NOVEL GPR40 AGONIST CPL-207-280 INDEPENDENTLY IMPROVES GLYCAEMIA AND MITIGATES NEUROPATHIC PAIN IN DIABETIC RODENTS.

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INTRODUCTION

GPR40 is a receptor for medium and long free fatty acids (FFA), which mediates signal through Gaα proteins. It is mostly expressed in pancreas and central nervous system (CNS). Activation of it in β-cells of pancreas in early T2D may improve glycemic control through enhancement of glucose-stimulated insulin secretion (GSIS). This results from activation of PLC, which gives rise to IP3 and DAG production followed by Ca2+ release from the intracellular stores and PKC activation, respectively. This mechanism ensures amplification of insulin secretion only at the activating glucose concentrations thereby minimizes the risk of hypoglycemia. The similar signaling pathway has been proposed to operate in neurons regulating pain sensation, constituting endogenous pain inhibitory system in CNS. Activation of GPR40 by endogenous lipids in supra-spinal area has recently been shown to ameliorate chronic pain sensation in various pathology states. Neuronal pain receptors possess up to 20% of diabetic population and is present in more than 50% of patients with neuropathy. It may occur already in pre-diabetic state manifesting itself commonly by allodynia, which transforms innocuous stimuli into painful experiences. Thus, the expression pattern of the receptor opens an opportunity for potential GPR40 agonists to act on the frontiers of both diabetes and its complication – neuropathy.

The most developed and evaluated in clinical studies GPR40 synthetic agonist was TAK-875, which proved effective secretagogue, but its development was terminated during the 3rd phase due to toxic effects in the liver. We designed and synthesized a novel, low molecular, GPR40 specific agonist aiming to overcome TAK-875’s drawback. We studied its capacity to improve GSIS in Wistar and diabetic ZDF rats, tested its safety in the liver cells and finally interrogated its effect on allodynia in STZ-treated diabetic mice.

RESULTS

Fig. 1. Efficacy of CPL-207-280 and TAK-875 in activation of GPR40 and effects on insulin secretion in vitro
(A) Schematic presentation of the mechanism, through which activation of GPR40 enhances GSIS. (B) CPL-207-280 is more potent to liberate intracellular Ca2+ than TAK-875. (C) Cells stably overexpressing human GPR40 were treated with ascending concentrations of tested agonists and intracellular Ca2+ efflux was assessed based on luminescence before binding to counterstained with [Ca2+]i-labeled cells. (D) CPL-207-280 is a stronger secretagogue than TAK-875. Met cells were treated with 20μM glucose in Krebs buffer in the presence of different concentrations of agonists and spontaneous was protocoted for insulin. *, p<0.05, **, p<0.01, *** p<0.001, **** p<0.0001

Fig. 2. Effects of test compounds in hepatocytes. (A) Dose dependent test effect of agonists in hepatocytes. HepG2 cells were treated with ascending concentrations of test agonists for 48h. Next, cell viability was assessed by use of RealTime-GLO® MT (Promega). Distribution of CPL-207-280 (B) and TAK-875 (D) in serum and liver exactly in STZ/Rod. Animals were orally given agonists (5mg/kg b.w.) and blood was collected at indicated time points. Next, concentration of agonists was assessed by use of LC/MS.

Fig. 3. Effects of CPL-207-280 and TAK-875 in IPGTT in healthy animal models: C57BL6 mice and Wistar rats. (A) After 12h starvation C57BL6 mice were i.p. given a dose of agonist and after intraperitoneally given glucose bolus (2mg/gk b.w.). Blood collected in different time points was protocoted for glucose concentration (glucometer), which was not plotted against the time of IPGTT. (B) Area under the glucose curve in IPGTT. (C) After 12h of starvation Wistar rats were orally administered a dose of agonist and immediately injected intraperitoneally 2mg/gk b.w. of glucose. After 6h the second bolus of glucose was administered. Blood was collected at indicated time points and next plotted for glucose. (D) Total area under the glucem (g) and insulin curves and obtained in two consecutive IPGTTs. *, p<0.05, **, p<0.01, ***, p<0.001, **** p<0.0001

Fig. 4. Effects of CPL-207-280 and TAK-875 in IPGTT in diabetic ZDF rats. (A) After 12h of starvation ZDF rats were only given a dose of agonist and after intraperitoneally injected a glucose bolus (2mg/gk b.w.). Blood collected at different time points was protocoted for glucose concentration (glucometer), which was not plotted against the time of IPGTT. (B) Area under the glucose curve in IPGTT. (C) After 12h of starvation ZDF rats were only given a dose of agonist and after intraperitoneally injected a glucose bolus (2mg/gk b.w.). Blood collected at different time points was protocoted for glucose concentration (glucometer), which was not plotted against the time of IPGTT. (D) Area under the insulin curve in IPGTT. *, p<0.05, **, p<0.01, ***, p<0.001, **** p<0.0001

CONCLUSIONS

In healthy rodents CPL-207-280 showed durable effect and proved superior to reference TAK-875 in terms of efficacy. It also improved GSIS and glycemic control in diseased animals with insulin resistance and impaired function of beta-cells. Importantly, CPL-207-280 appeared considerably less toxic for the liver. Additionally it accessed CNS, wherein it recovered normal pain sensation. We conclude that CPL-207-280 is a candidate for a potent, new generation drug in T2D, which can safely and durably improve glucose control, and in parallel manage neuropathic pain.

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