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 $G\alpha_q$ 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 glycaemic control through enhancement of glucose-stimulated insulin secretion (GSIS). This results from activation of PLC, which gives rise to IP_3 and DAG production followed by Ca^{2+} 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 hypoglycaemia. The similar signalling pathway has been proposed to operate in neurons regulating pain sensation, constituting endogenous pain inhibitory system in CNS. Activation of GPR40 by endogenous ligands in supra-spinal area has recently been shown to ameliorate chronic pain sensation in various pathology states. Neuropathic pain affects up to 20% of diabetic population and is present in more than 50% cases of 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 ZDSD rats, tested its safety in the liver cells and finally interrogated its effect on allodynia in STZ-treated diabetic mice.

RESULTS

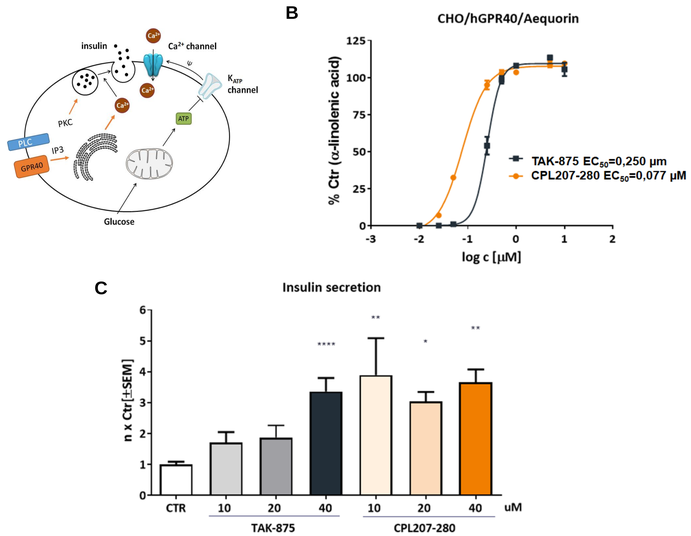

Fig.1. Efficacy of CPL-207-280 and TAK-875 in activation of GPR40 and effects on insulin secretion *in vitro*.
(A) Schematic presentation of the mechanism, through which activation of GPR40 enhances GSIS. **(B)** CPL-207-280 is more potent to liberate intracellular Ca2+ than TAK-875. CHO cells stably overexpressing human GPR40 were treated with ascending concentrations of tested agonists and intracellular Ca2+ efflux was assessed based on luminescence after binding to coelenterazine-h. **(C)** CPL-207-280 is stronger secretagougue than TAK-875. MIN6 cells were treated with 20mM glucose in Kreb's buffer in the presence of different concentrations of agonists and supernatant was probed for insulin. *; p<0,5, **; p<0,01, ***; p<0,001, ****; p<0,0001

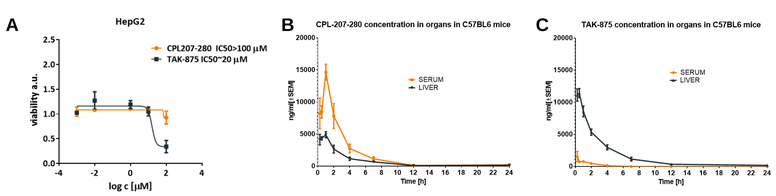

Fig.2. Effects of test compounds in hepatic cells.
(A) Dose dependent toxic effect of agonists in hepatocytes. HepG2 cells were treated with ascending concentrations of test agonists for 48h. Next, cell viability was assesed by use of RealTime-Glo™ MT (Promega). Distribution of CPL-2017-280 **(B)** and TAK-875 **(C)** in serum and liver in C57BL6 mice. Animals were orally given agonists (3mg/kg b.w.) and blood was collected at indicated time points. Next, concentration of agonists was assesed by use of LC/MS.

RESULTS

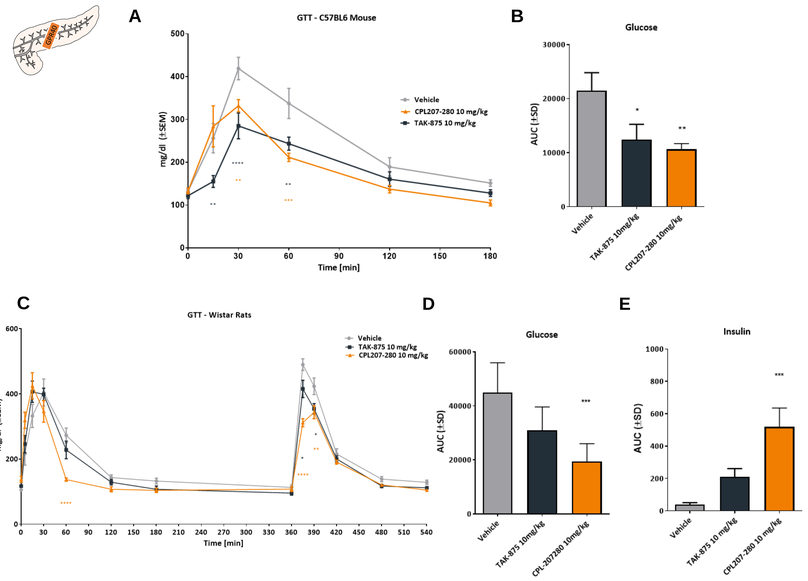

Fig.3. Effects of CPL-207-280 and TAK-875 in IPGTT in healthy animal models: C57BL6 mice and Wistar rats.
(A) After 12h starvation C57BL6 mice were i.v. given a dose of agonist and right after intraperitonealy given glucose bolus (2mg/kg b.w.). The blood collected at different time points was probed for glucose concentration (glucometer), which was next plotted against the time of IPGTT. **(B)** Area under the glucose curve in IPGTT. **(C)** After 12h of starvation Wistar rats were oraly administered a dose of agonist and immediately injected intraperitoneally 2mg/kg b.w. of glucose. After 6h the second bolus of glucose was administered. Blood was collected at indicated time points and next probed for glucose. **(D)** Total area under the glucose and **(E)** insulin curves and obtained in two consecutive IPGTTs. *; p<0,5, **; p<0,01, ***; p<0,001, ****; p<0,0001

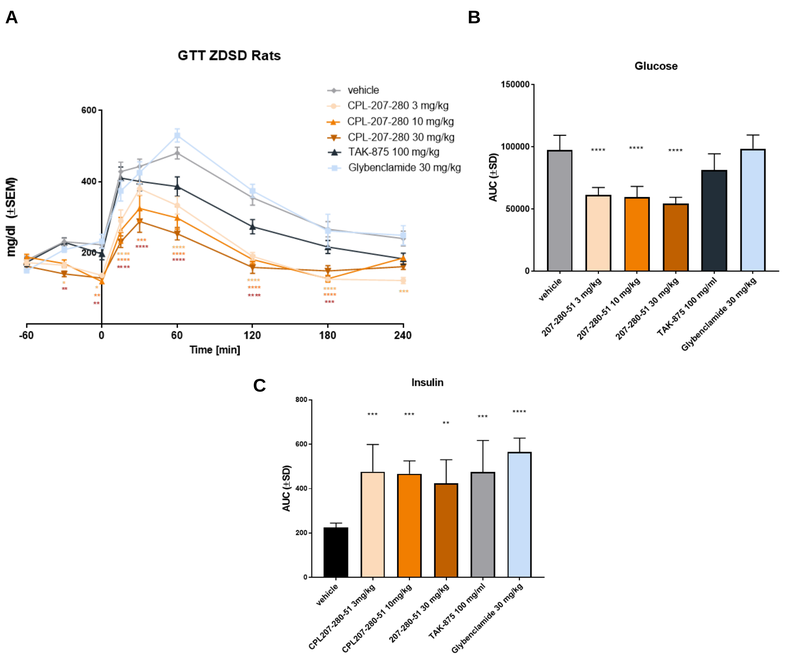

Fig.4. Effects of CPL-207-280 and TAK-875 in IPGTT in diabetic ZDSD rats.
(A) After 6h of starvation ZDSD rats were oraly given a dose of agonist and 1h after intraperitonealy injected a glucose bolus (2mg/kg b.w.). The blood collected at different time points was probed for glucose concentration (glucometer), which was next plotted against the time of IPGTT. **(B)** Area under the glucose curve in IPGTT **(C)** Area under the insulin curve in IPGTT. *; p<0,5, **; p<0,01, ***; p<0,001, ****; p<0,0001

RESULTS

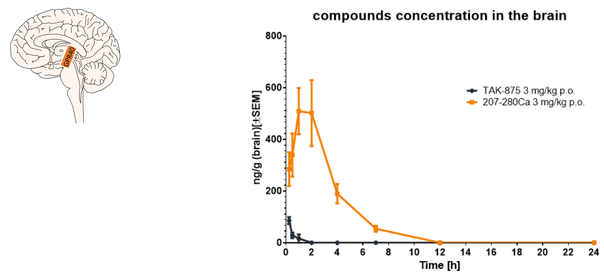

Fig.5. Pharmacokinetic of test agonists in C57BL6 mouse brain.
After 12h of starvation, mice were orally administered test compounds. Next, blood was collected at indicated time points and compounds concentration was measured in serum by use of LC/MS. The concentration is expressed as weight of the compound per weight of brain tissue.

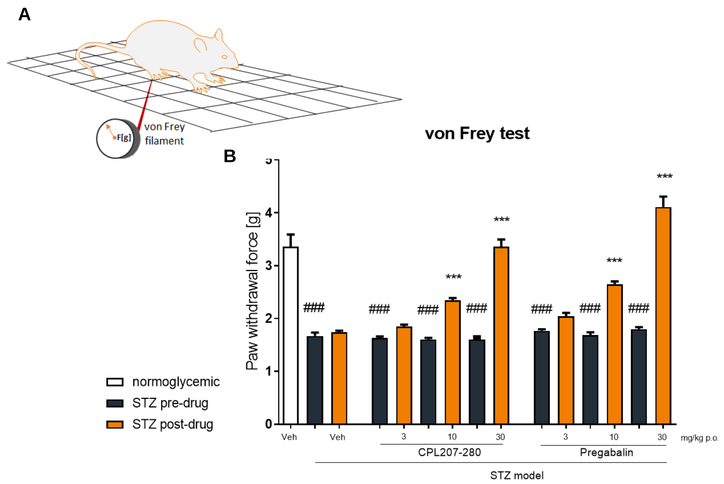

Fig.6. The effect of CPL-207-280 on diabetes (STZ) induced allodynia in C57BL6 mice.
(A) Schematic presentation of evaluation of allodynia in von Frey test. The subject is placed on the wire mesh and the filament is applied at the increasing force against the plantar surface of the hind paw. The degree of allodynia is assessed based on the force value, at which the subject withdraws the paw - „paw withdrawal force". **(B)** Amelioration of allodynia by CPL-207-280 and reference pregabalin in STZ treated mice. C57BL6 mice were given streptozotocin (STZ; 200 mg/kg b.w.) and have been probed for blood glucose concentration for the following 20 days. At days 21 those, which developed hyperglycemia (>300 mg/dl) were subjected to von Frey test. Mice, which exhibited decreased paw withdrawal force were considered neuropathic and were orally administered CPL-207-280 or pregabalin at increasing doses. After 1h the von Frei test was performed again to reveal therapeutic effect. ***, ###; p<0,001.

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 glycaemic 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 CPL207-280 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.

FUNDS

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