Synthetic Farnesoid X Receptor agonist PX20606 demonstrates anti-atherosclerotic effects and lowers cholesterol in HDL₂ but not in HDL₃ subfractions

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Abstract

The Farnesoid X Receptor (FXR, NR1H4) is a member of the nuclear receptor superfamily that functions as an endogenous sensor for bile acids and regulates – amongst other effects on metabolism – cholesterol and fatty acid metabolism. We studied the impact of different FXR agonists on cholesterol homeostasis, plasma lipoprotein profiles and transhepatic cholesterol efflux. Habruman et al., J Pharmacol Exp Ther. 2012 Aug 23 epub.

In C57Bl/6J mice on high fat diet, the synthetic FXR agonists FXR-450 and PX20606 demonstrated potent plasma cholesterol lowering activity that affected all lipoprotein species, whereas GW4066 and 6-ECDDA showed only limited effects. In FXR wildtype but not FXR–/– mice the more efficacious FXR agonists increased fecal cholesterol excretion and reduced intestinal cholesterol (re)uptake. Further we examined the effect of FXR activation on the development of atherosclerosis. In CETPtg LDLR–/– mice FXR agonist PX20606 potently lowered plasma total cholesterol as well as HDL cholesterol. Despite the apparent HDL lowering, PX20606 caused a highly significant decrease in atherosclerotic plaque size. FXR activation by PX20606 and 6-ECDDA led to similar cholesterol reductions in normolipidemic Cynomolgus monkeys. Lipoprotein separation by ultracentrifugation showed that the same FXR agonist Px20606 affects the cholesterol-rich HDL₃ subclass and shows anti-atherosclerotic effects in a new and highly interesting finding and sheds a new light on the FXR effects on HDL lowering which are so far perceived as a major limitation for clinical development of FXR agonists.

High fat high cholesterol diet animal model

Study Protocol: 6-week-old male C57BL/6J or FXR–/– mice (n=10) were maintained on a High Fat (HF) diet (Ssniff: 60 kcal% fat, 20 kcal% carbohydrate, 20 kcal% protein) designed as a high fat high cholesterol (HFD) diet. Animals were on this diet for 4 weeks and the upper 75% of animals in terms of body weight were randomly assigned into 3 treatment groups. The test compounds were gavaged once daily into the diet and homogenised.

Effect on Plasma total Cholesterol and Triglycerides:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Total Cholesterol (mg/dL)</th>
<th>Triglycerides (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>vehicle</td>
<td>200 ± 5</td>
<td>150 ± 10</td>
</tr>
<tr>
<td>PX20606 (50 mg/kg)</td>
<td>150 ± 5</td>
<td>100 ± 10</td>
</tr>
<tr>
<td>6-ECDDA (10 mg/kg)</td>
<td>180 ± 5</td>
<td>120 ± 10</td>
</tr>
</tbody>
</table>

Summary

• Treatment with different FXR agonists in HFHC diet fed C57Bl/6J mice resulted in different pharmacological effects on
  - Reduction of plasma cholesterol and triglycerides
  - HDL-derived cholesterol clearance into feces
  - Induction of liver target genes involved in the transport of cholesterol from the periphery via the liver into intestines.

In CETPtg LDLR–/– mice those differences between i.e. PX20606 and 6-ECDDA seem to account for the differences in aortic plaque formation in the CETPtg LDLR–/– mouse model. While PX20606 caused a significant decrease in atherosclerotic plaque area treatment with 6-ECDDA showed no effect.

In normolipidemic Cynomolgus monkeys a similar cholesterol reduction was observed upon PX20606 treatment. Suggesting that the basic mechanisms of FXR-mediated HDL cholesterol clearance are conserved in mice and non human primates. The depletion of HDL cholesterol with unchanged absolute levels of ApoAI induced by a synthetic FXR agonist is a novel finding

The concerted regulation of different physiological mechanisms upon FXR activation thus might be a means to increase the overall flux through the reverse cholesterol pathway by opening the drainage from HDL particles into feces, a mechanism so far not being addressed pharmacologically.

Atherosclerosis animal model

Study Protocol:

- C57BL/6J mice were fed a Western diet (21% milk fat, 0.15% cholesterol) containing PX20606 (5 or 15 mg/kg) or 6-ECDDA (10 mg/kg) for 16 weeks. Animals were sacrificed after a 4-hour fasting.
- CETPtg LDLR–/– mice were fed a Western diet (21% milk fat, 0.15% cholesterol) containing PX20606 (5 or 15 mg/kg) or 6-ECDDA (10 mg/kg) for 16 weeks. Animals were sacrificed after a 4-hour fasting.

Analysis of HDL subfractions:

- Pooled plasma samples of male or female Cynomolgus monkeys after four weeks of p.o. gavage treatment with PX20606 (20, 50 or 100 mg/kg) were separated by ultracentrifugation.

Summary

- ApoAI and cholesterol content of fractions after ultracentrifugation
  - Plasma radioactivity after single i.v. injection of [14C]-cholesterol and [3H]-sitostanol into C57BL/6J or FXR transgenic mice

Literature

