Synthetic Farnesoid X Receptor agonist PX20606 demonstrates anti-atherosclerotic effects and lowers cholesterol in HDL_2 but not in HDL_3 subfractions

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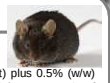
Abstract

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 *(*Hambruch 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 GW4064 and 6-ECDCA 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-ECDCA led to similar cholesterol reductions in normolipidemic Cynomolgus monkeys. Lipoprotein separation by ultracentrifugation showed that PX20606 specifically lowered HDL_{2c} but not HDL_{3c} nor ApoAI protein.
That the same FXR agonist Px20606 affects the cholesterol-rich HDL_2 subclass and shows anti-atherosclerotic effects is a new and highly interesting finding and sheds a new light on the FXR effects on HDLc lowering which are so far perceived as a major limitation for clinical development of FXR agonists.

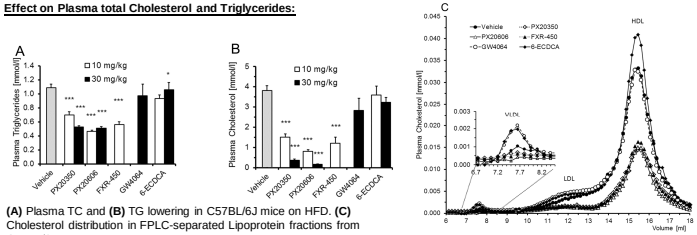
High fat high cholesterol diet animal model



Study Protocol:

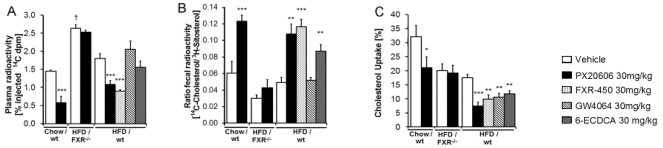
8 week old male C57BL/6J or FXR $^{-/-}$ mice (n=10) were maintained on a High Fat (HF) diet (Ssniff :60 kcal% fat) plus 0.5% (w/w) cholesterol designated as High fat High cholesterol (HFHC) diet. Animals were prefed on this diet for 4 weeks and the upper 75% of animals in terms of FPG, TC, TG and body weight were then randomised into treatment groups. The test compounds were ground with mortar and pestle into the diet and homogenized.

Effect on Plasma total Cholesterol and Triglycerides:



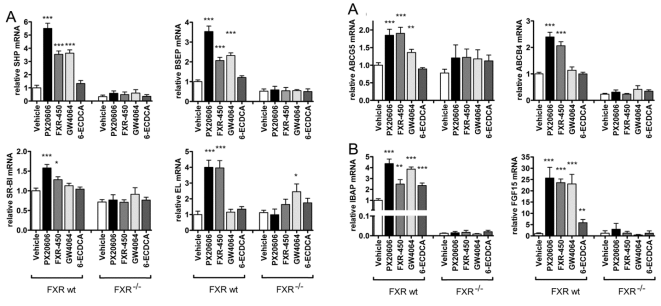
(A) Plasma TC and **(B)** TG lowering in C57BL/6J mice on HFD. **(C)** Cholesterol distribution in FPLC-separated Lipoprotein fractions from C57BL/6J on HFD.

Cholesterol uptake and Efflux experiments:



(A) Plasma radioactivity after single i.v. injection of [14C]-cholesterol and [3H]-sitostanol into C57BL/6J or $FXR^{-/-}$ mice. **(B)** Ratio of fecal radioactivity of [14C]-cholesterol over [3H]-sitostanol.
(C) Fraction cholesterol absorbed in [%] 72 h after an oral gavage of [3H]-cholesterol and single i.v. injection of [14C]-cholesterol into C57BL/6J or $FXR^{-/-}$ mice.
Values are expressed as mean ± SEM. $^{***}p<0.001$, $^{**}p<0.01$, and $^{*}p<0.05$.$^{\dagger}p<0.05$ for comparison of vehicle-treated wt to $FXR^{-/-}$ mice on the same HFD.

Gene Regulation:

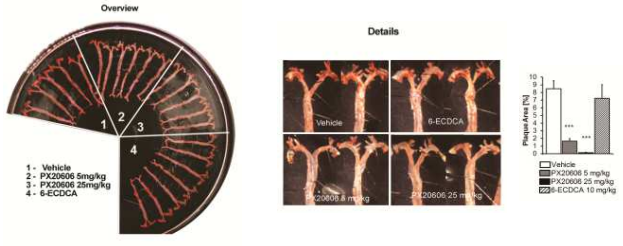


Tissues were snap frozen and ground to fine powder. FXR-triggered gene expression in **(A)** liver and **(B)** ileum was measured using Quantitative-real time PCR (Taqman®) analysis. Values are expressed as mean ± SEM. $^{***}p<0.001$, $^{**}p<0.01$, and $^{*}p<0.05$.

Atheriosclerosis animal model

Study Protocol:

CETPtg-$LDLR^{-/-}$ mice were generated by breeding CETPtg mice with $LDLR^{-/-}$ mice both on a C57BL/6J background. Starting at eight weeks of age, male CETPtg; $LDLR^{-/-}$ mice were fed a Western diet (21% milk fat and 0.15% cholesterol) containing PX20606 (5 or 25 mg/kg) or 6-ECDCA (10 mg/kg) for 16 weeks. Animals were sacrificed after a 4-hour fasting.

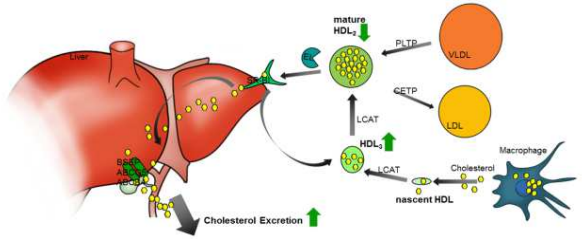


Sudan IV stain of aortae from FXR agonist or vehicle treated CETPtg-$LDLR^{-/-}$ mice together with the results of the quantitative image analysis depicted as plaque loaded area as % of total aortic area.
Values are expressed as mean ± SEM. $^{***}p<0.001$, $^{**}p<0.01$, and $^{*}p<0.05$

Summary

- Treatment with different FXR agonists in HFHC diet fed C57Bl/6J mice resulted in different pharmacogical 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 live into intestine
- In CETPtg-LDLR-/- mice these differences between i.e. PX20606 and 6-ECDCA seem to account for the differences in aortic plaque formation in the CETPtg LDLR–/– mouse model. While PX20606 caused a significant decrease in arteriosclerotic paque area treatment with 6-ECDCA 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_2 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.

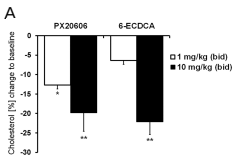


Normolipidemic Cynomolgus monkeys

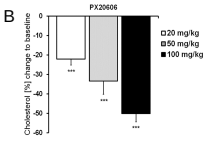
Study Protocol:

Cynomolgus monkeys (male/female, age 2- 3 yrs., body weight 2-3 kg) which were maintained on primate growth and maintenance feed under standard conditions for non-human primate animal husbandry (AAALAC guidance) were allocated to either protocol A of dose-escalation (n=6 male monkeys per group, 1 and 10 mg/kg treatment) or to protocol B for continuous 28 days testing (n=3 male and 3 female animals per dosing group). In both protocols, the test compounds were administered by oral gavage at the indicated doses after suspending them in standard vehicle [0.5% (w/v) Polyvinylpyrrolidone, 0.1% (v/v) Tween 80, in PBS pH 7.4].

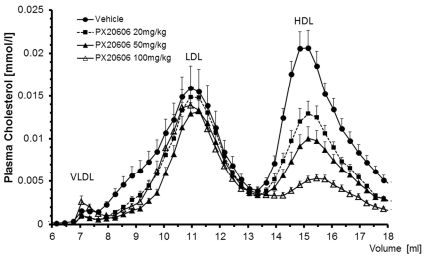
Plasma Cholesterol and Lipoprotein profile:



(**A**) Plasma total cholesterol lowering in *Cynomolgus* monkeys after one week of p.o. gavage treatment with PX20606 or 6-ECDCA.



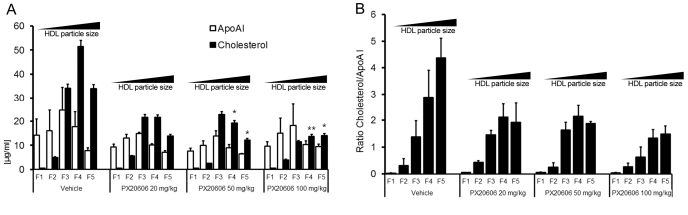
(**B**) Plasma total cholesterol lowering in *Cynomolgus* monkeys after 28 days of p.o. gavage treatment with PX20606.



(C) Cholesterol distribution in FPLC-separated Lipoprotein fractions from *Cynomolgus* monkeys after four weeks of p.o. gavage treatment with PX20606 at the indicated doses. Each plot represents mean values of three male and three female animals.

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 100mg/kg) were separated by ultracentrifugation.



ApoAI and cholesterol content of fractions after ultracentrifugation (A). Ratio of cholesterol normalized to ApoAI protein amount (A). Values are expressed as mean ± SEM. $^{***}p<0.001$, $^{**}p<0.01$, and $^{*}p<0.05$

Literature

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