Novel LXR inverse agonists demonstrate anti-steatotic effects in human hepatocytes and in rodent models of NAFLD

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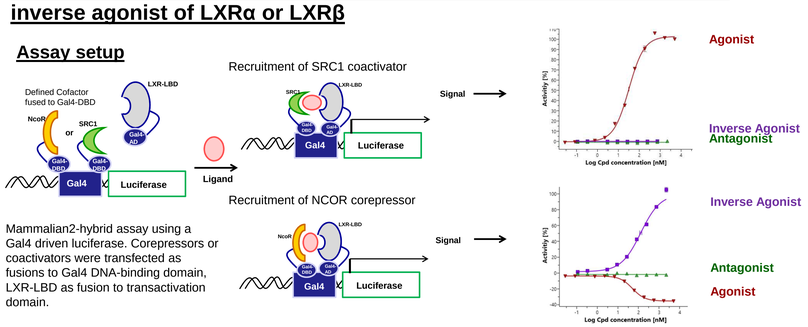
BACKGROUND

Several mechanisms are currently evaluated as potential pharmacotherapies for the spectrum of non-alcoholic fatty liver disease (NAFLD), including modulators of nuclear receptors such as PPARα/γ/δ or FXR. Activation of Liver X Receptor (LXR) in the liver by potent, synthetic agonists is known to result in severe steatosis and hypertriglyceridemia in various animal models[1,2] and in humans[3]. Thus, we have designed and synthesized LXR inverse agonists with the aim to inhibit LXR´s pro-steatotic transcriptional activity. The pharmacological effects of these LXR inverse agonists were evaluated in human hepatocytes and in a mouse and a rat steatosis model. These first results confirm the findings by another group[4,5], that synthetic LXR inverse agonists can reduce liver fat content which may provide a new mechanism for the treatment of NAFLD / NASH.

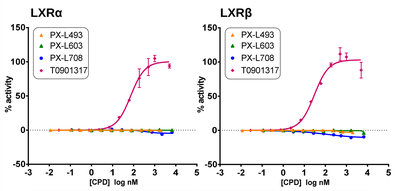
IN VITRO ACTIVITIES

Cellular reporter assay differentiating between agonist, antagonist and inverse agonist of LXRα or LXRβ

Assay setup

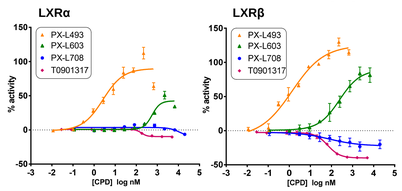


Recruitment of SRC1 coactivator peptide



SCR1 recruitement	PX-L493	PX-L603	PX-L708	T0901317
AC_{50} [nM] LXRalpha	inactive	inactive	inactive	72.7
AC_{50} [nM] LXRbeta	inactive	inactive	inactive	29.9

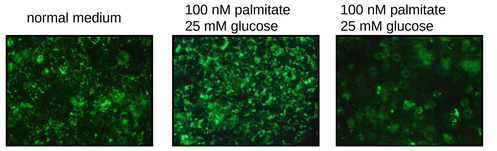
Recruitment of NCOR coactivator peptide



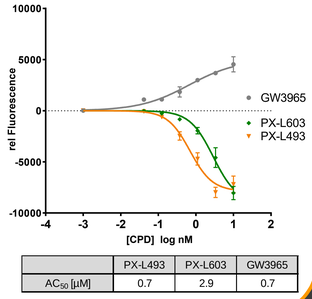
NCOR recruitement	PX-L493	PX-L603	PX-L708	T0901317
AC_{50} [nM] LXRalpha	5.3	966	inactive	177.1
AC_{50} [nM] LXRbeta	1.4	326	inactive	66.2

Compounds PX-L493 and PX-L603 were characterized in cellular reporter assays as inverse agonists of LXRα and LXRβ [AC_{50} for LXR(α/β) in NCoR recruitment mammalian 2-hybrid assay: PX-L493 (5.3/1.4 nM); PX-L603 (966/326 nM)]

Anti steatotic effect in primary human hepatocytes



Human primary Upcyte hepatocytes were cultivated in the presence of 100 nM palmitate and 25 mM glucose for 5 days. The addition of LXR inverse agonists PX-L603 and PX-L493 dose-dependently reduced the hepatocyte lipid load as determined by Bodipy staining and fluorescence measurements.

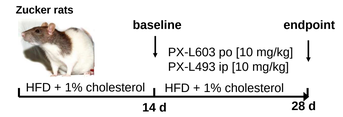


	PX-L493	PX-L603	GW3965
AC_{50} [µM]	0.7	2.9	0.7

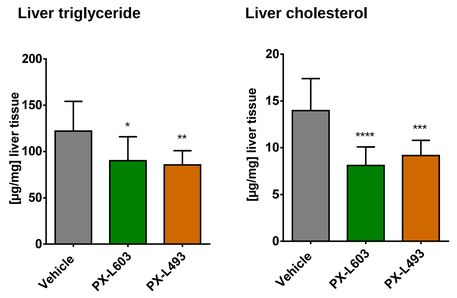
RODENT STUDIES

LXR inverse agonists show liver lipid lowering in high fat diet fed Zucker fa/fa rats

Study design

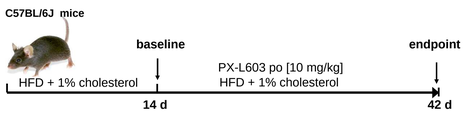


Zucker (fa/fa) rats were maintained on a Survit-type high fat diet with 1% cholesterol for two weeks. 10 mg/kg LXR inverse agonists or vehicle were administered for the following 2 weeks on the same diet.
Analysis of the hepatic lipid content showed a significant reduction of triglycerides as well as total cholesterol in the livers of animals treated with PX-L603 or PX-L493 compared to vehicle treatment.

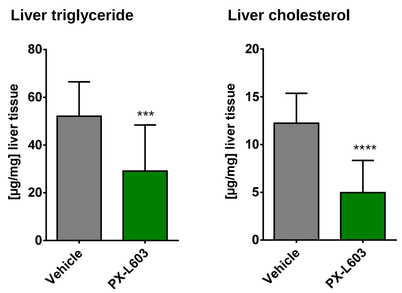


LXR inverse agonists show liver lipid lowering in high fat diet fed mice

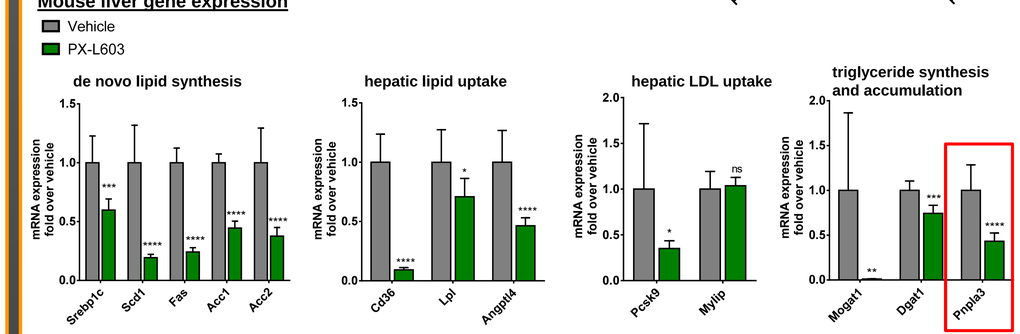
Study design



C57Bl/6 mice were maintained on a Survit-type high fat diet with 1% cholesterol for two weeks. PX-L603 (10 mg/kg, po) LXR inverse agonists or vehicle were administered for the following 4 weeks on the same diet.
Hepatic triglyceride content was significantly reduced from 52.1(±19.3) µg/mg to 29.1 (±14.4) µg/mg. Total cholesterol in the livers of animals treated with PX-L603 was 5.0 (±3.4) µg/mg compared to 12.2 (±3.1) µg/mg of vehicle treated animals.

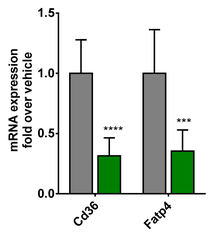


Mouse liver gene expression



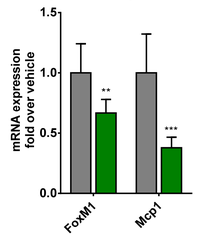
Gene expression analysis of the liver tissue from the mouse study revealed that LXR inverse agonist PX-L603 repressed known LXR target genes involved in fatty acid synthesis and lipid homeostasis such as Scd1, Fas, Cd36 or Angptl4.
It is noteworthy, that PX-L603 treatment reduced the mRNA expression of **Pnpla3** with high significance. Pnpla3 expression is highly associated with human NAFLD development.

Intestinal lipid absorption



Gene expression analysis of mouse intestinal segments revealed that LXR inverse agonist PX-L603 repressed Cd36 and Fatp4 mRNA expression. Both are genes invovled in intestinal lipid uptake.

Gene regulation beyond steatosis



LXR also modulates genes beyond lipid control:
PX-L603 repressed the proliferative cell cylce control gene FoxM1 as well as the proinflammatory cytokine Mcp1 (Ccl2) in the HFD mouse liver.

PK DATA

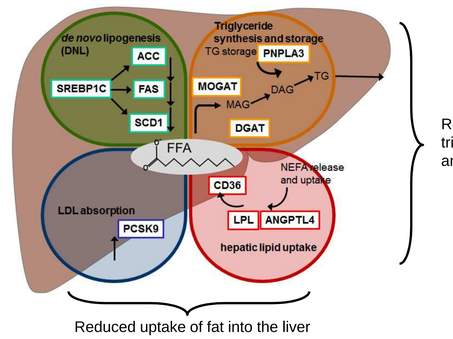
PK / ADME properties of LXR inverse agonists PX-L603

Animal Species	mouse	
Strain	C57BL/6J	
Gender	male	
Dose route	iv	po
Dosage (mg/kg)	5	20
$T_{1/2}$ (h)	7.0	4.4
F (%)	100	6
Vz (L/kg)	1.2	0.7
CL (ml/(min*kg))	2.0	2.0

- oral bioavailable
- metabolically stable (RLM $T_{1/2}$ 180 min)
- low clearance
- hepatotropic characteristic

CONCLUSIONS

Anti-Steatotic effects of LXR inverse agonist



Inhibition of LXR´s transcriptional activity by synthetic inverse agonists results in:

- Inhibition of *de novo* lipogenesis (DNL)
- Reduction of FFA release from chylomicrons and reduced FFA uptake
- Reduced triglyceride synthesis through downregulation of Mogat and Dgat
- Downregulation of Pnpla3 expression, an enzyme with proven clinical significance in NASH patients

→ ultimately resulting in reduced liver fat.

→ This opens a new avenue for the exploration of LXR inverse agonists for the treatment of NAFLD / NASH.

CONTACT INFORMATION

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