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


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) is the most clinically advanced GPR40 agonist compound, found to have potent anti-diabetic effects but was terminated due to idiosynratic 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-FA to differentiate from a partial agonist JNJ-GPR40-A. 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-A (partial agonist) in an oral glucose tolerance test (OGTT) in diabetic ZDF rats. This enhanced in vivo efficacy was accomplished 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. JNJ-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 most advanced, TAK developed and investigated for its anti-diabetic properties. GPR40 was originally deorphanized as a medium to long chain free fatty acid receptor and later was shown to be upregulated in adipocytes in response to high glucose exposure (4). GPR40 is a medium to long chain free fatty acid receptor that is able to mediate glucose stimulated insulin secretion (GSIS) from pancreatic β-cells and incretin release from enteroendocrine cells. Tak-875 (fasiglifam, Takeda), was clinically effective in two Phase II dose-ranging clinical trials, one in Central/Northern America and the other in Japan(5,6). The change in least square mean in HbA1c at week 12 from baseline was 1.27% in Japan and 1.11% in Central/Northern America population. In both cases, similar glucose-lowering to gliclazide was observed with little to no propendancy for hypoglycemia or weight gain(5,6). However, fasiglifam was discontinued due to concerns of liver toxicity(5,6).

Fasiglifam is a partial agonist at the GPR40 receptor as it does not demonstrate maximal efficacy for IPn and GSIS 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, hypertension with full GPR40 agonist has been reported pre-clinically with the Aemgen full agonist AM-1638(8).

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 inositol responses whereas full agonists signaled through Gq and Gs with robust inositol responses(9). Full agonists are differentiated from partial agonists by their maximal efficacy on IPn accumulation as well as their ability to stimulate CAMP. The enhanced signal 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

![Graph showing in vitro potencies in over-expressing cell lines for JNJ-GPR40-FA and JNJ-GPR40-A.](image)

**Table 1:** In vitro potencies of JNJ-GPR40-FA and JNJ-GPR40-A are assessed by calcium mobilization. **JNJ-GPR40-FA** demonstrated a 1.5 fold greater potency over **JNJ-GPR40-A** on human main pancreatic islet cell line NCI-H716, with an EC50 of 0.77 ± 0.09 µM and 1.27 ± 0.10 µM respectively. **Amplicon expressed GPR40-WT** demonstrated a 2.7 fold higher potency over **JNJ-GPR40-A** on human main pancreatic islet cell line NCI-H716, with an EC50 of 0.89 ± 0.07 µM and 2.55 ± 0.20 µM respectively. **Amplicon expressed GPR40-WT** was then cloned into and the cells incubated for another 24 hours. **Full agonist** was then added to the cells incubated for another 18 minutes at 37°C. Filtrated by 15 minutes of room temperature protection from the light. The end plate and a plate of glass slides were washed, fixed with Carnoy fixation, and the formalin in situ immunohistochemistry was then performed. Immunohistochemical staining was performed and the fluorescence intensity of each well was read at 1 second intervals for 8 minutes and outputs the data for analysis in an Excel.

**Differential of Full and Partial Agonists**

![Graph showing differential of full and partial agonists.](image)

**Figure 1:** Full and Partial GPR40 Agonist are differentiated by IPn. Human GPR40-KO-β cell expressing rCasa was plated into 24 well plate at a seeding density of 50000 cells/well and cultured 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 cells were 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.

**Figure 2:** Gut and Partial GPR40 Agonist are differentiated by CAMP. Human GPR40-KO-β cell expressing rCasa was plated into 24 well plate at a seeding density of 50000 cells/well and cultured overnight in 5% CO2 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’s protocol (CISBIO IPone Tb kit, Cat #62IPAPEC, or CISBIO cAMP Dynamic kit Cat #62AM4PEC).

**CONCLUSIONS**

• GPR40 full agonists can be differentiated from partial agonists based on their signaling properties with 3P騰 and CAMP.

• Full GPR40 agonists demonstrate superior glucose lowering to partial agonists in preclinical studies due to increased insulinotropic (IPn) and GLP-1 secretory effects.

• Superior glucose stimulated insulin secretion by the GPR40 full agonist is consistently observed in islets from human donors.

• GPR40 full agonist also potentiates basal insulin secretion in vivo.

• GPR40 agonists have the potential to be a complementary mechanism to other oral anti-diabetic therapies.

REFERENCES