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PXL770, a Novel Direct AMPK Activator, Inhibits Hepatic *de novo* Lipogenesis for the Treatment of Metabolic Disorders

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Background

- Most patients with type 2 diabetes have additional cardiovascular risk factors such as excess body weight, insulin resistance, lipid abnormalities and/or non-alcoholic fatty liver diseases (NAFLD)[1]
- Adenosine monophosphate-activated protein kinase (AMPK) is a key enzyme involved in energy metabolism.[2] It regulates energy metabolism by affecting energy-consuming pathways such as *de novo* lipogenesis (DNL), which includes fatty acid and cholesterol synthesis, as well as energy-producing pathways such as lipid oxidation and glucose uptake[3]
- PXL770 is a new oral anti-hyperglycemic agent that directly activates AMPK, thus improving glycemic control and decreasing blood and liver lipids as well as body weight[4]
- PXL770 has been shown to decrease lipogenesis and more particularly DNL. This suggests that PXL770 could play an important role in the management of patients with type 2 diabetes and a high cardiovascular risk profile

Objectives

The aim of this study was to evaluate the effects of PXL770 on hepatic DNL.

Research Methods

Effect of PXL770 on lipogenesis in human and mouse primary hepatocytes

- Primary mice hepatocytes were isolated by a modified version of the collagenase method[5] from randomly selected, fed, adult mice
- Hepatocytes were incubated for 3 h with 0.6 µCi/mL [1-^{14}C] acetate in M199 medium with various concentrations of PXL770 (0.1, 1, 3, 10, 30, or 100 µM) and reference compounds, such as A-769662 (30 µM, a reference drug as a direct AMPK activator) or 5-tetradecyloxy-2-furoic acid (TOFA; 10 µM, a competitive inhibitor of acetyl-CoA carboxylase (ACC))
- Incorporation of [1-^{14}C]-acetate into saponifiable lipid fractions to probe fatty acid synthesis was then monitored
- The same method was used to investigate the effect of PXL770 on cryopreserved human hepatocytes

Effect of PXL770 on lipogenesis in wild-type (WT) and AMPKα1α2-null hepatocytes

- WT and AMPKα1α2-null primary hepatocytes were incubated for a total of 20 h in M199 medium-containing antibiotics, 10% fetal bovine serum, 100 nM dexamethasone, and either 5 mM glucose (basal condition) or 25 mM glucose + 100 nM insulin (lipogenic condition) with one of the following compounds: PXL770 (10, 25, 50, or 100 µM), 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR; 100 µM), A-769662 (30 µM) or cerulenin (a fatty acid synthase inhibitor; 25 µM)
- Intracellular triglyceride (TG) content was measured by assay using a commercial kit (Dyasis) after an acetone extraction step

Effect of PXL770 on hepatic DNL in C57BL/6J mice in vivo

- C57BL/6J mice were fasted for 24 h and then re-fed a high-carbohydrate diet for 12 h to stimulate hepatic DNL. They were then given vehicle, 35 mg/kg or 75 mg/kg PXL770 three times orally during the fasting/re-feeding period (Figure 1)
- A 24-h-fasted and vehicle-treated mice group (n=5) was also included in the study as a low-lipogenesis control
- The hepatic rate of lipid synthesis was assessed by measuring the incorporation of ^3H_2O into hepatic lipids 1 hour after the intraperitoneal injection of 0.25 mL physiological saline containing 150 µCi ^3H_2O (Figure 1)
- Liver samples were saponified and lipids were extracted using a modified Folch method.[6] The radioactivity of ^3H_2O in the extracted lipids was quantified by liquid scintillation counting. Rates of fatty acid synthesis were calculated as numbers of ^3H incorporated into lipids per milligram of liver per hour

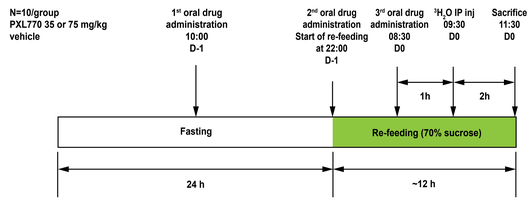

Figure 1. Study design for effect of PXL770 on hepatic DNL in C57BL/6J mice
IP inj, intraperitoneal injection

Results

PXL770 dose-dependently decreased DNL in human and mouse hepatocytes

- A significant dose-dependent inhibition of [1-^{14}C]-acetate incorporation was observed in human and mouse hepatocytes treated with PXL770 (Figure 2, 3a and 3b)
- PXL770 induced a significant inhibition (P<0.001) of fatty acid synthesis reaching –40%, –43%, –70%, –99%, –100% at 1, 3, 10, 30 and 100 µM, respectively (Figure 2). At 0.1 µM PXL770, lipogenesis was not inhibited (Figure 2)
- A-769662 (30 µM, a direct activator of AMPK), showed an inhibition in fatty acid synthesis of –80%, which is comparable to 10 µM PXL770. Thus, PXL770 appears to inhibit lipogenesis with a higher potency than the reference compound A-769662 (Figure 2)
- Hepatocytes treated with 10 µM TOFA, a competitive inhibitor of a key enzyme of lipogenesis (acetyl-CoA carboxylase), showed an inhibition of fatty acid of –75% that was similar to that seen with 10 µM PXL770 (Figure 2)
- Fatty acid synthesis was inhibited in human and mouse primary hepatocytes with an IC50 of 3 µM and 2.8 µM, respectively (Figure 3a and 3b)

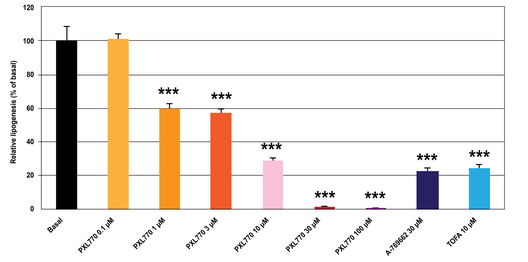

Figure 2. PXL770 dose-dependently decreases DNL in primary mouse hepatocytes
TOFA, 5-tetradecyloxy-2-furoic acid
***P<0.001 vs Basal condition

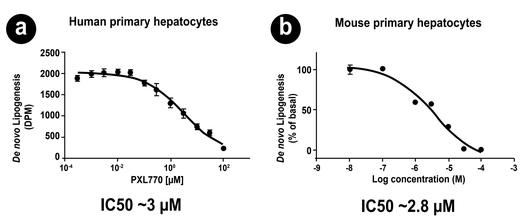

Figure 3a and 3b. PXL770 dose-dependently decreases DNL in cryopreserved primary human hepatocytes and primary mouse hepatocytes
FA, fatty acid; DPM, disintegrations per minute

PXL770 inhibits DNL in an AMPK-dependent manner

- In both WT and AMPKα1α2-null hepatocytes, TG accumulation was highly increased after incubation with 25 mM glucose + 100 nM insulin compared with 5 mM glucose, reflecting a similar capacity for increasing lipogenesis (Figure 4a and 4b)
- TG accumulation was abolished in both WT and AMPKα1α2-null hepatocytes in the presence of cerulenin, a fatty acid synthase inhibitor, indicating that the increase in intracellular TG content is due to stimulation of lipogenesis (Figure 4a and 4b)
- In WT hepatocytes, TG accumulation was decreased (P<0.05) in a dose-dependent manner, achieving –10%, –18%, –26% and –35% at 10, 25, 50 and 100 µM of PXL770, respectively (Figure 4a)
- In parallel, the reference drugs AICAR (100 µM) and A-769662 (30 µM) decreased TG accumulation by –55% and –35%, respectively (data not shown)
- In contrast, in AMPKα1α2-null hepatocytes the effects of PXL770 and the reference drugs AICAR (100 µM) and A-769662 (30 µM) were severely blunted, demonstrating an AMPK-dependent action of these compounds on lipogenesis inhibition (Figure 4b)

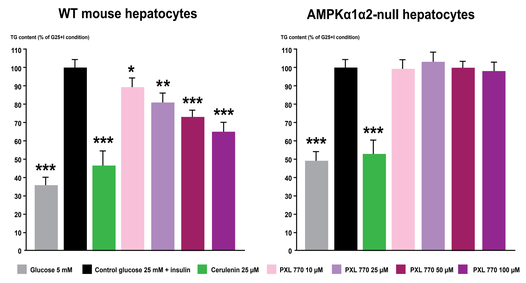

Figure 4a and 4b. Intracellular TG accumulation was decreased in a dose-dependent manner in WT hepatocytes but not in AMPKα1α2-null hepatocytes
TG, triglyceride; WT, wild-type
*P<0.05, **P<0.01, ***P<0.001 compared to control glucose 25 mM + insulin alone

PXL770 strongly decreases hepatic DNL in C57BL/6J mice

- Comparison between the fasted control group and the fasted then re-fed control group showed a 15-fold induction of ^3H_2O incorporation in the liver, indicating a strong stimulation of hepatic DNL induced by the fasting/re-feeding transition
- There was a dose-dependent reduction in the incorporation of ^3H_2O into lipids in the liver of fasted/re-fed mice treated orally with PXL770 compared with control fasted/re-fed mice (–45%, P=0.0022 and –71%, P=0.000079 after 35 mg/kg and 75 mg/kg PXL770, respectively) (Figure 5)

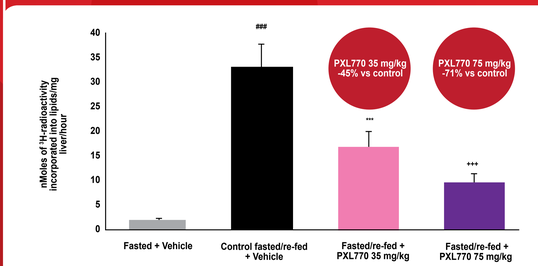

Figure 5. 35 mg/kg and 75 mg/kg PXL770 decreases liver DNL in C57BL/6J mice
###P<0.001 compared with control fasted mice; ***P=0.00222 compared with control fasting/re-fed mice; +++P=0.000079 compared with control fasting/re-fed mice

Conclusion

- PXL770 is a potent inhibitor of hepatic DNL, as demonstrated *in vivo* by the strong reduction of ^3H_2O incorporation into lipids in the liver of C57BL/6J mice. These results are consistent with the decrease in fatty acid synthesis in response to PXL770 measured *in vitro* in human and mouse primary hepatocytes
- PXL770 inhibition of hepatic DNL occurs through an AMPK-dependent pathway
- These data provide evidence for the therapeutic potential of PXL770 in hepatic lipid metabolism disorders, such as NAFLD
- PXL770 may be a promising treatment for patients with type 2 diabetes, particularly for those with high cardiovascular risk. This will need to be confirmed in a clinical setting during the development of PXL770

Conflicts of Interest

Sophie Hallakou-Bozec and Sebastien Bolze are Poxel employees.

Professor Michael Roden is a member of the Poxel scientific advisory board.

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