LXR inverse agonists inhibit de novo lipogenesis and reduce intestinal lipid and cholesterol absorption in a NAFLD mouse model

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THE INTERNATIONAL LIVER CONGRESS™ 2018

APRIL 11-15 PARIS FRANCE

THU-495

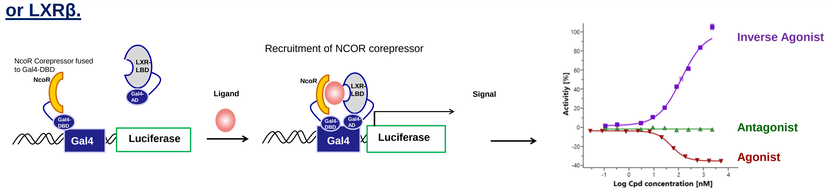
INTRODUCTION

Nuclear receptors LXR alpha and beta are both involved in the control of de novo lipogenesis (DNL) and of lipid and fatty acid uptake in various tissues including small intestine and liver.

We have developed potent synthetic LXR inverse agonists, PX-L25593 (5593) and PX-L25788 (5788), representing two different structural classes, which block transcriptional activity of both LXRs. Both compounds display anti-steatotic effects in a mouse NAFLD model. Through stable and radioactive isotope labeling we could demonstrate that these effects are exerted through a combined inhibition of liver and intestinal de novo lipogenesis which also results in a reduced intestinal lipid and cholesterol uptake. This offers a new opportunity to address NAFLD clinically through LXR inverse agonists.

IN VITRO ACTIVITIES & PK

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

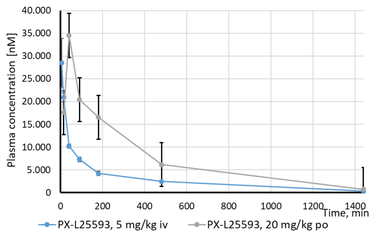


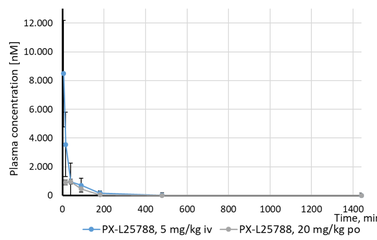
Mammalian2-hybrid assay using a Gal4 driven luciferase. Corepressors or coactivators were transfected as fusions to Gal4 DNA-binding domain, LXR-LBD as fusion to transactivation domain.

Cpd. #		5788	5593
Cellular Gal4 M2H (NCOR) [nM/%eff]	LXR α	7.9 / 159	2.0 / 122
	LXR β	8.6 / 132	1.2 / 115

PX25593 and PX25788 were both characterized as potent synthetic LXR inverse agonists (LXRα/β EC50s: '593= 2nM/1.2nM; '788 =17nM/19nM)

PK properties were established in C57BL/6J mice for LXR inverse agonists 5593 and 5788.





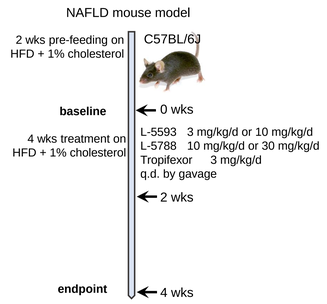
PK properties	5593		5788	
	5 mg/kg iv	20 mg/kg po	5 mg/kg iv	20 mg/kg po
t1/2, h	5.6	4.8	1.3	1.2
AUC 0-t, µM*h	68.0	176.2	4.7	23.3
AUC 0-inf_obs, µM*h	71.0	181.3	4.7	31.4
Cl_obs, L/Kg/h	0.12		1.7	
Vss_obs, L/Kg	0.71		1.5	
absolute bioavailability	64%		9%	

Exposure	5593	5788
Liver exposure	44 µM (4h aa of 20 mg/kg)	1.3 µM (4h aa of 20 mg/kg)
Liver/blood ratio	Ratio: 3	Ratio: 34
	non liver selective	liver selective

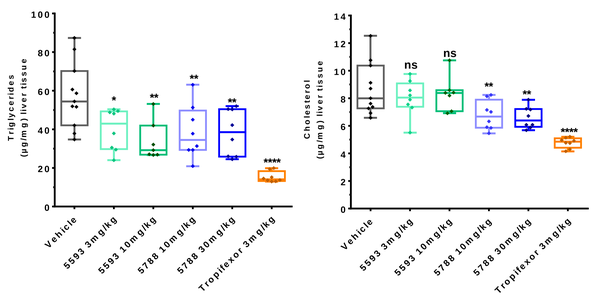
A key difference between these compounds is their distribution between liver and plasma.[liver]/[plasma] ratio at 4 h after oral administration: 5593=3 and 5788=34. Hence, 5788 can be regarded as a liver-selective LXR inverse agonist, whereas 5993 has a longer blood residence time.

IN VIVO RESULTS I

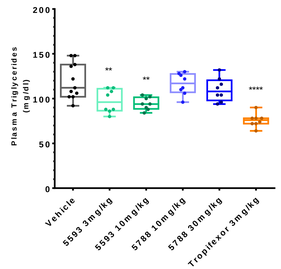
HFD study: C57BL/6J mice which were prefed on a 60%kcal Survit-type high fat diet for 14 days and then dosed with PX25593 (3 and 10 mg/kg/d), PX25788 (10 and 30 mg/kg/d) and a clinical stage potent FXR agonist (Tropifexor, 3 mg/kg/d) for 28 days.



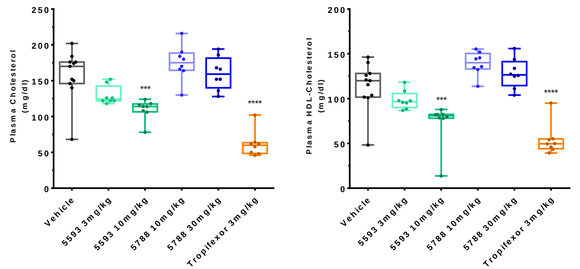
Liver triglycerides and cholesterol reduction



Plasma triglycerides

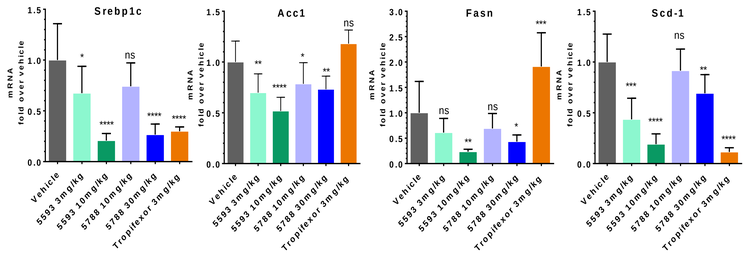


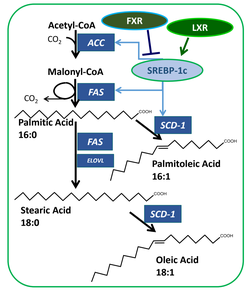
Plasma total cholesterol and HDL cholesterol



Liver triglycerides were reduced by all compounds (5593 3mg/kg: -33%±7; 5593 10mg/kg -41%±7; 5788 10mg/kg -31%±10; '788 30mg/kg -30%±8; Tropifexor -73%±2). Plasma lipid and lipoprotein analysis yielded a differential reduction in HDLc by the treatments (5593 3mg/kg: -13%±3; 5593 10mg/kg -35%±8; 5788 10mg/kg +24%±4; 5788 30mg/kg +14%±5; Tropifexor -52%±6). Plasma triglyceride reduction was observed to varying extents (5593 3mg/kg: -19%±11; 5593 10mg/kg -21%±6; 5788 10mg/kg -3%±10; 5788 30mg/kg +8%±10; Tropifexor -37%±7).

Impact of LXR on *de novo* lipogenesis



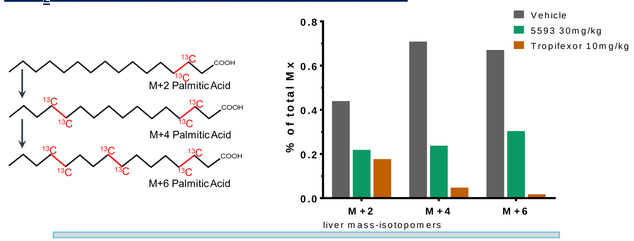


Gene expression analysis in liver tissue after 4 weeks of treatment showed reduction in crucial genes for hepatic DNL.

IN VIVO RESULTS II

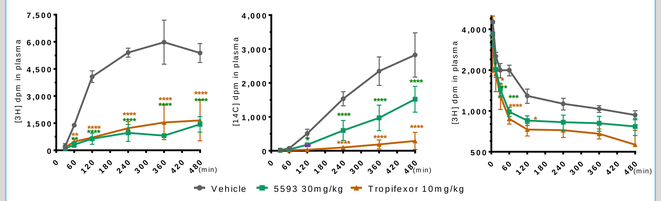
Short term mechanistic study in C57BL/6J mice with four days treatment on HFD + 1% cholesterol with LXR inverse agonist 5593 and Tropifexor as comparator. Animals were given $[^{13}C]_2$-acetate in drinking water to asses hepatic DNL. $[^{13}C]_2$-labelled fatty acids from liver tissue were identified by MIDA (mass isotope distribution analysis).

$[^{13}C]_2$-acetate incorporation as a proxy for DNL



To asses intestinal uptake of triglycerides and cholesterol, animals were given an oral bolus of [^{3}H]-triolein and [^{14}C]-cholesterol. For cholesterol excretion, animals were injected with [^{3}H]-cholesterol. After application, plasma samples were taken over 8 h and radioactivity was counted.

[^{3}H]-Triolein intestinal uptake | **[^{14}C]-Cholesterol intestinal uptake** | **[^{3}H]-Cholesterol plasma (excretion)**



LXR inverse agonists as well as Tropifexor reduced intestinal lipid and cholesterol uptake as well as inhibited liver-borne DNL.

CONCLUSIONS

LXR inverse agonists, similar to FXR agonists are capable of reducing liver fat in animal models. Like FXR agonists they reduced DNL in intestine and liver and markedly reduced intestinal lipid and cholesterol uptake. Thus, LXR inverse agonists might offer a new treatment option for NAFLD/NASH which potentially lacks the FXR-associated liabilities like HDLc lowering

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