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A Novel Acyl-CoA: Diacylglycerol Acyltransferase 1 (DGAT1) Inhibitor, GSK2973980A, Inhibits Postprandial Triglycerides and Reduces Body Weight in a Rodent Diet-induced Obesity Model

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SK, JA, VGT, DT and GB designed and interpreted the experiments. Authors AA, VBJ, PA, MY, SM, ST, TW and DT conducted the experiment and analyzed the data. Authors RST and MC designed the inhibitors and authors SK, JA and VGT wrote the manuscript. All authors discussed the results, reviewed the manuscript critically and approved the final manuscript.

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ABSTRACT

Background and Aim: Acyl-CoA: diacylglycerol acyltransferase 1 (DGAT1) is a key enzyme catalyzing the final step of triglyceride (TG) synthesis and is implicated in fat absorption and disposition. However, development of several DGAT1 inhibitors has been hampered due to unacceptable gastrointestinal (GI) tolerability observed in clinical trials. Our aim is to develop a novel, potent and selective DGAT1 inhibitor that reduces plasma TG levels and body weight with acceptable GI tolerability in a rodent model of obesity.
Study Design: *In vitro* enzyme and cellular assays as well as *in vivo* experimental studies in mice and rat examining the effect of drugs on triglycerides, fecal fat, body weight and food intake.
Place and Duration of Study: Virtual proof of concept, GSK USA & Cellzome, GSK Germany and Collaborative research, GVK, India between 2011-2017.
Methodology: A radiometric assay of TG formation evaluated DGAT activity and a thermal shift assay determined target specificity. Fasted mice received an oral corn oil bolus to model hypertriglyceridemia. Intralipid administration in fasted rats was used to evaluate triglyceride clearance. Mice were fed a high fat diet to induce obesity.
Results: Herein, we describe GSK2973980A as a novel, potent and selective DGAT1 inhibitor that reduced plasma TG levels in postprandial lipid excursion and impaired clearance studies in rodents and reduced body weight and food intake in obese mice. Interestingly, robust plasma TG reduction was accompanied by varying degrees of fecal lipid excretion. Alternate dosing via the subcutaneous route maintained a significant effect on plasma TG reduction with no altered fecal lipid excretion, suggesting GI tolerability may be modulated by limiting local GI exposure of DGAT1 inhibitors.
Conclusion: Our data suggest that the impaired TG clearance model in the rat can be used to identify DGAT1 inhibitors with potentially better GI tolerability.

Keywords: Fatty acid; DGAT; triglycerides; lipid droplets; obesity.

ABBREVIATIONS

GI: gastrointestinal; FFA: free fatty acids; NASH: non-alcoholic steatohepatitis; MGAT: monoacylglycerol acyltransferase; HFD: high fat diet; GLP-1: glucagon-like peptide 1; PYY: peptide YY; DIO: diet-induced obesity; C10-CoA: decanoyl CoA; C10-DAG: didecanoyl acylglycerol; QD: quaque die (once daily); BID: bis in die (twice daily); CETSA: cellular thermal shift assay; EPXH: epoxide hydrolase; PK: pharmacokinetic; SDR39U1: epimerase family 2; AST: aspartate transaminase; ALT: alanine transaminase; PO: per os (orally); IV: intravenous; SC: subcutaneous; PGC-1α: peroxisome proliferator-activated receptor gamma; DGAT1: Acyl-CoA: diacylglycerol acyltransferase 1; ACAT: acyl-coenzyme A:cholesterol acyltransferase; HPMC: hydroxypropyl methylcellulose; sf9: Spodoptera frugiperda; CB1: cannabinoid receptor 1; TCHO: total cholesterol; ANOVA: analysis of variance; SEM: standard error of mean.

1. INTRODUCTION

Dietary fats are digested into free fatty acids (FFA) and 2-monoacylglycerol and absorbed at the intestinal wall and sequentially esterified by monoacylglycerol acyltransferase (MGAT) and DGAT to re-synthesize triglyceride (TG) in the enterocytes. TG in various tissues has been linked to many metabolic diseases such as obesity and non-alcoholic steatohepatitis (NASH). DGAT1 catalyzes the final and only committed step of TG synthesis and is expressed widely in all tissues examined, with the highest expression levels in the small intestine [1]. Genetic and pharmacologic studies suggested an important role of DGAT1 in fat disposition, food intake and the control of weight gain.

Beneficial metabolic effects of inhibiting DGAT1 became evident in dgat1-null mice that showed reduced plasma and tissue TG levels associated with increased sensitivity to insulin [2] and resistance to diet-induced obesity and hepatic steatosis [3,4]. Reduced intestinal TG synthesis and post-absorptive chylomicronemia following an oral lipid challenge were also observed [5]. Interestingly, transgene-mediated expression of DGAT1 only in the intestine in an otherwise dgat1-null background reversed the dgat1-deficient phenotype [6]. Conversely, over-

expression of DGAT1 in the intestine led to weight gain, indicating a critical role of intestinal DGAT1 in obesity. A second enzyme in this family, DGAT2, is highly expressed in the liver, exhibits little or no sequence homology with DGAT1 and is the key enzyme catalyzing de novo TG synthesis in the liver. Showing reduced TG, dgat2- null mice are significantly smaller and die shortly after birth [7]. These data provide a strong rationale for targeting DGAT1 for the treatment of metabolic diseases such as obesity and NASH [8].

Several companies have developed DGAT1 inhibitors that cause reduction in weight gain in diet-induced obesity (DIO) and protect against hepatic steatosis [9,10]. However, development of two DGAT1 inhibitors from AstraZeneca and Pfizer [11] has been discontinued due to GI tolerability issues related to diarrhea [12,13], whereas Novartis' DGAT1 inhibitor Pradigastat is progressing in phase III clinical studies and appears to have acceptable GI tolerability (ClinicalTrials.gov Identifier: NCT01514461) [14]. Therefore, a preclinical model that incorporates GI tolerability along with efficacy is essential to the careful selection of promising candidates with minimal GI tolerability issues.

In the present study, we describe the identification and characterization of a novel DGAT1 inhibitor, GSK2973980A, in models of hypertriglyceridemia and diet-induced obesity. In addition, the effect of DGAT1 inhibition on fecal lipid excretion in rodents was studied to understand and correlate the preclinical data to clinical GI effects. Using this model, we also demonstrate the potential of an alternate dosing route to limit GI intolerability effects while maintaining efficacy. These data suggest novel ways of evaluating DGAT1 inhibitors in the clinical setting to limit GI intolerability.

2. MATERIALS AND METHODS

2.1 Reagents

Decanoyl Coenzyme A (C10-CoA), was obtained from Sigma (Bangalore, India). Didecanoylacylglycerol (C10-DAG) was obtained from Caymen via Genetix Biotech Asia Pvt. Ltd, (Hyderabad, India). C10-Co A, [Decanoyl-1-14C] and Microscint E were from Perkin Elmer (Singapore). C10-Co A, [Decanoyl-1-3H] and [14C] Sodium Oleate (ARC0297) were from American Radiolabeled Chemicals, (St. Louis, MO). Partisil K6 60Å TLC plates were from Whatman, via Apex Chromatography Pvt Ltd. (Secunderabad, India). C2C12 mouse myoblast cells were obtained from ATCC. Triglyceride Kit was from ERBA Diagnostics Mannheim GmbH (Mannheim, Germany). GSK2973980A [(S)-2-(6-(5-(3-(3,4-difluorophenyl)ureido)pyrazin-2-yl)-1-oxo-2-(2,2,2-trifluoroethyl)-1,2,3,4-tetrahydronaphthalen-2-yl)acetic acid] [15] was synthesized at GVK Biosciences, Hyderabad, India. GSK2646224A (PF-04620110) and Pradigastat are the published DGAT1 inhibitor from Pfizer and Novartis, respectively [11,16] and were synthesized at GVK Biosciences and used as reference compounds [11,16]. Orlistat was obtained from Transgene Biotech Limited (Hyderabad, India). Rimonabant was obtained from Shanghai Me-too PharmaTech Co., Ltd (Shanghai, China).

2.2 Radiometric Human DGAT1 and DGAT2 Assays

The radiometric assay directly measures radiolabeled TG formation as a measure of human DGAT1 or DGAT2 activity using tritium [3H] labeled C10-CoA (decanoyl CoA) as one of the substrates with 25 µM C10-DAG (didecanoyl acylglycerol) and 0.05 µg/mL human DGAT1. The newly formed TG was extracted into organic phase and counted using a MicroBeta multiplate reader from Perkin Elmer (Downers Grove, IL, USA). Recombinant human and rat DGAT1 and DGAT2 were expressed in Sf9 (Spodoptera frugiperda) insect cells using a baculovirus system.

2.3 Thermal Shift Assay for Target Engagement

The experiments were performed as previously described [17,18]. An in-house version of software was used for the quantification [19]. The thermal stability of a protein was considered reproducibly altered when a > 1 log2 fold change compared to the respective vehicle control was observed at two consecutive temperatures close to the respective melting point.

2.4 Cell-based DGAT1 Assay

This assay measures the TG formation by endogenous DGAT1 using labeled [14C]-Oleic acid as a substrate and tracer during the TG neo-synthesis in C2C12 mouse myoblast cells. Cells were incubated with GSK2973980A followed by

20 min incubation with [14C] sodium oleate with 0.001% fatty acid free BSA. Labeled TG was resolved by TLC and quantified using a phosphorimager (Bio-Rad, Quarry Bay, Hong Kong).

2.5 Mouse Model of Acute Postprandial Hypertriglyceridemia

Overnight-fasted Swiss Albino mice were challenged with an oral bolus of corn oil (5 mL/kg) 30 min post compound administration [20]. Plasma was collected for TG estimation by a colorimetric glycerophosphate oxidase method (Transasia Bio-medicals Ltd., India) and read on a Spectramax Plus 384 (Molecular Devices, Sunnyvale, CA, USA).

2.6 Rat Model of Impaired TG Clearance

Intralipid was used as a high fat meal (10 mL/kg, single oral dose) [16]. Triton was used to stabilize the TG levels by impairing hydrolysis or the tissue uptake of TG. Overnight-fasted Sprague Dawley rats were orally dosed with Intralipid 30 min post compound administration. Triton WR-1339 (100 mg/kg) was administered intravenously (IV) 30 min later. Plasma was collected for TG estimation and read on a Spectramax Plus 384.

2.7 Fecal Lipid Analysis

Fecal samples from animals of each group from 0 to 240 min were collected, pooled and air dried overnight. Fecal total lipid was extracted using the Folch method [21]. The pooling of fecal fat from each cohort of the study was necessitated by the requirement of sufficient fecal fat for measurement since the amount of fecal fat from a single rat was not sufficient. For this reason, no statistical analysis was performed on fecal fat data. Instead the fecal fat from n=8 mice represent pooled fat content representing the whole cohort.

2.8 Diet-induced Obesity Model

Following one week of acclimation, six-week old male C57BL/6J mice were fed a high fat diet (HFD) (D12492, Research Diet, New Brunswick, NJ) [22] (caloric contribution: protein, 20%; fat, 60%; carbohydrates, 20%) ad lib for 13 weeks for obesity induction. Mice continued to be fed HFD and were randomized by body weight into the following 9 treatment groups (n=8): Group 1 was administered vehicle (0.5% HPMC; hydroxypropyl methylcellulose and 2% tween 20) once daily (QD); Group 2 was administered 3 mg/kg Rimonabant QD; Group 3 was administered 1 mg/kg GSK2973980A twice daily (BID); Group 4 was administered 3 mg/kg GSK2973980A BID; Group 5 was administered 10 mg/kg GSK2973980A QD; Group 6 was administered 10 mg/kg GSK2973980A BID; Group 7 was administered 30 mg/kg GSK2973980A QD; Group 8 was administered 30 mg/kg GSK2973980A BID; and Group 9 was pair fed to match Group 6 (10 mg/kg GSK2973980A BID). Oral dosing continued for 21 days with daily body weight and food consumption measurements. Data were plotted as means ± SEM (standard error of the mean). Statistical significance was evaluated by one-way ANOVA (analysis of variance) followed by Dunnett's test using SPSS statistical software. Blood was collected prior to dosing and on day 21 blood and plasma were prepared before being stored at -20°C. Plasma TG levels were measured according to manufacturer's instructions (Shanghai Ronghsheng Biotech Co., Ltd., Shanghai, China).

All animal studies were conducted in accordance with the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals and were reviewed by the respective Institutional Animal Care and Use Committee according to the guidelines established by AAALAC. Male Swiss Albino mice were obtained from Bioneeds Ltd. (Bangalore, India). Male Sprague Dawley rats were obtained from Gentox Bioservices Pvt. Ltd. (Hyderabad, India). Test compounds and lipid were administered orally by gavage. Six-week old male C57BL/6J mice were obtained from Shanghai Laboratory Animal Center (Shanghai, China).

3. RESULTS

3.1 Identification of GSK2973980A as a Novel, Potent and Selective DGAT1 Inhibitor

GSK2973980A is a novel DGAT1 inhibitor [15]. It is a potent inhibitor of DGAT1 with an IC50 value of 3.3 nM and 13 nM in human (Fig. 1A) and rat (not shown) enzyme assays, respectively with >2900-fold selectivity for DGAT2 as well as Acyl-CoA: cholesterol acyltransferase (ACAT) 1 and ACAT2 enzymes (data not shown). In a cell based assay using C2C12 mouse myoblast cells,

GSK2973980A inhibited TG synthesis in a concentration-dependent manner with an IC50 value of 75 nM (Fig. 1A).

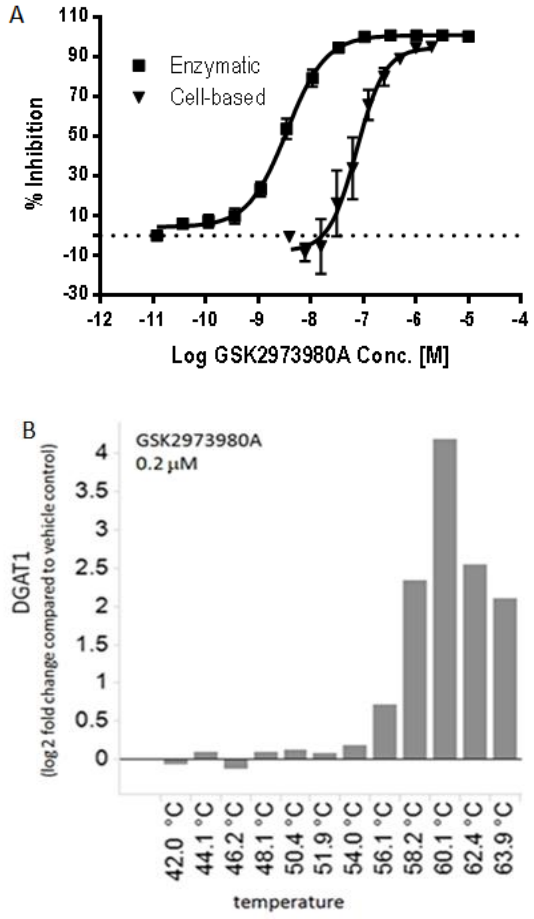


Fig. 1. Activity profile of GSK2973980A.
A. Activity profile of GSK2973980A in radiometric human enzymatic and C2C12 mouse myoblast cell-based assays. Data shown here represent mean ± SEM. obtained from 10 (enzyme) and 3 (cell) independent experiments. B. Target engagement of DGAT1 protein with GSK2973980A in HepG2 cells using cellular thermal shift assay (CETSA) showing stabilization of the DGAT1 protein as compared to untreated cells

Evidence of target engagement of DGAT1 by GSK2973980A in HepG2 cells was demonstrated using a cellular thermal shift assay combined with multiplexed quantitative mass spectrometry. After treatment of HepG2 cells with 0.2 μM of GSK2973980A, DGAT1 displayed an increase in thermal stability as compared to vehicle at temperatures between 54°C and 63.9°C (Fig. 1B). Out of the 7103 quantified proteins, in addition to DGAT1, the thermal stability of only two other proteins, namely epoxide hydrolase (EPHX2) and the epimerase family protein (SDR39U1), was reproducibly altered, (Supplementary Fig. 1) indicating high selectivity.

3.2 GSK2973980A Shows a Favorable Pharmacokinetic Profile

We next conducted pharmacokinetic (PK) studies in mice and rats. GSK2973980A showed clearance of 13.25 mL/min/kg, 2.24 h half-life, and 41% bioavailability in mouse; and clearance of 3.32 mL/min/kg, 2.64 h half-life, and 48% bioavailability in rat (Supplementary Table S1). We also determined the tissue-to-plasma ratio of GSK2973980A in various rat tissues following oral administration. Interestingly, jejunum and duodenum had ~2-4-fold higher levels of the drug compared to plasma, whereas the ratio for liver to plasma was 0.8 (Supplementary Table S2). The favorable PK profile allowed an assessment of the effect of DGAT1 inhibition in both mice and rats.

3.3 GSK2973980A Reduced Plasma TG Levels in a Mouse Model of Lipid-induced Acute Postprandial Hypertriglyceridemia

Acute postprandial hypertriglyceridemia was used as a pharmacodynamic model to assess the effect of enterocyte DGAT1 inhibition on TG synthesis in vivo. Plasma TG levels were reduced in a dose-dependent manner by oral administration of GSK2973980A, with a good correlation between increasing plasma concentrations of GSK2973980A and decreasing plasma TG levels with an estimated ED_{50} of 0.088 mg/kg (Fig. 2A). This inhibition occurred at a low plasma concentration of GSK2973980A (approximately 17 ng/mL, or ~32 nM at 0.1 mg/kg).

Since this effect on TG excursion is largely due to DGAT1 inhibition in the enterocytes where the inhibitor concentration is likely to be significantly higher during drug absorption following oral dosing, it was of interest to determine the duration of action of GSK2973980A in reducing TG levels. In this experiment, lipid challenge occurred either 4 or 15 h after drug treatment allowing for all the drug to be absorbed such that enterocytes would have to be supplied with drug only through the systemic circulation. The effect

of GSK2973980A on postprandial TG levels was persistent even when the compound was dosed at 10 mg/kg either 4 or 15 h prior to lipid challenge with approximately 87%, and 77% reduction, respectively (Fig. 2B). Plasma concentrations of GSK2973980A measured 15 h post-dose (approximately 240 ng/mL) were sufficiently higher than the IC50 value observed in the in vitro mouse cell based DGAT1 assay (~39 ng/mL). These results clearly suggest that the plasma concentrations of GSK2973980A continued to significantly reduce plasma TG levels up to 15 h after dosing.

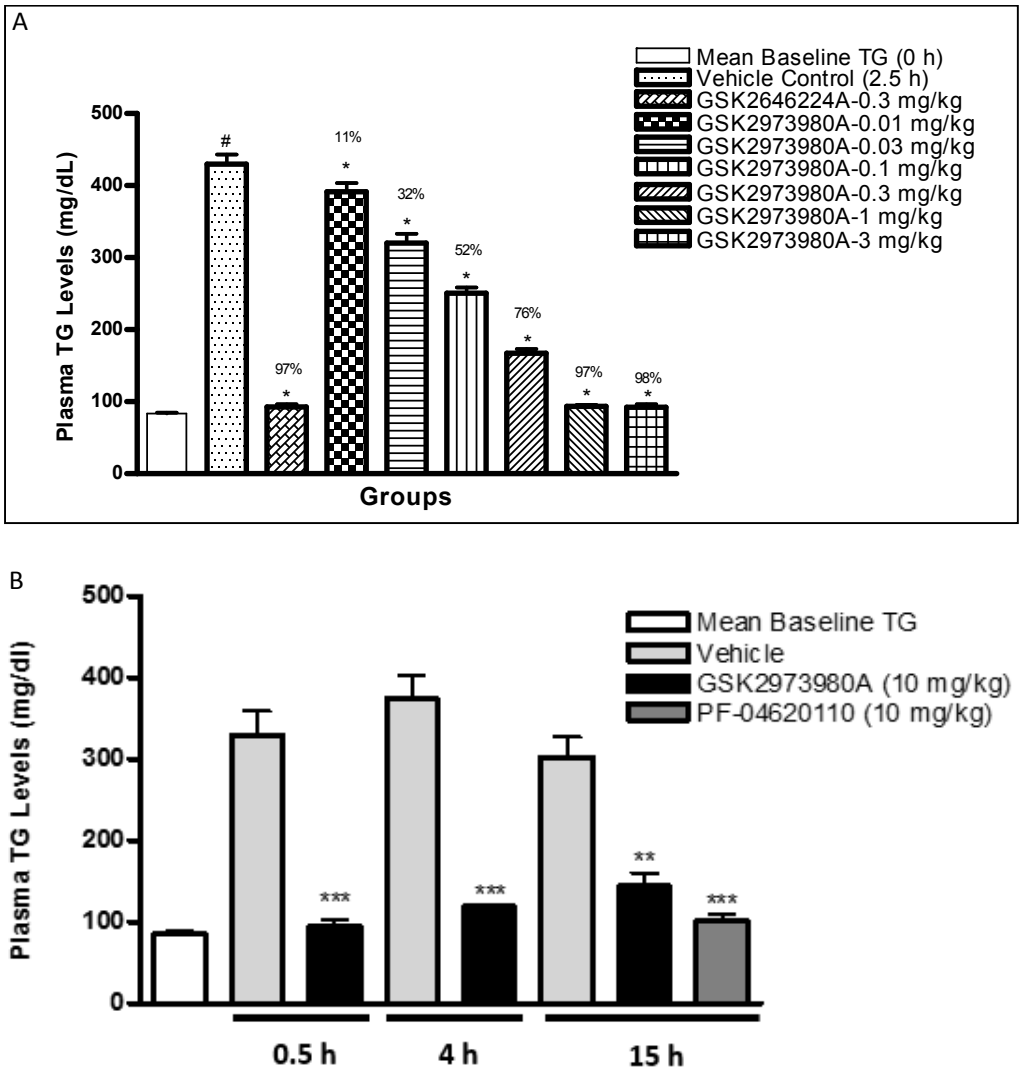
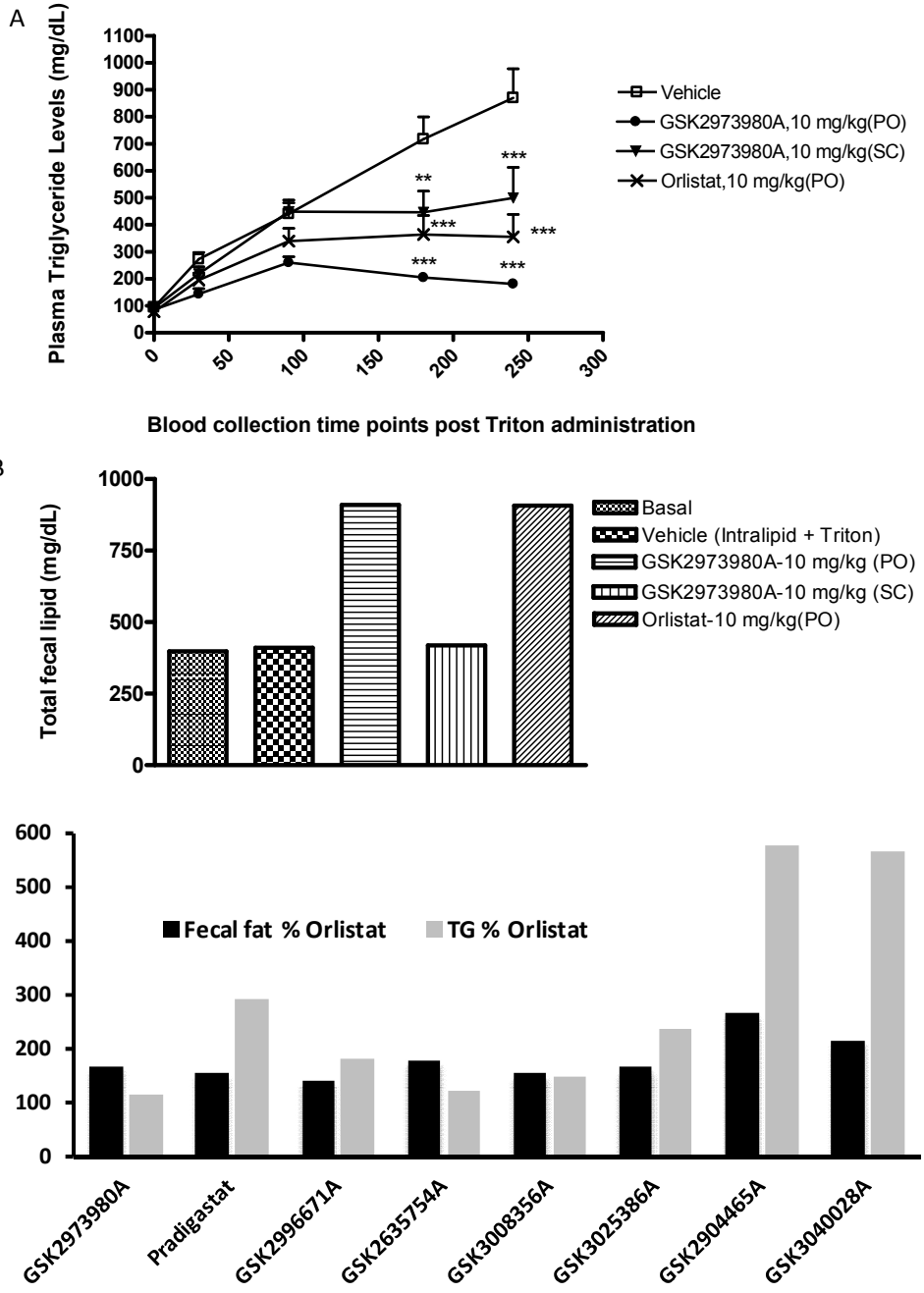


Fig. 2. Effect of GSK2973980A in a mouse model of postprandial hypertriglyceridemia

A. *Various doses of compound were administered orally to male Swiss Albino mice 30 min prior to an oral bolus of corn oil. Plasma TG levels were measured at 2.5 h post corn oil challenge. Values above the bar represent percent inhibition.* compared with Vehicle control (2.5 h), p<0.001, # compared with Mean Baseline control (0 h), p<0.001. GSK2646224A (PF-04620110) is the published DGAT1 inhibitor from Pfizer and was used as a reference compound [11].* ***B.*** *For the duration of action study, vehicle or test compounds (10mg/kg) were dosed orally at 15, 4, and 0.5 h prior to corn oil challenge. Plasma TG levels were measured at 2.5 h post corn oil challenge. Statistical analysis was performed using one-way ANOVA followed by Bonferroni post-hoc test. **p=0.003 vs. vehicle, ***p<0.0001 vs vehicle.*

3.4 TG Reduction in a Rat Model of Impaired TG Clearance by GSK2973980A

Use of Triton WR-1339 after Intralipid dosing results in a high plasma TG excursion comparable to that observed in familial chylomicronemia. A significant and steady TG excursion was observed in the plasma of vehicle-treated animals reaching up to 870 mg/dL by 240 min that was significantly inhibited by orlistat used as a reference compound. Oral dosing of GSK2973980A significantly inhibited Intralipid-induced TG excursion (~88% at 240 min) (Fig. 3A). To determine if bypassing gut would also be efficacious, we utilized the subcutaneous route (SC) of administration of the drug. SC dosing of GSK2973980A also resulted in significant but slightly less efficacy compared to oral dosing (Fig. 3A).



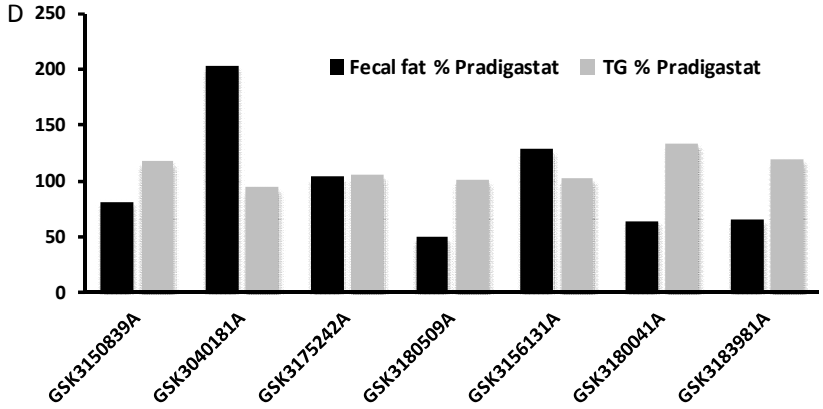


Fig. 3. Effect of GSK2973980A in Rat model of impaired TG clearance

A. *Compounds were administered either orally (Per os: PO) or subcutaneously (SC) to male SD rats 30 min prior to an oral bolus of intralipid. Thirty min later, an IV dose of Triton WR-1339 (100 mg/kg) was administered via lateral tail vein. Plasma TG levels were then measured at various time points post Triton WR-1339 administration. Statistical significance was assessed using Repeated measures two-way ANOVA followed by Tukey's post-hoc test. ** p <0.01, *** p<0.001 vs vehicle.* ***B.*** *Fecal samples from animals of each group from 0 to 240 min were pooled, followed by lipid extraction and lipid content estimation using the protocol described.* ***C.*** *Graph depicting fecal lipid content and TG normalized to that of Orlistat in the rat impaired TG clearance model for several DGAT1 inhibitors.* ***D.*** *Graph depicting fecal lipid content and TG normalized to that of Pradigastat in the rat impaired TG clearance model for several DGAT1 inhibitors. All experiments were conducted with n=8 per group. The pooling of fecal fat from each cohort of the study was necessitated by the requirement of sufficient fecal fat for measurement since the amount of fecal fat from a single rat was not sufficient. For this reason, no statistical analysis was performed on fecal fat data. Instead the fecal fat from n=8 mice represent pooled fecal fat content representing the whole cohort*

Given the GI intolerability observed with various DGAT1 inhibitors and to better understand the role of DGAT1 inhibition on intestinal lipid absorption, total fecal lipids were also measured. Oral dosing of GSK2973980A resulted in ~2-fold increase in fecal lipids, whereas SC dosing did not alter the fecal lipids compared to the vehicle. Orlistat caused a substantial increase in total fecal lipids compared to intralipid treatment alone (Fig. 3B). We extended the rat fecal lipid excretion assay to a set of compounds from 6 distinct chemical series to understand the extent of fecal lipid excretion and TG inhibition. As shown in Fig. 3C, all DGAT1 inhibitors caused an increase in fecal lipid excretion compared to Orlistat (~140 – 265%) with varying levels of TG inhibition ranging from 115 – 577% that of Orlistat. Since it was difficult to discriminate among compounds in relation to Orlistat, a set of compounds were tested using Pradigastat (Novartis) as a reference compound [23]. Several compounds showed profiles similar to or better than Pradigastat with respect to both fecal lipid excretion and TG reduction (Fig. 3D). DGAT1 inhibitors had variable impact on fecal lipid excretion (~49.7 – 204%) whereas TG inhibition ranged from 95 – 133% compared to that of Pradigastat. Notably, GSK3180509A, GSK3180041A, and GSK3183981A caused substantially less fecal fat excretion (50%, 65%, and 66% of Pradigastat) with comparable or slightly better TG inhibition (102%, 133%, and 120%, respectively) (Fig. 3D). These data suggest that the rat intralipid-Triton-induced postprandial TG model combined with the fecal fat measurement can be used to triage and prioritize compounds to identify those that may have acceptable GI tolerability profiles. Furthermore, alternative dosing routes that bypass the GI tract and minimize local drug concentration may also improve tolerability in the clinic, especially for patients on high fat diets.

3.5 GSK2973980A Causes Weight Loss in a Mouse Model of Diet-induced Obesity

Having demonstrated a significant effect on TG excursion, we next tested GSK2973980A in a chronic 21-day study in the mouse model of high fat diet-induced obesity. Cumulatively, animals in the vehicle group gained ~5.48% in body weight over the 21-day treatment period, while animals

administered GSK2973980A QD or BID at various doses or rimonabant lost significant body weight (Fig. 4A and Table 1). However, GSK2973980A was less efficacious than rimonabant, a cannabinoid receptor 1 (CB1) inverse agonist used as a positive control.

The reduction in body weight for all groups correlated with the reduction in the food intake and appear to be compound exposure related (Table 1). The pair fed group (pair fed with GSK2973980A 10 mg/kg BID group) had a cumulative food intake reduction (17.4 %) comparable to the GSK2973980A 10 mg/kg BID group (16.54%) and similar body weight reduction (Fig. 4B and Table 1). In a parallel PK study of GSK2973980A, the Cmax (maximum concentration) and AUC (area under the curve) of GSK2973980A increased in a dose-dependent manner. The 10 mg/kg BID group achieved greater body weight loss than the 10 mg/kg QD group with an AUC of 6477 h*ng/mL after 21-day treatment (Fig. 4A and Table 1). These data indicate that reduction in food intake is largely responsible for the weight loss and that BID dosing was more efficacious than QD dosing possibly suggestive of continued inhibition of the DGAT1 enzyme from BID dosing.

Although occasional changes were observed, none of the differences in any of the other parameters (perirenal fat weight, blood insulin, plasma AST (aspartate transaminase) / ALT (alanine transaminase) / total cholesterol or liver and muscle TG) was statistically significant (Supplementary Figs. 3-5). Significant TG reduction was observed in the GSK2973980A 1 mg/kg BID, GSK2973980A 30 mg/kg QD and pair fed groups (Fig. 4C). Treatment with GSK2973980A for 21 days also induced a substantial decrease in lipid vacuoles in the 3 mg/kg or 10 mg/kg BID groups of GSK2973980A compared to either vehicle or rimonabant-treated DIO mouse livers and jejunums suggesting a treatment-dependent effect (Fig. 4D).

5. DISCUSSION

5.1 GSK2973980A is a Potent and Selective DGAT1 Inhibitor

Synthesis of TG is a fundamental biochemical pathway important for nutrient utilization and energy storage. Excess TG, however, have been linked to human diseases such as obesity, insulin resistance, dyslipidemia, and NASH [20]. DGAT1 is a key enzyme responsible for disposition of TG following dietary intake of fat. In this study, we report the identification of a novel and structurally distinct DGAT1 inhibitor, GSK2973980A. In in vitro enzymatic and cell-based assays, GSK2973980A demonstrated potent activity on DGAT1 and high selectivity against not only DGAT2 but also ACAT1 and 2. Indeed, in a cellular thermal shift multiplexed quantitative mass spectrometry assay, GSK2973980A engaged only DGAT1 and two other proteins (EPHX2 and SDR39U1) out of 7103 proteins quantified in HepG2 cells.

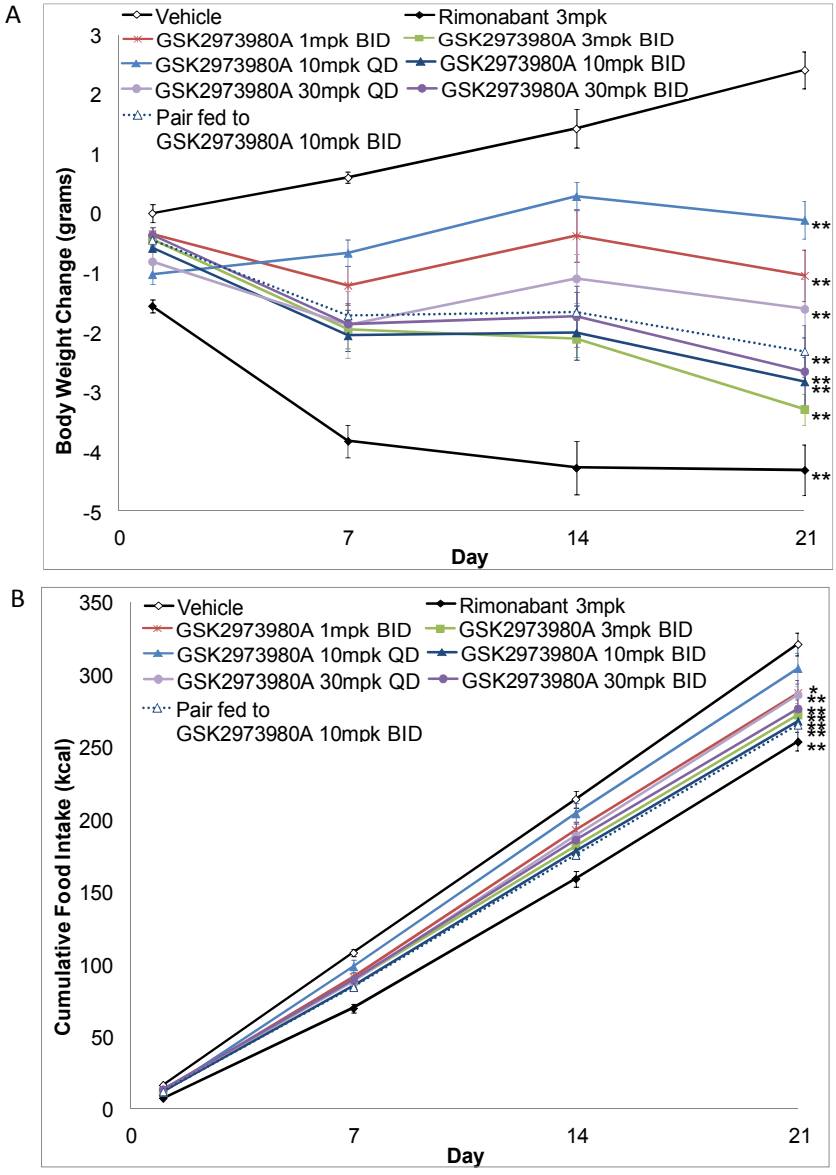
5.2 GSK2973980A Inhibits Acute Postprandial Hypertriglyceridemia

Dose-dependent reduction in plasma TG excursion was demonstrated in a model of lipid-induced acute postprandial hypertriglyceridemia

Table 1. Effect of GSK2973980A on Cumulative Body Weight and Food Intake in a Chronic 21-day DIO Model in Mice

Group	Cumulative body weight reduction (%)	Body weight P Value	Cumulative food intake reduction (%)	Food intake P value	AUC0-last (hr*ng/mL) of GSK2973980A	
					Day 1	Day 21
Rimonabant 3 mpk	-15.34	0.01	-21.04	0	ND	ND
GSK2973980A 1 mpk BID	-7.93	0.235	-10.52	0.012	1496	1702
GSK2973980A 3 mpk BID	-12.94	0.097	-15.19	0	7455	6207
GSK2973980A 10 mpk QD	-5.74	0.761	-5.19	0.423	7503	1603
GSK2973980A 10 mpk BID	-12.01	0.042	-16.54	0	11103	6477
GSK2973980A 30 mpk QD	-9.16	0.233	-10.9	0.004	18738	19816
GSK2973980A 30 mpk BID	-11.6	0.056	-13.92	0	62394	33640
Pair fed to GSK2973980A 10 mpk BID	-10.78	0.12	-17.4	0	ND	ND

with an estimated ED50 of 0.088 mg/kg. Such potent activity of GSK2973980A is likely related to its high levels in the enterocytes during the drug absorption process, as the plasma compound level is not sufficient to account for the observed efficacy. Optimal efficacy requires sustained and durable suppression of postprandial TG that was demonstrated when the lipid challenge was conducted either 4 or 15 h following a single dose of drug. At these time points, most of the administered drug is absorbed such that any detected enterocyte levels of the drug would most likely have to be supplied through the systemic circulation. In this experiment, the effective TG reduction was still maintained for up to 15 h. Thus a durable postprandial TG suppression for up to 15 h following a single dose is likely due to sustained exposure of GSK2973980A in the plasma. As expected, a much higher dose of the drug (10 mg/kg) is needed to maintain plasma levels at sufficient concentration for significant DGAT1 enzyme inhibition.



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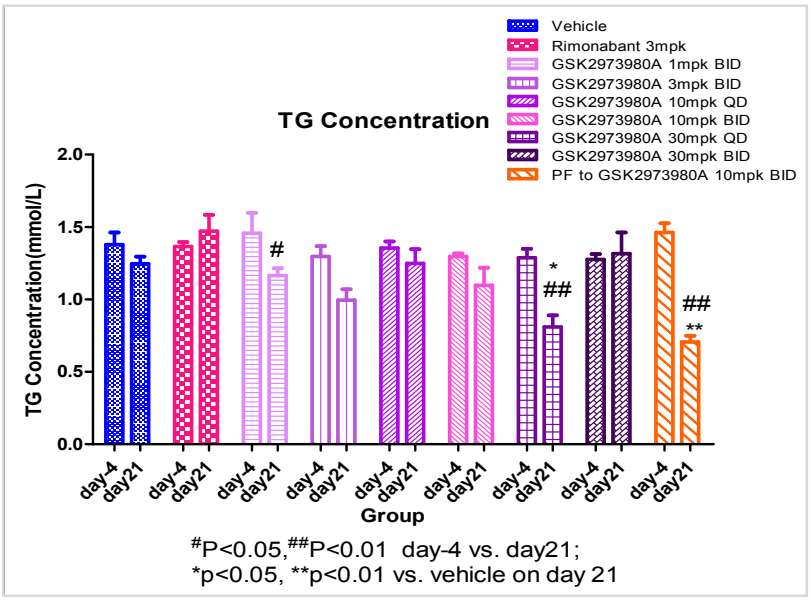

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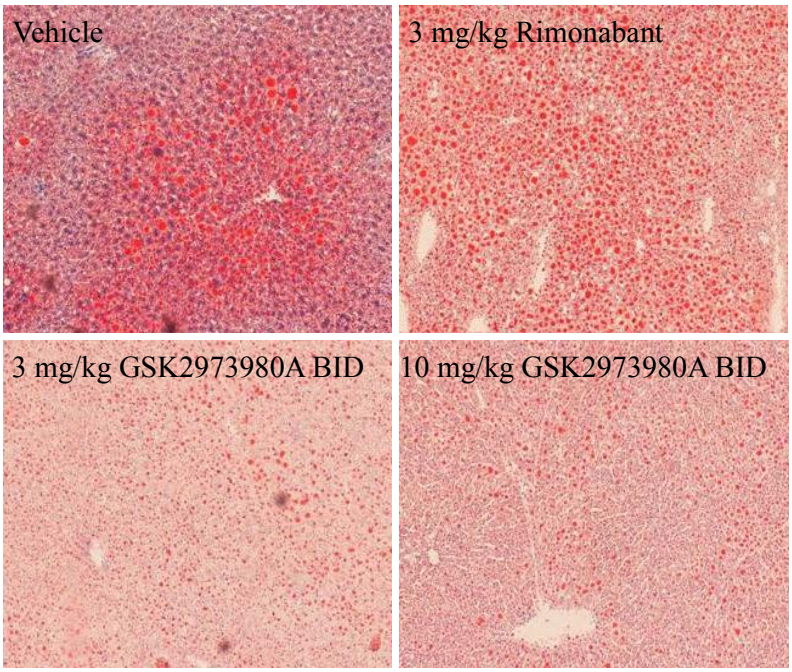

Fig. 4. Effect of GSK2973980A on cumulative body weight, food intake, plasma triglyceride, and liver vacuoles in DIO mice

Male C57BL/6J mice were made obese by HFD feeding for 13 weeks. Mice were randomized based on body weight and treated with vehicle, rimonabant (positive control) or various doses of GSK2973980A once (QD) or twice daily (BID). Body weight and food intake were measured daily. Data were plotted as means ± SEM. Statistical significance was evaluated by one-way ANOVA followed by Dunnett's test using SPSS statistical software. ***A.*** *Cumulative change in body weight.* ***B.*** *Cumulative food intake. *p<0.05 and **p<0.01 vs. vehicle.* ***C.*** *Plasma TG levels were measured on day -4 (prior to dosing) and day 21. n=8 per group. Values that are significant outliers by Grubb's test were removed from the analysis.* ***D.*** *Representative images of Oil Red O-stained liver sections. Images clockwise from upper left are from mice treated with vehicle, 3 mg/kg Rimonabant, 3 mg/kg GSK2973980A BID (twice daily), and 10 mg/kg GSK2973980A BID.*

5.3 Measurement of Fecal Fat as a Measure of GI Intolerability

GI intolerability of varying degrees has been observed in all clinical studies conducted with DGAT1 inhibitors to date, leading to discontinuation of development of two DGAT1 inhibitors from AstraZeneca and Pfizer. These adverse events are likely related to DGAT1 inhibition based on the recently reported genetic data showing loss of function DGAT1 mutations in 2 subjects with congenital diarrheal disorder [24,25]. Interestingly, these effects were not observed either in dgat1-null mice or when DGAT1 is pharmacologically inhibited in mice. Recent studies with radio labeled (^{14}C triolein) corn oil fed to mice showed that DGAT1 inhibition resulted in increased levels of FFAs more distally in the small intestine, and increased fecal excretion of lipids (mainly FFAs and DAGs) [13]. Gut mucosal exposure to increased amounts of FFAs and inflammation may play a role in steatorrhea and diarrhea [26]. These data suggest that complete inhibition of DGAT1 in enterocytes by oral dosing increases the FFA content in the distal part of the GI tract resulting in steatorrhea and diarrhea. Hence, for optimal clinical efficacy and tolerability, it may be important to not achieve complete DGAT1 inhibition in the enterocytes. Since oral dosing results in very high local exposure in enterocytes, an alternate parenteral route may allow for efficient modulation of DGAT1 inhibition in the enterocytes while minimizing enterocytes drug levels and thus GI intolerability. Therefore, we tested the effect of drug through a parenteral route. Indeed, SC dosing of GSK2973980A resulted in significant TG reduction although with slightly reduced efficacy compared to oral dosing. Interestingly, the plasma exposures following either oral or subcutaneous dosing were comparable. This suggests that high levels of drug concentration in the enterocytes during initial drug absorption contributes to the observed efficacy in the initial phase but for a durable TG inhibition, drug levels need to be maintained in plasma for sufficient plasma TG reduction. This is also supported by the tissue distribution studies in rats where jejunum and duodenum levels of the drug following oral dosing was 2-4 fold higher than in plasma.

Given the GI intolerability observed with several DGAT1 inhibitors, a preclinical model that incorporates GI tolerability along with efficacy is essential to carefully select promising candidates with minimal GI tolerability issues with excellent efficacy. As part of the rat Intralipid studies, we noticed changes in stool consistency with several compounds. Since GI intolerability has been observed with many of the DGAT1 inhibitors in the clinic, we measured fecal lipids to see if it could be used as a surrogate for diarrhea observed in the clinic. We used orlistat, a known fat absorption inhibitor, as a positive control at 10 mg/kg, which resulted in ~2-fold increase in fecal lipids. While oral administration of GSK2973980A resulted in a similar increase in fecal lipids, SC administration did not alter the fecal lipid content. Since SC dosing resulted in significant plasma TG reduction without an increase in fecal lipids, the data suggest that steatorrhea is driven by high enterocyte concentration of GSK2973980A when dosed orally and that it is possible to achieve TG reduction by bypassing the GI tract. Clinically, orlistat leads to a profound increase in lipid excretion [27] that was recapitulated preclinically in this model. GSK2973980A also resulted in a similar increase in fecal lipids following oral dosing, consistent with the reported clinical experience with DGAT1 inhibitors. We utilized this model to identify compounds with a profile comparable or superior to Pradigastat, a DGAT1 inhibitor currently in phase III clinical studies [23]. Several compounds (GSK3180509A, GSK3180041A, GSK3183981A) showed 35-50% less fecal fat excretion compared to Pradigastat while maintaining TG inhibition. Our data suggest that the rat model of impaired TG clearance combined with the fecal lipid measurement can be used to triage, identify, and prioritize compounds that may have an acceptable GI tolerability profile. Additionally, it is possible to limit lipid excretion while maintaining the efficacy by altering the route of administration through bypassing the GI tract.

5.4 GSK2973980A Reduces Body Weight in Diet-induced Obese Mice Model

Chronic inhibition of DGAT1 with GSK2973980A resulted in significant reduction in both cumulative body weight and cumulative food intake in a 21-day study in DIO mice. The reductions in body weight and food intake appeared to be greater with BID dosing compared to those with QD dosing. Furthermore, the data from the pair fed group suggest that the reduction in body weight is largely related to an effect on food intake, although a slight contribution of increased fatty acid oxidation as suggested by an increase in Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α expression; data not shown)

and/or an increase in GLP-1 (glucagon-like peptide 1) and PYY (peptide YY) cannot be completely ruled out. Pharmacological inhibition of DGAT1 has previously been shown to result in changes in intestinal lipid homeostasis in a temporal and spatial manner, which could contribute to the increase of GLP-1 release upon treatment [28]. Consistent with these reports, GLP-1 and PYY levels were increased in the acute mouse model of lipid-induced postprandial hypertriglyceridemia (Supplementary Fig. 2). Additionally, dietary fat sensing via fatty acid oxidation in enterocytes has also been suggested to play a role in the control of eating [29]. GSK2973980A treatment also induced substantial decreases in lipid vacuoles in DIO mouse livers and jejunums in this study suggesting a potential utility in fatty liver disease consistent with previous reports [4].

6. CONCLUSION

We have identified a novel, potent and selective DGAT1 inhibitor, GSK2973980A, that inhibits postprandial TG excursion in both mouse and rat models and also reduced food intake and body weight in a mouse model of diet-induced obesity along with substantial reduction in lipid vacuoles in liver and jejunum. While these data suggest potential utility for metabolic disorders such as obesity, chylomicronemia and NASH, clinical experience with DGAT1 inhibitors highlights species differences in tolerability to DGAT1 inhibitors. Our data suggest that appropriate preclinical models can still be used for evaluation of GI tolerability of DGAT1 inhibitors. This combined with alternate dosing routes to minimize drug concentration in local GI tissue has potential for improved tolerability in the clinic. However, more studies are warranted to understand the translatability of our preclinical approach to the clinic.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This study was approved by the respective IACUC. All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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COMPETING INTERESTS

The authors declare no competing interests regarding the publication of this article. Some of the compounds disclosed in this manuscript are part of one or more patents. Some of the authors from GSK have equity interest in GSK.

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