Hepatobiliary and Cardiovascular Effects Limit the Utility of Systemic TGR5 Agonists in Diabetes

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Abstract

There is considerable interest in the bile acid receptor TGR5 as a drug target for type 2 diabetes. Activation of TGR5 in intestinal enteroendocrine cells leads to increased cAMP and subsequent enhanced GLP-1 secretion, which is a clinically validated approach to control blood glucose levels. In addition, its expression and anti-inflammatory activity in monocytes/macrophages and dendritic cells suggested that it could also address the chronic inflammation characteristic of obesity and diabetes, and thus provide a better clinical benefit versus glucose lowering agents alone. We have identified a TGR5 agonist, FC-92-EC85, with suitable in vitro and pharmacokinetic properties. Acute non-clinical efficacy was demonstrated in both GLP-1 secretion and LPS-induced cytokine release models. However, treatment with FC-92-EC85 and other TGR5 agonists in rodents also led to increased bile volume, gallbladder wall thickening and hyperplasia. To determine whether these effects would be seen in higher species, we tested FC-92-EC85 in dogs. Unlike what was seen in mice, oral administration of FC-92-EC85 led to diminished GLP-1 response due to delayed gastric emptying. In addition, FC-92-EC85 delayed gallbladder emptying and induced acute wall thickening, as well as dose dependent increases in heart rate. These deleterious effects may limit the clinical application of TGR5 agonists in diabetes.

Keywords: TGR5; Gall bladder; Diabetes

Abbreviations: TGR5: Takeda G-protein-coupled Receptor 5; GLP-1: Glucagon-Like Peptide 1; cAMP: 3’-5’-cyclic Adenosine Mono Phosphate; GPBAR1: G-protein-Coupled Bile Acid Receptor 1; CFTR: Cystic Fibrosis Transmembrane Conductance Regulator; LPS: LipopolySaccharide; TNF: Tumor Necrosis Factor; FXR: Farnesoid X Receptor; PBMC: Peripheral Blood Mononuclear Cells

Introduction

TGR5 is a Gs-coupled GPCR activated by both primary and secondary bile acids (i.e. tauroliothocholic acid) encoded by the G protein-coupled bile acid receptor 1 (GPBAR1) gene. The biological role of TGR5 is a function of both its pattern of expression and its ability to increase cellular cAMP [1-3]. Activation of TGR5 in intestinal enteroendocrine cells leads to secretion of GLP-1 and other related incretins, through a mechanism similar to GPR119 [4]. In monocytes/macrophages and dendritic cells (Mo/Mφ/DC), TGR5 activation leads to decreases in LPS-induced Th1 cytokines like TNF-α and IL-12 [5]. Together, these functions make TGR5 an attractive target in the treatment of type 2 diabetes, where strategies to enhance GLP-1 [6] and mitigate chronic inflammation [7-9] are already demonstrating benefit in the clinic. While several pharmaceutical organizations have pursued TGR5 agonists, no TGR5 drug has been approved for diabetes or metabolic diseases indication.

TGR5 expression is not limited to the gastrointestinal and immune systems. In the mouse, the highest expression of TGR5 is in the gallbladder [10]. Stimulation of TGR5 in gall bladder epithelium leads to increased membrane permeability to chloride, bicarbonate and water, mediated in part by CFTR [11]. In this way, TGR5 serves as a sensor of bile acid concentration in the bile, enabling dilution if the concentration is too high. Considering the toxicity of high concentrations of bile acids on the liver, TGR5 sensor activity in the gall bladder is a key function. Evidence also suggests that TGR5 is localized to gall bladder smooth muscle, where its activation leads to inhibition
of contraction and subsequent gall bladder filling [12]. These functions may make development of a systemic TGR5 agonist challenging, especially in type 2 diabetics who are already prone to gall bladder dysfunction [13]. This may explain efforts by several companies to develop non-systemic TGR5 agonists, with the goal of activating TGR5 in the GI tract and avoiding gall bladder exposure [14-17]. However, this presents a challenging path for drug development, and it is still not clear if the distribution and function of TGR5 are the same in the gall bladder of human and the various pre-clinical species where studies have been conducted to date.

Considering the potential to treat type 2 diabetes, we identified systemic low molecular weight agonists of TGR5. Despite significant challenges in optimization of clinical candidates, potent compounds with suitable pharmacokinetic profiles were achieved [18]. The TGR5 agonist FC-92-EC85 strongly increased glucose mediated GLP-1 secretion and decreased LPS-induced TNF-α both in vitro and in vivo, as expected. However, acute treatment also led to increases in bile volume. Two week treatment with TGR5 agonists representing several chemo types induced changes in the gall bladder of mice, including increased wall thickness mostly due to mucosal hyperplasia. Subsequent acute testing of FC-92-EC85 in dogs showed a diminished GLP-1 response and delayed gastric emptying. In addition, acute changes in gall bladder morphology and function were seen by ultrasound imaging. Taken together, these data suggest that the clinical utility of systemic TGR5 agonists to treat type 2 diabetes may be limited.

Materials and Methods

**TGR5 cellular cAMP assay.** Jurkat cells overexpressing human or mouse TGR5 were seeded at 50,000 cells/25 μl/well in 384-well plates (Greiner) in AIM V media (Life Technologies) supplemented with 1 mM IBMX (Sigma) and 10 mM HEPES. Immediately after plating, 200 μL of compounds in DMSO were transferred using the Pintool (GNF systems). Following a 1 hr at 37 °C, cryptate and D2 conjugates from the cAMP HiRange HTRF kit (Cisbio) were added (12.5 μL each). Plates were then incubated 1 hr at room temperature and emission at 620 nM and 665 nM were read on the Envision (Perkin Elmer) as suggested by the manufacturer. Data was expressed as a ratio of emission at 665/620, and then normalized to a DMSO alone control. Dose-response data points were curve fitted using the standard logistic regression model, implemented with an in-house developed Matlab (mathworks.com) program. The resultant EC50 values correspond to the compound concentration that inhibited activity by 50%.

**FXR reporter gene assay.** 293T cells stably expressing GAL4-luciferase-FXR receptor were maintained in Dulbecco’s Modified Eagles Medium (Hyclone) containing 3% charcoal/dextran treated fetal bovine serum (Sigma) and 1% penicillin/streptomycin. Following 24 hour incubation at 37°C, 50 μL of compound diluted in DMSO was transferred by Pintool (GNF Systems) into the assay plate and incubated for 24 hours at 37°C. 25 μL Bright Glo (Promega) was added per well and flash luminescence was detected on the Viewlux (PerkinElmer Life Sciences). Dose-response data points were curve fitted using the standard logistic regression model, implemented with an in-house developed Matlab (mathworks.com) program. The resultant IC50 values correspond to the compound concentration that inhibited activity by 50%.

**GLUTag GLP-1 secretion assay.** Murine GLUTag cells were maintained in Dulbecco’s Modified Eagles Medium (Hyclone) containing 10% fetal bovine serum and 1% penicillin/streptomycin. On day one of the assay, cells from a confluent flask were detached using 0.25% trypsin (Life Technologies) and seeded in 96 well poly-D lysine plates (BD Biosciences) at 42,000 cells /150 μl/well in maintenance medium. After incubation at 37°C overnight, medium was changed to phenol red free DMEM with low glucose (Sigma) and then incubated for another 24hrs at 37°C. GLUTag cells were then washed and incubated in glucose free EBSS buffer with 0.1% BSA (Sigma) at 37 °C to starve, followed by two additional EBSS buffer washes. Following the last wash, EBSS buffer containing 15 mM glucose, DPP4 inhibitor PKF 275 (600 nM final concentration) and agonist compounds was added to the cells and incubated for another 2hr at 37 °C to allow for GLP-1 secretion. 6 μL of supernatant was then transferred to HEK293 cells stably expressing a GLP-1 luciferase reporter construct, seeded the morning of GLUTag cell treatment at 12,000 cells/40 μl/well in a 384-well plate (Greiner). After overnight incubation, 15 μL of 25% Bright Glo (Promega) was added to the reporter cells, incubated at room temperature for 2 min and luminescence was then read on Envision per the manufacturer’s instructions (Perkin Elmer). Data were normalized to a DMSO alone control. Dose-response data points were curve fitted using the standard logistic regression model, implemented with an in-house developed Matlab (mathworks.com) program. The resultant EC50 values correspond to the compound concentration that induced half-maximal activity.

**PBMC LPS assay.** Peripheral blood mononuclear cells (PBMC) were isolated from healthy donors using Ficoll Hypaque per the manufacturer’s recommendation (GE Healthcare). Cells were seeded at 30,000 cells/10 μL/well in a 384-well proxiplate (Perkin Elmer) in AIM V media (Life Technologies) supplemented with 3% FBS (Hyclone) and 1X Pen/Strep/Glutamine. Following an overnight incubation at 37 °C, 50 nL of compound in DMSO was added per well by Pintool (GNF systems). Plates were incubated for 30 min at 37 °C, followed by the addition of 2μL/well of 6X LPS (EC80 final, determined during LPS dose titration for each donor) and incubation...
Male C57BL/6 mice frozen tissues were transferred into 10% neutral buffered (NB) were saved frozen at -80°C after being separated from the liver. EC85 for 14 days. At the termination of the study gall bladders were fasted overnight and then dosed orally with 10, 30, or 100 mg/kg of FC-92-EC85 one hour prior E. Coli LPS challenge (40 μg/kg). After 30 minutes, animals were challenged with by 3 g/kg oral dextrose (Sigma). For assessment of GLP-1 secretion, plasma was collected at 2 min. post-challenge into EDTA coated tubes containing 8μL of a DPP4 inhibitor solution (EMD Millipore) and GLP-1 levels were measured by ELISA according to the manufacturer's instructions (Meso Scale Discovery). Data was analyzed using One-way ANOVA followed by Dunnett's post-test in Graphpad Prism. Values of p < 0.05 were considered statistically significant. Data was expressed as a ratio of emission at 665/620, and then normalized to a DMSO alone control. Data shown is the average +/- SEM of 8 animals.

Mouse in vivo GLP-1 secretion assay. Male C57BL/6 mice were fasted overnight and then dosed orally with 10, 30, or 100 mg/kg of FC-92-EC85. After 30 minutes, animals were challenged with by 3 g/kg oral dextrose (Sigma). For assessment of GLP-1 secretion, plasma was collected at 2 min. post-challenge into EDTA coated tubes containing 8μL of a DPP4 inhibitor solution (EMD Millipore) and GLP-1 levels were measured by ELISA according to the manufacturer's instructions (Meso Scale Discovery). Data was analyzed using One-way ANOVA followed by Dunnett's post-test in Graphpad Prism. Values of p < 0.05 were considered statistically significant. Data shown is the average +/- SEM of 8 animals.

Mouse in vivo LPS challenge assay. Male C57BL/6 mice were fasted overnight and then dosed orally with 10, 30, or 100 mg/kg of FC-92-EC85 one hour prior E. Coli LPS challenge (40 μg/kg Sigma). Serum was collected at 90 minutes post-challenge and TNFα or IL-10 levels were measured by ELISA according to the manufacturer's instructions (Meso Scale Discovery). Data was expressed as a ratio of emission at 665/620, and then normalized to an internal standard (36B4) to obtain Ct values were normalized to an internal standard (36B4) to obtain a delta Ct, and relative quantity was calculated according to the formula:  RQ = 2^- deltaCt. Data was then expressed as the average +/- standard deviation.

Samples of dog gall bladder, spleen and brain (portion of frontal cortex) were obtained from untreated or vehicle-control animals used for internal preclinical toxicity studies at Novartis Pharma AG (Basel, Switzerland). Tissues were immediately snap-frozen in liquid nitrogen followed by storage at -80 °C until processing. Total RNA was then isolated by Trizol extraction (Life Technologies) followed by purification on an affinity resin (RNeasy, Qiagen) according to the manufacturer’s instructions. mRNA samples for human and cynomolgus monkey were obtained commercially (Biochain). For human, mouse, and cynomolgus TGR5 analysis, quantitative RT-PCR was performed using the RNA to Ct kit (Life Technologies), run on the ABI Prism 7700 (Applied Biosystems) per the manufacturer’s instructions. Validated primer/probe master mixes were obtained from Life Technologies: human and cynomolgus Hs00544894_m1, mouse Mm00558112_s1. Ct values were normalized to an internal standard (36B4) to obtain a delta Ct, and relative quantity was calculated according to the formula:  RQ = 2^- deltaCt. Data was then expressed as the average +/- standard deviation.

Effect of FC-92-EC85 on metabolic endpoints in dogs. Male Beagles with reproducible postprandial triglyceride and glucose excursions were selected for the study. A cream-glucose-water-saline mixture (= liquid meal) was prepared with heavy cream (2.7 ml/kg, fat 0.9 g/kg), glucose (1 g/kg) and water (5.3 ml/kg). A solution of acetaminophen (20 mg/kg) in saline (2 ml/kg) was added to the mixture. The dogs were fasted (16h) and an initial baseline blood sample was obtained at 30 min before the meal (-30 min). Immediately thereafter the vehicle was administered via gavage and after 15 min a second baseline blood sample was drawn (-15 min). A third baseline blood sample was obtained before the meal (0 min) and the meal (10 ml/kg) was given via gavage. Subsequent blood samples were obtained after 10, 20, 30, 40, 50, 60, 75, 90, 105, 120, 180 and 240 min from the ingestion of the meal. After a washout period, FC-92-EC85 was tested using the same dogs and the same sampling regimen. The compound was administered orally at 50 mg/kg in PEG300 and dH2O in a proportion of 3:1. Blood samples (3 ml) were drawn from the cephalic vein via syringe and transferred into tubes with EDTA, aprotinin (Trasyol, 500 kallikrein inhibitor units/mL blood) and diprotin A (110 μmol/mL blood). Blood samples were placed on ice until centrifugation at 3,000 rpm for 20 min at 4°C. Plasma samples were divided into aliquots and stored at -70°C. Plasma acetaminophen was analyzed using an enzyme immunoassay procedure (Olympus Emit Tix Acetaminophen Assay, Olympus America Inc., Center Valley, PA) and absorbance was measured in an Olympus au4000e spectrophotometer. Plasma GLP-1 was measured by an ELISA per the manufacturer’s instructions (Meso Scale Discovery). The results were analyzed using one-way repeated measures ANOVA followed by Dunnett’s post-tests. The entire acetaminophen curves (0 to 240 minutes) of the vehicle and the FC-92-EC85 phases were compared to each other using paired T tests. Paired T tests were also used to compare isolated time points. Values of P<0.05 were considered statistically significant.

Effect of FC-92-EC85 on gall bladder morphology and function in dogs. Three male beagle dogs (body weight 9.5 to 14.7 kilograms) were dosed with vehicle on study day 1. A single dose of 10, 30, 100 or 300 mg/kg FC-92-EC85 in rising dose fashion was given on separate days with at least 5 days between doses. ECG waveforms were recorded using non-invasive jacket telemetry for 2h prior to dose and 24h post dose at pretest and each dose. Animals were acclimated to telemetry jacket procedures at least three times on separate days. ECG waveforms were recorded using the emka ECG system when animals were in their home cage. For heart rate and ECG intervals, the third 15-min period of every hour were reported for after each treatment to minimize the influence of TK blood sampling procedures on animals and for pretest. The heart rate and ECG intervals (RR, PR, QRS and QT intervals) were analyzed by emka ECG-Auto computer software. QT interval was corrected for heart rate using Van de Water QT correction formula QTc or QTcVW=QT-0.087 (RR-1000).

Results

FC-92-EC85 is a TGR5 agonist suitable for proof-of-concept studies. We began a campaign to develop oral low molecular weight agonists of TGR5. While a diverse array of chemotypes was identified from high throughput screening, several key challenges became evident during initial medicinal chemistry. Human and mouse TGR5 are highly homologous at the protein level (83%), yet the majority of compounds showed preferential activation at the human receptor. This type of profile makes preclinical efficacy and toxicity studies challenging, so the effort focused on chemotypes with some degree of cross-reactivity. Lead optimization led to the identification of FC-92-EC85, a...
potent and selective TGR5 agonist with no activity on the nuclear bile acid receptor FXR [18] (Figure 1A). FC-92-EC85 increased glucose dependent GLP-1 secretion both in vitro, as measured in GLUTag cells, and in vivo following glucose bolus (Figures 1B & 1C). In addition, FC-92-EC85 was able to decrease LPS-induced TNF-alpha in human PBMC and in vivo in mice (Figures 1D & 1E). In pharmacokinetic studies in mice, oral dosing resulted in moderate exposure and short half-life due to rapid clearance (Table 1). Because of its acute efficacy and adequate mouse exposure, FC-92-EC85 was deemed a suitable tool compound for pre-clinical efficacy studies.

Table 1: Pharmacokinetic parameters of FC-92-EC85.

<table>
<thead>
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<td>PO (20)</td>
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<td>F (%)</td>
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Figure 1A: C-92-EC85 potently induced cAMP in Jurkat cells over expressing human or mouse TGR5. Data reported is the average of more than 10 independent experiments. Minimal FXR activity was seen by reporter gene assay. Data reported is the average of three replicates.

Figure 1B: C-92-EC85 potently induced GLP-1 secretion from GLUT ag cells as assessed by ELISA. Data shown is the average of more than 10 independent experiments.

Figure 1C: C-92-EC85 induced GLP-1 secretion in vivo following oral glucose challenge, as measured by ELISA. Data shown is the average +/- SEM of 8 animals.

TGR5 agonists induce changes in gall bladders of obese mice. As shown for other TGR5 agonists in the literature [20], oral dosing of FC-92-EC85 led to a 2-3 fold increase in bile volume (Figure 2). This finding was less of a concern since bile volume changes 3-4 fold during regular periods of fasting and refeeding in humans [21] and such a change can be easily tracked by ultrasound. However, following 14 days of dosing in diet-induced obese (DIO) mice, animals dosed with FC-92-EC85 had distended, yellow, thickened gallbladders (Supplemental Figure 1). In control animals, gall bladders were small, their walls were relatively simple with occasional folds and their epithelial lining was uniformly low columnar, and there was no evidence of a cellular infiltrate in the lamina propria.

By contrast, gall bladders from animals in the high dose group had a thicker wall than controls, were more complex, and contained multiple in-folding/villus-like structures and occasional inflammatory cells in the lamina propria. Epithelial cells appeared to be tall columnar and nuclei were somewhat crowded; generally the elongated oval nuclei were oriented along their long axis. The morphology of gall bladders from the low dose animals was intermediate between controls and high-dose (Figure 2B). Analysis of actin levels via immunostaining and fiber type using Sirius red revealed an increased fibromuscular supportive tissue in animals that were dosed with FC-92-EC85. Additionally, an increased immune-reactivity to Ki67 was recognized at the base of the mucosal folds even without the benefit of image analysis (Figure 2C) [22]. Other TGR5 agonists representing different chemotypes had similar, albeit less severe changes (Figure 2C).
Expression of TGR5 in the mouse gall bladder dwarfs that of other tissues [10], which may explain why such marked changes in gall bladder morphology with FC-92-EC85 were observed. Interestingly, the mRNA levels in gall bladder of other non-rodent
species are more comparable to the spleen, suggesting there may be species differences in response (Figure 3). Hamsters dosed with FC-92-EC85 showed expected increases in GLP-1 but without significant bile volume increases, further supporting the idea of species differences (Supplemental Figure 2). However, TGR5 has been detected in human gall bladder epithelium [11]. Because it was unclear whether the mouse findings would translate to higher species, we decided to test FC-92-EC85 in dogs, looking for acute effects both on metabolic endpoints and on gall bladder morphology and contractility.

**TGR5 agonist FC-92-EC85 decreases gastric motility in dogs.** Pharmacokinetic profiling of FC-92-EC85 in dogs showed adequate oral exposure for acute studies (data not shown). Oral administration of FC-92-EC85 led to an increase in pre-challenge basal GLP-1 levels (165% prior to meal challenge), but greatly diminished GLP-1 levels in response to mixed meal challenge (61% decrease at 20 minutes post-meal; (Figure 4A). Acetaminophen was added to the meal challenge and tracked over time to determine if changes in gastric motility were responsible for this altered profile. Analysis of plasma acetaminophen levels showed significant delays in absorption following FC-92-EC85 administration (Figure 4B). These findings are consistent with recent data showing decrease in gastric smooth muscle contractility with TGR5 agonists [23] (Figure 4).

**Figure 3:** Levels of TGR5 mRNA in gall bladder, spleen and brain across species.

**Figure 4A:** Levels of GLP-1 were measured at the times indicated by ELISA. FC-92-EC85 induced a significant increase in GLP-1 pre-meal (165% at time zero) but a decrease post-meal (-61% at 20 minutes). Data shown is the average of 4 animals +/- SEM.
TGR5 agonist FC-92-EC85 impedes gall bladder contractility in dogs. In order to evaluate hepatobiliary effects in dogs, a method using ultrasound to measure gall bladder volume and morphology was developed. During fasting, the gall bladder stores bile in preparation for the next meal. When a meal is ingested, the gall bladder contracts to release the stored bile into the duodenum, thus solubilizing nutrients for absorption. To validate the canine ultrasound methodology, gall bladder volume was measured following overnight fasting, where the volume should be largest, and then again following a liquid meal challenge, where gall bladder contraction should decrease overall volume. Normal changes in gall bladder size were demonstrated by ultrasound during fasting and re-feeding, with decreasing volume in response to food challenge over a one hour time period (Figure 5a). Having validated the methodology, we next determined the effects of FC-92-EC85 on this process. Single administration of FC-92-EC85 at 30 mg/kg produced gall bladder wall thickening/edema and fluid around the gallbladder starting at 1h post-dose and were more apparent at 3h post dose in all animals (Figure 5B). The wall edema and surrounding fluid disappeared in all animals by 24h after administration of FC-92-EC85, suggesting a quick recovery. FC-92-EC85 also produced a marked decrease in meal induced gallbladder emptying suggesting impaired contractile function of the gall bladder (Figure 5C).

In addition, FC-92-EC85 was associated with apparent enlargement of the gallbladder at approximately 48h post dose, compatible with an increase in overall bile volume. Meal induced gall bladder emptying was partially recovered at 48 hours post dose. Perhaps more troubling, dose dependent increases in heart rate were also noted with FC-92-EC85, even at the lowest dose tested (Figure 5D). Together, these effects may limit the utility of systemic TGR5 agonists for metabolic disease (Supplemental Figure 3).
Figure 5B: (B and C) Male beagles were dosed with FC-92-EC85 followed by a mixed meal. Gall bladder size and morphology were assessed by ultrasound prior to FC-92-EC85 dose, after FC-92-EC85 dose pre-meal, and post-meal. Images shown are representative of 3 animals. Gallbladder wall thickening/edema and fluid (indicated by arrow) around the gallbladder were observed starting at 1 hour and were more apparent at 3 hours post dose in all animals.

Figure 5D: ECG waveforms were recorded pre-dose and for 24 hours after dosing of FC-92-EC85. Dose dependent changes in heart rate were observed with FC-92-EC85. Data shown is the average of 3 animals.

Figure 5: Gall bladder effects of TGR5 agonist FC-92-EC85 in dogs.
Discussion

There has been considerable interest in the potential of TGR5 as a drug target for type 2 diabetes. Activation of TGR5 in gastrointestinal enterendocrine cells leads to increased cAMP and subsequent enhanced GLP-1 secretion, which is a clinically validated approach to control glucose levels. These effects can be accomplished without the inconvenience of protein injections and induces levels higher than those seen with DPP4 inhibitors. However, because of a wealth of treatments already available to decrease glucose levels, agents entering the clinic must demonstrate either superior chronic efficacy or bring additional benefits beyond glucose control. In the case of TGR5, its expression and anti-inflammatory activity in myeloid cells suggests it could also address the chronic inflammation characteristic of obesity and diabetes, and thus provide a benefit versus glucose lowering agents alone. This promising profile has led to the pursuit of systemic TGR5 agonists by several pharmaceutical companies.

Shortly after the role of TGR5 in GLP-1 secretion was published, there were reports that suggested that systemic TGR5 activation could have negative consequences. Tissue expression analysis shows widespread expression of the receptor in tissues beyond the GI tract and myeloid compartments, most notably in gall bladder, the heart, and the brain [10]. It has also been demonstrated that administration of bile acids leads to increases in bile volume that is not solely mediated by FXR [24], suggesting that the gall bladder expression may serve a physiological function at steady state. More recently, several authors have described that TGR5 agonists can mediate smooth muscle relaxation, both in the gall bladder and the GI tract [1,22,23]. Since TGR5 is a Gs-coupled receptor that increases cellular cAMP, this fits with studies showing that general elevations in cAMP lead to smooth muscle relaxation [25,26]. Dilation of gall bladder has been shown to cause mucosal hyperplasia [27]. Consistent with these findings, we found profound effects of chronic TGR5 activation on the mouse gall bladder, including hyperplasia and increased wall thickness. These effects were seen with multiple scaffolds, suggesting this may be a general consequence of TGR5 activation. However, they were more pronounced in mice on high fat diet compared to wild type mice.

Together with the lack of bile volume effect in hamsters, we questioned whether the gall bladder effects were species specific. Despite differences in absolute quantity of TGR5 mRNA in the gall bladder, these deleterious effects could also be witnessed acutely in dogs by ultrasound and were accompanied by defects in contractility. Decreased contractility was also noted in the gastrointestinal tract, where delayed gastric emptying was seen following FC-92-EC85 administration.

The gall bladder findings shed considerable doubt on the utility of systemic TGR5 agonists in type 2 diabetes, a population that already exhibits an increase in gall bladder dysfunction. Moreover, dogs treated with FC-92-EC85 showed elevated heart rate and QT prolongation. While a compound specific effect is conceivable due to the in vitro hERG inhibition, Piotrowski et al. has recently reported similar effects on heart rate and blood pressure with two TGR5 agonists from different compound classes and concluded that the observations were an on-target effect [28]. More recently, Fryer et al. reported that TGR5 activation shows a strong and pronounced effect on arterial vasodilation in a Kca1.1 dependent manner [29]. The cardiovascular effects observed with FC-92-EC85 in our studies are consistent with these reports and demonstrate that potent, bioavailable agonists will likely have low therapeutic indices.

To address the issues with systemic agonists, several small companies are pursuing GI-restricted agonists of TGR5 [14-17]. These would hopefully activate GLP-1 secretion in the intestine without significant systemic exposure. This concept faces several challenges. First, TGR5 is enriched in specific segments of the intestine, including the jejunum, ileum, and colon. Ensuring that enough compounds is delivered selectively and retained long enough in this location represents a significant challenge. Second, we have witnessed effects on bile volume at compound exposures below what is required for GLP-1 secretion. This suggests that a gastrointestinal-restricted compound may need to avoid all absorption. Since it is unclear whether TGR5 resides most in the luminal or basal membranes of the enterendocrine cells, it remains to be seen whether strict luminal exposure will be efficacious. Third, regardless of where it is delivered, chronic delivery of TGR5 agonists could lead to loss of receptor expression from the cell surface and subsequent loss of activity, also known as tachyphylaxis. Indeed, studies with our more optimized candidates have demonstrated a loss of activity during chronic treatment [30,31]. Thus, even a gastrointestinal-restricted agonist will need to be carefully selected to avoid desensitization. Finally, focusing only on the GLP-1 secretion aspect of TGR5 agonism reduces it to being just another glucose lowering agent, a profile that does not meet the current patient needs. Coupled with the severity of the on-target side effects seen with systemic exposure, these challenges suggest that systemic TGR5 agonists may never realize their clinical potential.

Supplementary Figure 1: Gross morphology of gall bladders following FC-92-EC85 treatment. Male diet-induced obese (DIO) mice were dosed with 3, 10, or 30 mg/kg of FC-92-EC85 twice a day for 14 days. Animals were then sacrificed, and gall bladders were collected. Shown are representative gall bladders from each group (one of 8 each).

Supplemental Figure 2: Effects of FC-92-EC85 on GLP-1 secretion and bile volume in hamsters. (A) Male Golden Syrian hamsters were fasted overnight and then dosed with 30 or 100 mg/kg of FC-92-EC85 followed by 3 g/kg glucose challenge. For assessment of GLP-1 secretion, plasma was collected at 10 minutes post-challenge and GLP-1 levels were measured by ELISA. Data shown is the average of 8 animals. (B) Hamsters were deprived of food one hour prior to administration of FC-92-EC85 (10, 30 or 100 mg/kg). Two hours after dosing, gall bladders were isolated and their volume assessed. Data shown is the average of 8 animals.

Supplemental Figure 3: Characteristics of TGR5 agonists TA-80-NY46 and VA-72-MP94. Compounds were added in dose response to Jurkat cells overexpressing human or mouse TGR5, and agonistic activity was determined by measuring induction of cAMP. Data reported is the average of more than 5 independent experiments. To determine FXR activity, FC-92-EC85 was added to 293T cells overexpressing a luciferase reporter gene under the control of the FXR promoter. Data reported is the average of three replicates.

References


