Differentiation of Partial and Full GPR40 Agonists for the Treatment of Type 2 Diabetes

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ABSTRACT

GPR40 is a clinically validated therapeutic target for the treatment of diabetes due to its ability to mediate glucose-stimulated insulin secretion (GSIS) from pancreatic β-cells and incretin release from enteroendocrine cells. Tak-875 (fasiglifam©, Takeda) the most clinically advanced GPR40 agonist compound, was found to have potent anti-diabetic effects but was terminated due to idiosyncratic liver toxicity. Fasiglifam is a partial GPR40 agonist and potentially does not realize the full magnitude of efficacy possible via this receptor. Therefore, we examined the ability of a potent and selective full agonist JNJ-GPR40-A to differentiate from a partial agonist JNJ-GPR40-B. The full and partial agonists displayed similar potencies at the GPR40 receptor in calcium flux assays. However, the full agonist was determined to clearly differentiate from the partial agonist in vitro in cell-based assays for IP-1 accumulation. In addition, in the same cell line the full agonist promoted cAMP accumulation whereas the partial agonist did not. JNJ-GPR40-FA (full agonist) demonstrated superior acute glucose lowering compared to JNJ-GPR40-PA (partial agonist) in an oral glucose tolerance test (OGTT) in diabetic ZDF rats. This enhanced in vivo efficacy was accompanied by GLP-1 secretion and augmented insulin secretion during the OGTT confirming the ability of full agonists to engage the enteroinsular axis. Importantly, superior glucose-stimulated insulin secretion from the full agonist was consistently observed in human islets from multiple human islet donors. GPR40 full agonists may provide an additional opportunity for the treatment of diabetes.

INTRODUCTION

GPR40 is a medium to long chain free fatty acid receptor that is highly expressed in pancreatic beta cells and to a lesser extent in enteroendocrine K and L cells[1]. GPR40 agonists induce glucose-stimulated insulin secretion (GSIS) and stimulate incretin release[1,2]. Numerous GPR40 ligands have been developed and investigated for their anti-diabetic actions[3]. The most advanced, TAK-875 (fasiglifam©, Takeda), was clinically effective in two Phase II dose-ranging clinical trials, one in Central/North America and the other in Japan[4,5]. The change in least square mean in HbA1c at week 12 from baseline was 1.27% in the Japanese group and 1.12% in Central/North American population. In both cases, similar glucose-lowering to glimepiride was observed with little to no propensity for hypoglycemia nor weight gain[4,5]. However, fasiglifam was discontinued due to concerns of liver toxicity [6].

Fasiglifam is a partial agonist at the GPR40 receptor as it does not demonstrate maximal efficacy for IPone accumulation when compared to full agonists. Its efficacy in the clinic indicates that targeting partial agonists of GPR40 would be an attractive approach to lower glucose in T2DM as a stand alone therapy or in combination with other oral anti-diabetic drugs[7]. However, it is possible that partial agonists at the receptor may not realize the maximal efficacy via GPR40 activation. In fact, superior efficacy with a full GPR40 agonist has been reported pre-clinically with the Amgen full agonist AM-1638[7].

GPR40 was originally deorphanized as a medium to long chain free fatty acid receptor primarily signaling through Gq. However, recent evidence indicates that GPR40 has the propensity to couple to Gq only or both Gq and Gs depending on the ligand. In general, partial agonists signaled through Gq only and resulted in small incretin responses whereas full agonists signaled through Gq and Gs with robust incretin responses[8]. Full agonists are differentiated from partial agonists by their maximal efficacy on IPone accumulation as well as their ability to stimulate cAMP. The enhanced signaling of full agonists results in superior glucose-lowering in an oGTT in ZDF rats as well as augmented GSIS in islets from multiple human donors.

RESULTS

In vitro Potencies in Over-Expressing Cell Lines

	Human GPR40	Rat GPR40	Mouse GPR40
JNJ	**EC50 nM**	**EC50 nM**	**EC50 nM**
GPR40-FA	1.4 ± 0.6 (n=11)	7.7 ± 0.9 (n=7)	37.4 ± 12.2 (n=2)
GPR40-PA	31 ± 3 (n= 35)	39 ± 4 (n= 24)	181 ± 32 (n= 17)

[a] Values represent the mean ± standard deviation, number of replicate experiments are in parenthesis.

Table 1: In vitro potency of JNJ-GPR40-FA and JNJ-GPR40-PA as assessed by calcium mobilization in GPR40 Over-expressing Cell Lines. Human, rat or mouse GPR40-HEK293 cells were plated into poly-D-lysine coated 384-well plates at a seeding density of 5000 cells/well and cultured overnight in a 37ºC humidified tissue culture incubator. On the day of the experiment, the culture media is replaced with assay buffer (HBSS, 20 mM HEPES, 0.1% BSA) and the cells were incubated at 37ºC for 1 hour. Calcium-sensitive fluorescent dye (Fluo 8 No-Wash Calcium Dye, ABD Bioquest # 36316) is then added and the cells incubated for another 30 minutes at 37ºC followed by 15 minutes at room temperature protected from the light. The cell plate and a plate of diluted compounds are loaded into a fluorescent plate reader, and the fluorescence intensity of each well is read at 1 second intervals for 8 minutes and outputs the data for analysis in an Excel.

Differentiation of Full and Partial Agonists

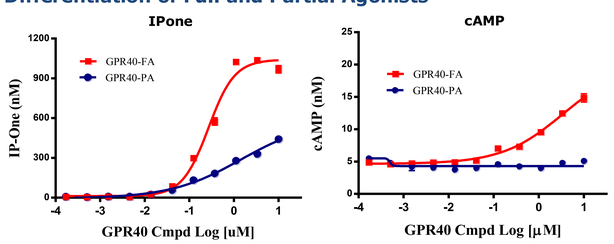

Figure 1: Full and Partial GPR40 Agonist are differentiated by IPone. Human GPR40-CHO-K1 low expressing cells were plated into poly-D-lysine coated 384-well plates at a seeding density of 5000 cells/well and cultured overnight in a 37ºC humidified tissue culture incubator. On the day of the experiment, the culture media is replaced with assay buffer and compounds were added and incubated with cells at 37ºC for 30 min with 500 mM IBMX (cAMP) or without IBMX for 90 min (IPone). Analytes were detected according to the manufacturer protocol (CISBIO IPone Tb kit, Cat #62IPAPEC, or CISBIO cAMP Dynamic kit Cat # 62AM4PEC).

Acute Oral Glucose Tolerance Test (OGTT) in ZDF Rats

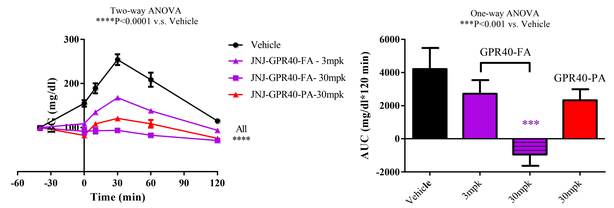

Figure 2: GPR40 Full Agonist Displays Enhanced Efficacy Compared to Partial Agonist During an Oral Glucose Tolerance (OGTT) Test in Zucker Diabetic Fatty (ZDF) Rats. 6-7 week old ZDF rats were fasted overnight and randomized by blood glucose and body weight. Rats were orally dosed with vehicle or compound and an OGTT (1g/kg: 20% glucose, 5ml/kg) was performed. Blood glucose was measured at the indicated time points via tail-vein. N=8/group.

Plasma Insulin During the OGTT in ZDF Rats

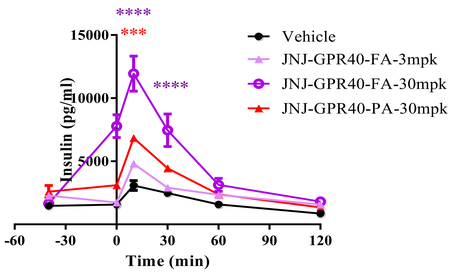

Figure 3: GPR40 Full Agonist Promotes Greater Insulin Secretion Compared to the Partial Agonist during an Oral Glucose Tolerance Test. Blood samples (25~30µl) were collected via tail-vein bleeding into EDTA tubes at the indicated time points. Insulin levels were then determined from plasma samples using the MesoScale Discovery (MSD) Mouse/Rat Insulin Kit (Cat # K152BZC-2).

GLP-1 Secretion in ZDF Rats

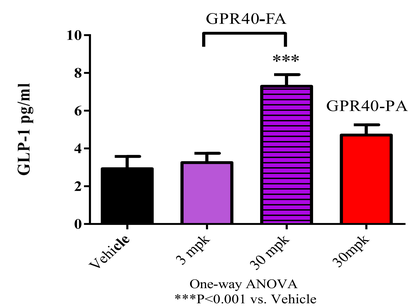

Figure 4: GPR40 Full Agonist Promotes Enhanced GLP-1 Secretion Compared to Partial Agonist in ZDF Rats. 6-7 week old ZDF rats were fasted overnight and randomized by blood glucose and body weight. Rats were orally dosed with vehicle or compound for 40 minutes and then orally dosed with 1g/kg (20% glucose, 5ml/kg). Ten minutes after glucose dosing 70 -80µl was collected via tail-vein bleeding into EDTA tubes containing 5µl of an aprotinin/DPP-IV inhibitor solution. Total GLP-1 was then determined from the plasma samples using the MesoScale Discovery Total GLP-1 (ver. 2) kit (cat #K150JVC-2).

Glucose Stimulated Insulin Secretion in Human Islets

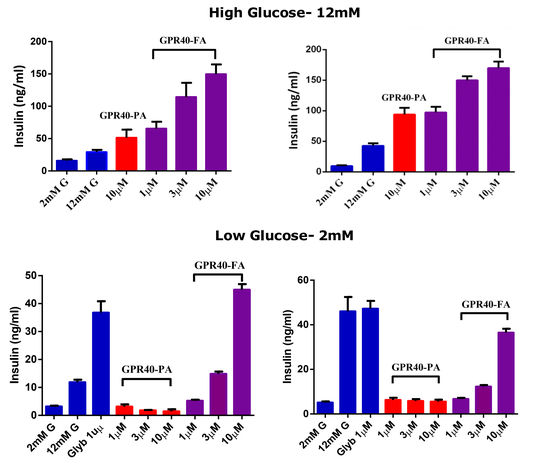

Figure 5: GPR40 Full Agonists Promote Enhanced Insulin Secretion in Both Low and High Glucose in Human Islet Donors. Human islets were dispersed with accutase for 10 minutes at 37 °C. 15,000 cells were plated in V-bottom 96-well plates in complete medium and incubated overnight. The next day medium was replaced with assay buffer and the indicated concentrations of compound were tested in either 2mM or 12mM glucose for 2 hrs. The supernatant was then tested for insulin using the CISBIO HTRF Insulin assay kit (cat# 62INSPEC).

CONCLUSIONS

- GPR40 full agonists can be differentiated from partial agonists based on their signaling properties with IPone and cAMP.
- Full GPR40 agonists demonstrate superior glucose lowering to partial agonists in pre-clinical species due to increased insulin and GLP-1 secretion.
- Superior glucose stimulated insulin secretion by the GPR40 full agonist is consistently observed in islets from human donors.
- GPR40 full agonist also potentiate basal insulin secretion in low glucose.
- GPR40 agonists have the potential to be a complementary mechanism to other oral anti-diabetic therapies.

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