progression of NASH. Unfortunately, they did not show efficacy in large randomized, clinical trials. Insulin-sensitizing agents, such as pioglitazone, and antioxidant agents, such as vitamin E, have shown some promise in improving liver histology in patients with NASH, but the long-term benefit of these medications has not been demonstrated.

The peroxisome proliferator-activated receptors (PPARs) are nuclear receptors that play key roles in the regulation of metabolic homeostasis, inflammation, cellular growth, and differentiation. In type 2 diabetes, PPAR agonists are used as lipid-lowering agents and oral hypoglycemic agents. It has recently been proposed that they may also have liver-protective actions. In the liver, PPAR-α is expressed at high levels in hepatocytes and plays a major role in regulating fatty acid transport and β-oxidation. PPAR-α also modulates gluconeogenesis and inflammatory responses. A protective role for PPAR-α against liver steatosis and inflammation in NASH has been suggested by the increased susceptibility to NASH of PPAR-α knockout (KO) mice. The human apolipoprotein E2 knock-in (hApoE2 KI) mouse is a model of mixed dyslipidemia that develops minimal liver steatosis and inflammation upon Western diet (WD) feeding. In this mouse model, PPAR-α deficiency has also been shown to aggravate liver steatosis and inflammation, indicating a protective role of PPAR-α. Similar to PPAR-α, PPAR-δ also governs hepatic glucose utilization and lipoprotein metabolism and has an important anti-inflammatory activity in the liver through actions in parenchymal and extraparenchymal cells, including Kupffer cells (KCs).

Based on the known functions of PPAR-α and PPAR-δ, a mixed PPAR agonist has the potential to address multiple biological processes involved in the pathogenesis of NASH, as well as the more global-associated metabolic and cardiovascular risk factors. GFT505 is a novel PPAR modulator that shows a preferential activity on PPAR-α and concomitant activity on PPAR-δ. In phase II studies in abdominally obese patients with either combined dyslipidemia or prediabetes, a 1-month treatment with GFT505 (80 mg/day) significantly improved lipid and glucose homeostasis. Moreover, a significant improvement of liver function markers was observed in GFT505-treated patients, illustrated by decreases in gamma-glutamyl transpeptidase (GGT), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) levels. Together, these clinical data suggest the potential of GFT505 for the treatment of NAFLD/NASH associated with metabolic syndrome (MetS).

In the present study, effects of GFT505 were assessed in a range of animal models that reflect NAFLD disease progression, from simple liver steatosis and inflammation (induced by a WD in hApoE2 KI mice) to advanced liver steatosis associated with inflammation (induced by a methionine- and choline-deficient [MCD] diet in db/db mice) and, finally, to chemically induced liver fibrosis in rats. The study also aimed to delineate the liver-protective roles of PPAR-α and PPAR-δ activation by the use of WD-fed hApoE2 KI/PPAR-α KO mice. Finally, a combined analysis of multiple clinical studies on the effects of GFT505 on liver dysfunction markers was performed. Preclinical and clinical results support the therapeutic potential of GFT505 in NAFLD/NASH.

Materials and Methods

For additional details on the materials and methods used, see the Supporting Materials.

Animal Treatments. All formulations of rodent food were supplied by ssniff Spezialdiäten GmbH (Soest,
Germany). hApoE2 KI20 and hApoE2 KI/PPAR-α KO mice16 were fed a WD (TD.88137) for 6 weeks in parallel with daily oral gavage with GFT505 (30 mg/kg) or vehicle only (0.1% Tween 80 and 1% carboxymethyl cellulose in 98.9% distilled water). db/db mice were fed either an MCD diet (TD.90262) or a nutritionally equivalent control diet. Sprague-Dawley (SD) rats were fed a standard rodent chow diet (E15000). Rats received a twice-weekly intraperitoneal (IP) injection of CCl4 (2 mL/kg, 1:2 in olive oil) or olive oil at 2 mL/kg. For the db/db mouse and SD rat experiments, GFT505 was incorporated into the appropriate diet at a percentage corresponding to an estimated dose of 1, 3, 10, or 30 mg/kg/day.

**Clinical Data Analysis.** Atherogenic dyslipidemic, prediabetic, or diabetic patients were treated for periods from 4 to 12 weeks with GFT505 (80 mg/day) or placebo in four phase II clinical trials (ClinicalTrials.gov identifiers: NCT01271751, NCT01275469, NCT01275469, and NCT01271777).

For details of analyses, see the Supporting Materials.

**Statistical Analysis.** Details of statistical analysis can be found in the Supporting Materials.

**Results**

**GFT505 Displays a Hepatotropic Tissue Distribution and Undergoes Extensive Enterohepatic Cycling After Oral Administration.** Tissue distribution of 14C-GFT505 was determined in rats after a single oral administration. Blood and major organs were collected, and radioactivity was measured. High concentrations of GFT505 were measured in the liver (Supporting Fig. 1A). In contrast, GFT505 concentrations were very low in white adipose tissue (Supporting Fig. 1A). In contrast, GFT505 concentrations were very low in white adipose tissue (Supporting Fig. 1A) and undetectable in skeletal muscle.

Biliary excretion and enterohepatic cycling were also examined in rats. A single oral dose of 14C-GFT505 was administered, and bile was collected over a 24-hour period for radioactivity quantification (Supporting Fig. 1B). The majority of radioactivity was excreted in bile (60% of the administered dose during the first 4 hours and 71% over the 24-hour collection period). The 0-4-hour bile samples were injected into the intestine of naïve rats. Bile was collected over a further 24-hour postinjection, and radioactivity was quantified. Once again, a large percentage of radioactivity was found in bile (73% of the dose after 24 hours), demonstrating substantial intestinal reabsorption and enterohepatic cycling of GFT505.

**GFT505 Protects From WD-Induced Fatty Liver in a PPAR-α-Deficient Early Mouse Model of NASH.** The PPAR-α-independent effect of GFT505 (30 mg/kg/day by oral gavage for 6 weeks) on plasma lipids and fatty liver was determined in an early model of NASH deficient for the PPAR-α gene, the WD-fed hApoE2 KI/PPAR-α KO mouse.15 In these studies, groups of PPAR-α-expressing WD-fed hApoE2 KI mice were included for comparison.

Treatment of PPAR-α-expressing hApoE2 KI mice with GFT505 significantly reduced plasma total cholesterol, triglycerides (TGs), and free fatty acids (FFAs) and strongly increased high-density lipoprotein (HDL) cholesterol levels (Fig. 1A-D). In hApoE2 KI/PPAR-α KO mice, GFT505 failed to influence plasma TGs (Fig. 1A). However, in this strain of mice, GFT505 still decreased plasma FFAs and total cholesterol and increased HDL cholesterol, albeit to a lesser extent (Fig. 1B-D). These data suggest that GFT505 may have favorable effects on plasma lipids that are independent of activation of PPAR-α. In contrast, in a similar study, the PPAR-α reference agonist, fenofibrate (100 mg/kg/day), did not show any lipid-modulating effects in hApoE2 KI/PPAR-α KO mice (Supporting Fig. 2A-D).

As expected in rodents exposed to a PPAR-α agonist,11 GFT505 significantly increased liver weight in hApoE2 KI mice, but not in hApoE2 KI/PPAR-α KO mice (Fig. 1E), illustrating the hyperresponsiveness of rodents to PPAR-α-induced peroxisomal proliferation and hepatomegaly. Similar findings were observed with fenofibrate (data not shown).

Microscopic examination of livers revealed both macro- and microsteatosis in WD-fed hApoE2 KI/PPAR-α KO mice, whereas PPAR-α-expressing hApoE2 KI mice were relatively resistant to WD-induced steatosis (Fig. 2A-C). In hApoE2 KI/PPAR-α KO mice, GFT505 administration reduced both diet-induced macro- and microsteatosis (Fig. 2A-C) and significantly reduced circulating levels of the liver dysfunction markers, aspartate aminotransferase (AST) and ALT (data not shown). Interestingly, GFT505 reduced WD-induced increased cellularity in sinusoids (KCs) in both hApoE2 KI and hApoE2 KI/PPAR-α KO mice (Fig. 2D). In contrast, fenofibrate had no effect on cellularity of sinusoids in ApoE2 KI/PPAR-α KO mice (Supporting Fig. 2E,F). These results suggested that GFT505 has liver-protective effects through combined PPAR-α-dependent and -independent mechanisms.

In hApoE2 KI mice, GFT505 provoked a significant reduction in hepatic expression of proinflammatory genes, such as interleukin-1 beta (IL-1β) and tumor necrosis factor alpha (TNF-α), the macrophage marker, F4/80, and the fibrosis genes, transforming growth factor beta (TGF-β) and tissue inhibitor of metalloproteinase (TIMP)–2 (Supporting Table 2). In
hApoE2 KI/PPAR-α KO mice, these genes were also reduced by GFT505, with significant down-regulation of additional profibrosis markers, such as collagens (Supporting Table 2). In contrast, fenofibrate significantly reduced the expression of proinflammatory and profibrotic genes in hApoE2 KI mice, but had little effect in hApoE2 KI/PPAR-α KO mice. In keeping with PPAR-α agonist-induced hepatomegaly in rodents (Fig. 1E), GFT505 and fenofibrate strongly increased the hepatic expression of the peroxisomal genes, acyl-CoA (coenzyme A) oxidase and enoyl-CoA hydratase/3-hydroxyacyl CoA dehydrogenase in hApoE2 KI, but not in hApoE2 KI/PPAR-α KO mice (Supporting Table 2).

In another experiment performed in WD-fed hApoE2 KI/PPAR-α KO mice, the pure PPAR-γ agonist, rosiglitazone, had no effect on inflammatory and fibrosis gene expression, whereas the pure PPAR-δ agonist, GW501516, showed a similar profile to GFT505 (Supporting Table 2). These results further suggest that, in hApoE2 KI/PPAR-α KO mice, GFT505 likely acts through activation of PPAR-δ in the liver.
GFT505 Prevents the Development of MCD Diet-Induced Steatohepatitis. To evaluate the effect of GFT505 on later stages of fatty liver disease, we next studied a model of advanced steatosis with strong inflammation induced by an MCD diet. In two independent experiments, insulin-resistant db/db mice were fed the MCD diet for 7 weeks and concomitantly treated with vehicle or 1, 3, 10 (experiment 1), or 30 mg/kg/day (experiment 2) of GFT505.

The MCD diet provoked a significant increase of plasma ALT levels, associated with intrahepatic accumulation of cholesterol and TGs (Fig. 3A-C). Upon histological examination, a marked macrovesicular steatosis induced by MCD diet feeding was accompanied by increased inflammation and weak fibrosis (Fig. 4A-D). In mice concomitantly treated with GFT505, intrahepatic cholesterol and TG content were significantly reduced in a dose-dependent manner to reach levels comparable to those in mice fed the control diet (Fig. 3B,C).

Microscopic examination showed that GFT505 administration at 10 mg/kg/day completely prevented MCD diet-induced macrovesicular steatosis and inflammation (Fig. 4B,C). The weak hepatic fibrosis observed in MCD diet-fed mice was not significantly reduced by GFT505 treatment (Fig. 4D). Consistent with liver protection by GFT505, plasma ALT activity was reduced to levels comparable to the control diet group (Fig. 3A), and liver weight was also significantly reduced (Fig. 3D).

In a study performed at 30 mg/kg/day of GFT505 and giving similar results, transcriptomic analyses
showed that the MCD diet-induced increased expression of hepatic inflammatory and profibrosis genes (IL-1β, TNF-α, TGF-β, and collagens) was blocked by GFT505 (Supporting Table 3). Moreover, hepatic expression of macrophage markers CD11b and F4/80 was significantly decreased by GFT505 treatment (Supporting Table 3).

**GFT505 Prevents CCl4-Induced Liver Fibrosis in SD Rats.** The effect of GFT505 on liver fibrosis was studied in a rat model induced by repeated IP injections of CCl4. Rats were injected with CCl4 or vehicle twice-weekly for 7 weeks, with parallel oral treatment with 30 mg/kg/day of GFT505 or vehicle. CCl4 administration induced a strong liver fibrosis with the formation of collagen bridges between veins (Fig. 5A), associated with an increased number of macrophages (KCs; Fig. 5B) and activated hepatic stellate cells (HSCs) expressing alpha smooth muscle actin (αSMA; Fig. 5C). These histological changes were accompanied by a significant increase in hepatic collagen, as measured by hydroxyproline content (Fig. 5E).

GFT505 treatment prevented CCl4-induced fibrosis, as demonstrated by the significantly decreased fibrotic surface (−54% versus CCl4 control group; Fig. 5A,D) and hepatic collagen content (Fig. 5E), and the reduced quantity of macrophages (Figure 5B) and activated HSCs (Fig. 5C). In keeping with the histological findings, expression of hepatic genes involved in the inflammatory response and fibrosis development (e.g., TGF-β, collagens, TIMP-2, or αSMA) was strongly reduced by GFT505 (Table 1). Other genes involved in the inflammatory response, but not induced by CCl4 injection (IL-1β and chemokine [C-C motif] ligand 5 [CCL5]), were also down-regulated by GFT505 treatment (Table 1).

**GFT505 Reverses Established CCl4-Induced Liver Fibrosis in SD Rats.** To assess the effect of GFT505 on the progression of established hepatic fibrosis,
fibrosis was induced in rats by twice-weekly CCl₄ injections for 2 weeks. GFT505 (30 mg/kg/day) or vehicle was then orally administered for 4 weeks to animals concomitantly with continued CCl₄ injections. Alternatively, CCl₄ injections were discontinued and GFT505 was orally administered to animals for 1 or 2 further weeks.

Microscopic quantification of fibrosis demonstrated that GFT505 stopped the progression of established liver fibrosis (Fig. 6A) and accelerated liver recovery (Fig. 6B). In both these studies, GFT505 treatment reversed the up-regulation of genes involved in the inflammatory and profibrotic response (Table 1).

**GFT505 Reduces Plasma Levels of Liver Markers in Humans.** The clinical efficacy of GFT505 has been evaluated in MetS patients in four independent phase II clinical studies. In these studies, GFT505 treatment significantly reduced circulating levels of the liver dysfunction markers, ALT, GGT, and ALP (Fig. 7A-C). Quartile analysis demonstrated that, for all three parameters, the effect size of GFT505 was greater for patients with the highest baseline values.

**Discussion**

The present study describes the effects of oral administration of GFT505 in experimental NAFLD/NASH rodent models of increasing severity. GFT505 is a dual PPAR-α/δ modulator that has previously demonstrated therapeutic efficacy on plasma lipids, insulin resistance (IR), and glucose homeostasis while decreasing inflammatory markers and liver enzymes.¹⁹ In addition, its pharmacokinetics profile of liver targeting and extensive enterohepatic cycling makes GFT505 an ideal candidate for the treatment of liver disease.
The MCD diet-fed rodent is a well-recognized animal model of steatohepatitis. In the present study, MCD diet-fed db/db mice treated with GFT505 were protected against the development of liver steatosis and inflammation. Moreover, GFT505 treatment prevented intrahepatic lipid accumulation, reduced liver enzymes, and repressed liver expression of proinflammatory and -fibrotic genes. GFT505 also had both prophylactic and curative effects on CCl4-induced liver fibrosis in rats. The antifibrotic effect of GFT505 correlated with a concomitant repression of proinflammatory and profibrotic genes in the liver. The relative contribution of PPAR-\(\gamma\) and PPAR-\(\delta\) to the liver-protective effects of GFT505 was examined in dyslipidemic hApoE2 KI and hApoE2 KI/PPAR-\(\gamma\) KO mice, which develop liver steatosis and inflammation when fed a WD (this study and an earlier study\(^{16}\)). Interestingly, GFT505 reduced WD-induced steatosis in hApoE2 KI/PPAR-\(\gamma\) KO mice, as well as reducing cellularity in sinusoids and hepatic expression of inflammatory markers in both mouse strains. Moreover, the protective effect of GFT505 on the expression of profibrotic genes was more pronounced in livers of hApoE2 KI/PPAR-\(\gamma\) KO mice, suggesting that GFT505 exerts liver-protective
effects that likely involve the activation of PPAR-δ. This hypothesis is further supported by the demonstration that the pure PPAR-δ agonist, GW501516, exerts similar effects in hApoE2 KI/PPAR-α KO mice.

The exact mechanism(s) of the liver-protective effects of GFT505 and the relative roles of PPAR-α and PPAR-δ activation remain to be clearly elucidated. However, studies using rodent models of liver disease converge toward a beneficial effect of PPAR-α in preventing steatosis, inflammation, and fibrosis. PPAR-α is highly expressed in rodent hepatocytes, where it prevents TG accumulation through induction of genes involved in mitochondrial and peroxisomal fatty acid β-oxidation. Moreover, the PPAR-α agonist, Wy-14,643, showed similar liver protective effects as GFT505 in MCD diet-fed C57BL/6 mice. Recently,

Table 1. Effects of GFT505 (30 mg/kg/day) on Hepatic Gene Expression After Preventive, Curative, and Reversion Treatments of SD Rats Injected With CCl4

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time (weeks)</th>
<th>IL-1β</th>
<th>TGF-β</th>
<th>Coll1α1</th>
<th>Coll1α2</th>
<th>TIMP-2</th>
<th>CCL5</th>
<th>αSMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevention</td>
<td>7 Ctrl</td>
<td>1.00 ± 0.46</td>
<td>1.00 ± 0.44</td>
<td>1.00 ± 1.13</td>
<td>1.00 ± 0.48</td>
<td>1.00 ± 0.49</td>
<td>1.00 ± 0.45</td>
<td>1.00 ± 0.41</td>
</tr>
<tr>
<td></td>
<td>CCl4</td>
<td>1.17 ± 0.58</td>
<td>2.65 ± 0.60</td>
<td>18.93 ± 11.41</td>
<td>5.54 ± 2.94</td>
<td>2.51 ± 1.59</td>
<td>0.75 ± 0.43</td>
<td>2.16 ± 1.10</td>
</tr>
<tr>
<td></td>
<td>CCl4 + GFT505</td>
<td>0.44 ± 0.15§</td>
<td>1.02 ± 0.49§</td>
<td>7.51 ± 7.99§</td>
<td>2.14 ± 1.71§</td>
<td>1.37 ± 0.94</td>
<td>0.39 ± 0.12³</td>
<td>1.02 ± 0.68</td>
</tr>
<tr>
<td>Curation</td>
<td>2+4 Ctrl</td>
<td>1.00 ± 0.45</td>
<td>1.00 ± 0.14</td>
<td>1.00 ± 0.23</td>
<td>1.00 ± 0.32</td>
<td>1.00 ± 0.22</td>
<td>1.00 ± 0.27</td>
<td>1.00 ± 0.61</td>
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<tr>
<td></td>
<td>CCl4</td>
<td>0.96 ± 0.49</td>
<td>1.33 ± 0.75§</td>
<td>10.99 ± 8.52§</td>
<td>5.81 ± 4.78§</td>
<td>2.23 ± 1.82</td>
<td>0.76 ± 0.16</td>
<td>2.30 ± 1.27</td>
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<tr>
<td></td>
<td>CCl4 + GFT505</td>
<td>0.38 ± 0.27§</td>
<td>0.39 ± 0.18§</td>
<td>2.57 ± 3.95§</td>
<td>1.24 ± 1.50§</td>
<td>0.55 ± 0.40§</td>
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<tr>
<td>Reversion</td>
<td>2+1 Ctrl</td>
<td>1.00 ± 0.32</td>
<td>1.00 ± 0.12</td>
<td>1.00 ± 0.78</td>
<td>1.00 ± 0.46</td>
<td>1.00 ± 0.31</td>
<td>1.00 ± 0.47</td>
<td>1.00 ± 0.32</td>
</tr>
<tr>
<td></td>
<td>CCl4</td>
<td>1.00 ± 0.39</td>
<td>1.24 ± 0.46</td>
<td>6.09 ± 7.19</td>
<td>2.84 ± 2.82</td>
<td>1.22 ± 0.70</td>
<td>1.30 ± 0.81</td>
<td>1.87 ± 1.57</td>
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<tr>
<td></td>
<td>CCl4 + GFT505</td>
<td>0.46 ± 0.28§</td>
<td>0.52 ± 0.10§</td>
<td>1.03 ± 0.70</td>
<td>0.71 ± 0.38</td>
<td>0.51 ± 0.19§</td>
<td>0.51 ± 0.16³</td>
<td>0.65 ± 0.21³</td>
</tr>
<tr>
<td></td>
<td>2+2 Ctrl</td>
<td>1.00 ± 0.33</td>
<td>1.00 ± 0.16</td>
<td>1.00 ± 0.34</td>
<td>1.00 ± 0.26</td>
<td>1.00 ± 0.28</td>
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<tr>
<td></td>
<td>CCl4</td>
<td>1.08 ± 0.26</td>
<td>1.12 ± 0.24</td>
<td>4.84 ± 4.87</td>
<td>2.16 ± 1.19³</td>
<td>1.37 ± 0.29³</td>
<td>1.31 ± 0.68</td>
<td>1.69 ± 0.69</td>
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<tr>
<td></td>
<td>CCl4 + GFT505</td>
<td>0.25 ± 0.11§</td>
<td>0.42 ± 0.21§</td>
<td>3.16 ± 4.20</td>
<td>1.12 ± 1.14</td>
<td>0.62 ± 0.42</td>
<td>0.22 ± 0.07³</td>
<td>0.83 ± 0.82</td>
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In the preventive protocol, rats were concomitantly injected with CCl4 and treated with GFT505 for 7 weeks. For the curative protocol, rats were pretreated with CCl4 for 2 weeks, followed for another 4 weeks with CCl4 injections and parallel GFT505 oral treatment. For the reversion protocol, rats were pretreated with CCl4 for 2 weeks, then CCl4 injections were discontinued and rats were orally treated with GFT505 or vehicle for a further 1 or 2 weeks. Values are expressed as fold change versus control group ± standard deviation; n = 6-12/group.

*P < 0.05; †P < 0.01; §P < 0.001 versus control (Ctrl). ¶P < 0.05; ††P < 0.01; §§P < 0.001 versus CCl4.

Abbreviations: Coll1α1, collagen type I, alpha 1; Coll1α2, collagen type I, alpha 2.

Fig. 6. GFT505 reverses established CCl4-induced liver fibrosis in SD rats. (A) For the curative protocol, rats were pretreated with olive oil (control) or CCl4 for 2 weeks, followed for another 4 weeks with CCl4 injections and parallel GFT505 (30 mg/kg/day) or vehicle treatment. Liver fibrosis was visualized by Masson’s trichrome staining, and liver fibrotic surface was quantified on 10 fields/animal. **P < 0.001, CCl4 at 2 + 4 weeks versus 2 weeks; ***P < 0.01 and ****P < 0.001 versus CCl4 at 2 + 4 weeks. (B) For the recovery protocol, rats were pretreated with olive oil (control) or CCl4 for 2 weeks, then CCl4 injections were discontinued and rats were treated with GFT505 (30 mg/kg/day) or vehicle for a further 1 or 2 weeks. Liver fibrosis was visualized by Picro-Sirius Red staining, and liver fibrotic surface was quantified on 10 fields/animal. **P < 0.001 versus olive oil at 2 weeks; *P < 0.05 and **P < 0.001 versus CCl4 plus vehicle at 2 + 1 or 2 + 2 weeks.
Wy-14,643 was also shown to improve steatosis and liver injury in high-fat–fed foz/foz diabetic/obese mice and decrease the number of infiltrating macrophages and neutrophils. Because PPAR-α is not expressed in rat KCs or in rodent HSCs, the anti-inflammatory and antifibrotic effects of pure PPAR-α agonists in rodents likely result from a cross-talk between parenchymal and nonparenchymal cells.

The liver-protective role of PPAR-δ activation is increasingly documented. In wild-type mice, the PPAR-δ agonist, KD3010, but, surprisingly, not GW501516, has protective effects against liver fibrosis induced by CCl4 injection or bile duct ligation. In contrast, GW501516 ameliorated hepatic steatosis and inflammation by an improvement in lipid metabolism and inhibition of inflammation in an MCD diet-induced mouse model. Similar to PPAR-α, PPAR-δ may contribute to the prevention of liver steatosis by stimulating hepatic fatty acid β-oxidation. In addition, PPAR-δ plays a role in KCs by regulating the polarization of classical proinflammatory M1 to alternative anti-inflammatory M2 macrophages. Indeed, mice deficient for PPAR-δ in hematopoietic cells display increased hepatosteatosis, with increased lipogenic gene expression and decreased anti-inflammatory M2 markers. PPAR-δ is also highly expressed in HSCs, and its expression is strongly induced during stellate cell activation and liver fibrogenesis. Taken together, these data suggest that both the PPAR-α and PPAR-δ activity of GFT505 may participate in its beneficial effects on steatosis and inflammation, whereas their role in fibrosis through effects on HSC activation remains to be clarified.

It is also possible that intestinal effects of GFT505 contribute to its hepatoprotective role in NAFLD/NASH. Indeed, PPAR-α activation in the intestine by agonists such as GFT505 has recently been shown to contribute to increased HDL production, indicating a potential role for intestinal PPAR-α in the regulation of whole-body lipoprotein metabolism. In view of its extensive enterohepatic cycling, GFT505 activation of PPARs in both the intestine and liver thus results in an improved lipid profile that would be beneficial in dyslipidemic NASH patients.

The PPARs have been proposed as targets of interest to treat NAFLD/NASH. Pilot studies with the thiazolidinediones in patients with NASH demonstrated improvements of IR, liver enzymes, and liver fat, but variable results on histological NASH features such as cellular injury, liver inflammation, and fibrosis. In two larger studies performed in patients with biopsy-proven NASH, long-term treatment with pioglitazone led to clear metabolic and liver histological improvement, but did not significantly improve fibrosis. Human studies performed with marketed PPAR-α agonists have generated inconsistent results on NAFLD/NASH. In a prospective study in patients with NASH, gemfibrozil demonstrated favorable effects on liver enzymes, whereas fenofibrate showed variable results. No PPAR-δ agonist is clinically available at present. However, treatment of overweight dyslipidemic patients with the PPAR-δ agonist, MBX-8025, for 8 weeks led to a reduction in liver enzymes. Moreover, after 2 weeks of treatment in moderately obese men, the PPAR-δ agonist, GW501516, reduced liver fat content by 20%, in conjunction with reductions in plasma GGT levels.

To assess the potential of GFT505 to ameliorate liver dysfunction associated with MetS, its effects on plasma markers of liver dysfunction were evaluated.
after 4-12 weeks of treatment at 80 mg/day in four independent phase II clinical studies performed in dyslipidemic, prediabetic, insulin-resistant, and/or diabetic patients. Quartile analysis showed that GFT505 significantly lowered liver dysfunction markers, such as ALT, GGT, and ALP. To confirm the therapeutic potential of GFT505 on histological features of NASH, a phase IIb study (ClinicalTrials.gov identifier: NCT01694849) in biopsy-proven NASH patients is currently ongoing.

In conclusion, together with its favorable effects on hepatic and peripheral insulin sensitivity, glucose homeostasis, and lipid metabolism, the present study shows the potential therapeutic effect of GFT505 for NASH treatment. By activating both PPAR-α and PPAR-δ, GFT505 acts on key cellular mechanisms involved in NAFLD/NASH pathogenesis, including TG accumulation, extracellular matrix synthesis, and inflammation. Furthermore, the specific distribution profile of GFT505, which accumulates predominantly in the liver, may play an important role in its beneficial efficacy profile.

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