FXR agonist Px-102 improves hepatic steatosis in NAFLD mouse models

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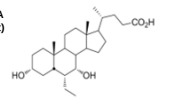
Abstract

FXR (NR1H4), a nuclear bile acid receptor, plays a major role in controlling hepatic lipid, cholesterol and carbohydrate metabolism (Ref 1,2). Currently FXR agonists are in advanced clinical studies as a novel pharmacotherapy for liver diseases such as NAFLD, NASH or Primary Biliary Cirrhosis (PBC).
We have developed a novel synthetic FXR agonist, Px-102, which displays drug-like pharmacokinetics in various animal models and humans. Px-102 is now entering Phase II clinical studies to determine whether this FXR agonist can reduce hepatic lipid accumulation in human NAFLD patients. Thus, we sought to analyze the impact of this drug in comparison to the other clinical stage FXR agonist 6-Ethyl CDCA (6-ECDCA or Obeticholic Acid) on liver lipid metabolism, hepatic steatosis and plasma clinical chemistry in mouse models of Metabolic Syndrome and NAFLD.
C57bl/6J mice were prefed on a High Fat High Cholesterol diet (HFHCD) or on a High Fat High Cholesterol diet plus Fructose (HFHCD+Fructose) and then treated with Px-102 and 6-ECDCA for either 2 weeks or 4 months. Px-102 and, to a lesser extent, 6-ECDCA showed substantial plasma cholesterol and triglyceride lowering paralleled by a massive reduction of hepatic triglycerides and cholesterol. Px-102 reduced the expression of SREBP-1c and Malic Enzyme 1 both of which are involved in hepatic de novo lipidogenesis, but at the same time increased expression of Fatty Acid Synthase (FAS) and genes involved in cholesterologenesis. Thus, it is unlikely that FXR agonists reduce steatosis only by liver-centric reduction of lipogenesis pathways but potentially also by downregulating intestinal lipid uptake.

In summary, Px-102, a novel synthetic FXR agonists improves Metabolic Syndrome associated liver steatosis and hyperlipidosis indicating that this drug might be a good candidate to treat human NAFLD.

FXR agonists and other compounds used in this study

6-Ethyl CDCA (FXR-Agonist) = 6-ECDCA



Px20606 (FXR Agonist) = Px-102

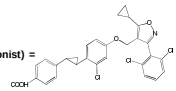


Fig 1: Human FXR assay data

	FRET		GAL4 reporter	
	EC50 [nM]	Efficacy [%]	EC50 [nM]	Efficacy [%]
6-ECDCA	90	98	150	95
GW4064	20	100	35	100
Px-102	100	103	85	80

Aim & Purpose :

Since we aim in developing an FXR agonist, Px-102 (old research code = Px20606) for the treatment of NAFLD and NASH, we investigated if Px-102 was able to reduce liver steatosis in mouse models of metabolic syndrome and fatty liver disease such as C57/bl6 mice on various High Fat Diets or CETPtg-LDLR$^{(-/-)}$ mice on a Western diet. All these animal models present with a significant degree of liver steatosis and varying degrees of plasma hyperlipidemia and hypercholesterolemia.

Px-102 (Px20606) has been tested in two Phase I studies in healthy volunteers and shows excellent human pharmacokinetics and no signs of enterohepatic circulation unlike the 6-ECDCA which is known to circulate for repeated cycles. 6-ECDCA (also called Obeticholic Acid) is an FXR agonist in advanced clinical development for Primary Biliary Cirrhosis (PBC) and NASH (Ref 3,4).

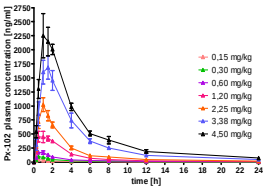


Fig 2: Human Px-102 plasma pharmacokinetics after single dose exposure ranging from 0,15 mg/kg to 4,5 mg/kg

Materials & Methods:

	„HFD model"	„HFD + Fructose model"	„Athero model"	„NOD model"
Mouse strain	C57bl/6J	C57bl/6J	CETPtg-LDLR$^{-/-}$	NOD
Diet	High fat high Cholesterol (HFHCD)	High fat high Cholesterol plus Fructose (HFHCD + Fructose)	Western diet	High fat diet (HFD)
Treatment period	2 weeks	4 months	3 months	2 weeks
Results shown in figures	Fig 3; 4; 7	Fig 3; 4; 6	Fig 3; 4	Fig 3; 4; 5

Additional Details:

HFD and HFD + Fructose model- Male C57bL/6J mice were purchased from Elevage Janvier (Rennes, France) at the age of 8 weeks. Animals were fed either a Surwit high-fat diet (HFD) (E15771, Ssniff, Germany) with 60 kcal% from fat plus 0.5% (w/w) extra cholesterol. Mice were prefed 4 weeks before treatment. In case of high fat – high fructose diet, drinking water was additionally supplemented with 42 g fructose/L (Ref 5). Px-102 or 6-ECDCA were given at the doses indicated by grinding the compounds into the high fat diet.
Athero model - B6.CBA-Tg(CETP)5203Tall/J)were obtained from the Jackson Laboratory. CETPtg-LDLR$^{-/-}$ mice were generated by breeding CETPtg mice with LDLR$^{-/-}$ mice on the same C57bl/6J background. At the age of 8 weeks mice were fed a Western diet (TD88137, containing 21% milk fat and 0.15% cholesterol, Harlan Teklad). Px-102 or 6-ECDCA were given at the doses indicated by grinding the compounds into the high fat diet.
NOD model – male NOD mice were prefed on a Surwit high-fat diet (HFD) (E15771, Ssniff, Germany) with 60 kcal% from fat plus 0.5% (w/w) extra cholesterol. Daily gavage treatment with FXR agonists started after 2 weeks on HFHCD and continued for 2 weeks.

Radiolipid uptake – Mice were given an oral bolus of a lipid based vehicle containing 40 µCi/kg [^{3}H]-labeled Triolein and 20 µCi/kg [^{14}C]-cholesterol. Additionally mice received an injection of Tyloxapol to prevent fast fading of [^{3}H] label due to lipoprotein lipase activity.

Conclusions:

FXR agonists such as Px-102 or 6-ECDCA exert potent plasma and liver cholesterol and triglyceride lowering effects in all tested mouse models. This correlates well with an improvement in liver histology. However, the exact mechanism of liver lipid reduction is unclear because some key enzymes for lipidogenesis are repressed (SREBP-1c, ME1) others are upregulated (Insig1 and 2, FAS, HMGCoR, Cyp 51). It might be that the cause for lipid reduction is extrahepatic and the dramatic plasma lipid lowering causes liver lipid depletion and induction of lipidogenesis at the same time.

It remains to be proven in human clinical trials whether these effects observed in mouse models will hold true in a human setting.

Results:

(1) FXR agonists reduce plasma cholesterol and triglycerides in C57bl/6J mice on HFD

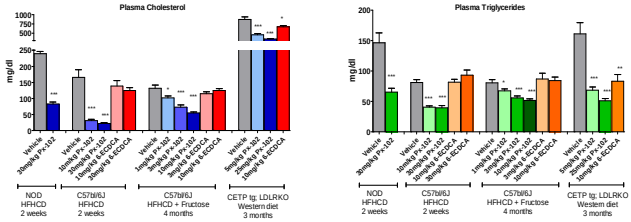


Fig 3: Time course of plasma cholesterol and triglycerides in different mice models on HFD, treated with various doses Px-102, 6-ECDCA or vehicle

(2) FXR agonists reduce liver cholesterol and triglycerides

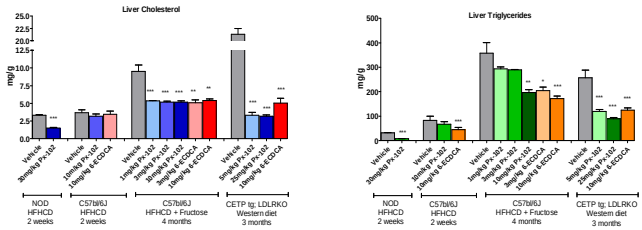
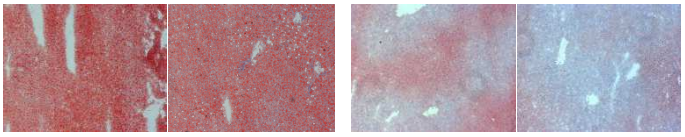


Fig 4: Liver cholesterol and triglycerides after 2 weeks of Px-102 treatment in C57bl/6J mice or 16 weeks Px-102 and 6-ECDCA treatment in C57bl/6J mice or and 12 weeks treatment in CETPtg-LDLR$^{(-/-)}$ mice.

(3) FXR agonists improve liver histology



HFHCD vehicle | HFHCD 30mg/kg Px-102

Fig 5: Reduction in liver triglycerides shown by OilRedO stains of NOD mice treated for 2 weeks with 30mg/kg Px-102.

(4) FXR agonist treatment results in mixed regulation of lipido- and cholesterologenesis enzymes

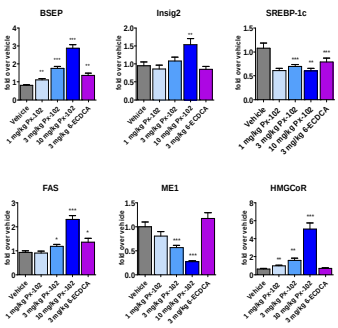


Fig 6: Gene expression analysis in the livers of C57bl/6J mice after 4 months on HFHCD+Fructose

(5) FXR agonists reduce lipid uptake after oral bolus

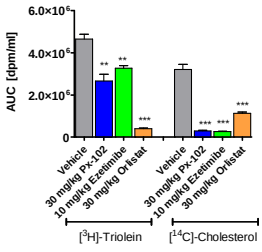


Fig 7: AUC (0-72h) of dpm counts in mouse plasma. After receiving a radiolipid bolus with [^{3}H]-labeled Triolein and [^{14}C]-labeled cholesterol.

References:

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