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

Ulrich Deuschle, Christian Gege, Olaf Kinzel, Thomas Schlüter, Johannes Fabian, Desiree Helen Krol, Manfred Birkel, Eva Hambrouch, Claus Kremoser*  
Phenex Pharmaceuticals AG, Heidelberg, Germany. * presenting author Dr. Claus Kremoser, email: claus.kremoser@phenex-pharma.com

**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 PPARγ or FXR. Activation of Liver X Receptor (LXR) in the liver by potent, synthetic agonists is known to result in severe steatosis and hyperglycemia in various animal models and in humans. 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 rat steatosis model. These first results confirm the findings by another group, 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**

- Complementary and modified assay using a Gq-coupled luciferase reporter gene containing the LXR response element.
- LXRβ at saturation to transactivate the reporter.

**Recruitment of SRC1 coactivator peptide**

**Recruitment of NCOR coactivator peptide**

Compounds PX-L493 and PX-L603 were characterized in cellular reporter assays as inverse agonists of LXRα and LXRβ ([6], for LXRβ in NGC reporter mammalian 2-hybrid assay: PX-L493 (5.3±1.4 μM); PX-L603 (966±326 μM).

**Anti steatotic effect in primary human hepatocytes**

Normal medium

- 100 mM palmitate 25 mM glucose
- 100 mM palmitate 35 mM glucose

2 μM LXR inverse agonist

**Intestinal lipid absorption**

**Gene expression analysis**

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

**Liver triglyceride and cholesterol parallel**

- Liver triglyceride and cholesterol levels were significantly reduced in PX-L603 treated mice compared to vehicle treated animals.

**Gene regulation beyond steatosis**

LXR also modulates genes beyond lipid control.

- PX-L603 repressed the proinflammatory cytokine Mip1α as well as the proinflammatory cytokine Ccl5 (Cx2) in the HFD mouse liver.

**CONCLUSIONS**

- 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 Pnpl3 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.

**REFERENCES**