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Saroglitazar, a PPAR-α/γ Agonist, for Treatment of NAFLD: A Randomized Controlled Double-Blind Phase 2 Trial

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SEE EDITORIAL ON PAGE 1730

BACKGROUND AND AIMS: NAFLD is characterized by insulin resistance and dysregulated lipid and glucose metabolism. Saroglitazar, a dual peroxisome proliferator activated receptor-α/γ agonist, improves insulin sensitivity, and lipid and glycemic parameters. Saroglitazar improved NASH histology in animal studies. In this randomized controlled clinical trial, we evaluated the efficacy and safety of saroglitazar in patients with NAFLD/NASH.

APPROACH AND RESULTS: A total of 106 patients with NAFLD/NASH with alanine aminotransferase (ALT) ≥ 50 U/L at baseline and body mass index ≥25 kg/m^2 were randomized in a 1:1:1:1 ratio to receive placebo or saroglitazar 1 mg, 2 mg, or 4 mg for 16 weeks. The primary efficacy endpoint was percentage change from baseline in ALT levels at week 16. Liver fat content (LFC) was assessed by MRI proton density fat fraction. The least-squares mean percent change from baseline in ALT at week 16 was −25.5% (5.8), −27.7% (5.9), and −45.8% (5.7), with saroglitazar 1 mg, 2 mg, and 4 mg, respectively, versus 3.4% (5.6) in placebo (P < 0.001 for all). Compared with placebo, saroglitazar 4 mg improved LFC (4.1% [5.9] vs. −19.7% [5.6]), adiponectin (−0.3 μg/mL [0.3] vs. 1.3 μg/mL [0.3]), homeostatic model assessment–insulin resistance (−1.3 [1.8] vs. −6.3 [1.7]), and triglycerides (−5.3 mg/dL [10.7] vs. −68.7 mg/dL [10.3]) (P < 0.05 for all). Saroglitazar 4 mg also improved lipoprotein particle composition and size and reduced lipotoxic lipid species. Saroglitazar was well-tolerated. A mean weight gain of 1.5 kg was observed with saroglitazar 4 mg versus 0.3 kg with placebo (P = 0.27).

CONCLUSIONS: Saroglitazar 4 mg significantly improved ALT, LFC, insulin resistance, and atherogenic dyslipidemia in participants with NAFLD/NASH. (ClinicalTrials.gov identifier: NCT03061721.) (Hepatology 2021;74:1809-1824).

NAFLD is the most common chronic liver disease worldwide, with an estimated prevalence of 25% globally.[1] The severity of NAFLD ranges from relatively benign isolated steatosis to NASH.[1-4] NASH is characterized by hepatocellular injury and inflammation with or without fibrosis, and may progress to cirrhosis, liver failure, and HCC.[2]

NAFLD is considered the hepatic component of the metabolic syndrome and other components of this syndrome such as atherogenic dyslipidemia, peripheral insulin resistance, obesity, type 2 diabetes mellitus (T2DM), and hypertension are commonly present in

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; APRI, aspartate aminotransferase–to–platelet ratio index; AST, aspartate aminotransferase; BL, baseline; CAP, controlled attenuation parameter; CK18, caspase-cleaved cytokeratin-18; CVD, cardiovascular disease; ELF, enhanced liver fibrosis; HbA1C, hemoglobin A1c; HDL-C, HDL-cholesterol; HOMA-IR, homeostasis model assessment–insulin resistance; LFC, liver fat content; LS, least-square; LSM, liver stiffness measurement; PDFF, proton density fat fraction; PPAR, peroxisome proliferator-activated receptor; QoL, quality of life; T2DM, type 2 diabetes mellitus; TG, triglyceride; VCTE, vibration-controlled transient elastography.

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patients with NAFLD.[3,4] Due to a rising prevalence of obesity and T2DM, NAFLD has emerged as a major public health threat worldwide.[5]

Metabolic stress is a hallmark of NAFLD.[6,7] It is characterized by insulin resistance and dysregulated lipid and glucose metabolism. There is significant influx of free fatty acids to the liver, which results in mitochondrial, peroxisomal, and endoplasmic reticulum stress in addition to generation of lipotoxic lipid species that induce inflammation, hepatocellular injury, apoptosis, and fibrosis. NAFLD is also characterized by atherogenic dyslipidemia, an important risk factor for cardiovascular disease (CVD),[8] which is the leading cause of death in patients with NAFLD.[9-12] Therefore, in addition to improving liver parameters and histology, therapeutic agents for NAFLD or NASH should preferably have favorable or neutral, but not negative, impact on CVD risk in these patients. At present, there are no approved drugs for the treatment of NAFLD or NASH.[13]

Ligand of the peroxisome proliferator-activated receptor (PPAR) nuclear receptors display a range of metabolic actions that modulate lipid, glucose, and energy homeostasis. These actions in addition to anti-inflammatory and antifibrotic effects make them an attractive class for treating NAFLD.[14-16] PPARs act as sensors of fatty acids and their derivatives with different body distribution, actions, and side-effect profiles.[15,17] PPAR-α is expressed primarily in the liver and brown adipose tissue and activates fatty acid oxidation; PPAR-β/δ is expressed ubiquitously and activates oxidative metabolism, whereas PPAR-γ is expressed predominantly in adipose tissue and macrophages and promotes adipogenesis and storage of fatty acids. Pioglitazone (PPAR-γ ligand), elafibranor (dual PPAR-α and β/δ ligand), and lanifibranor (pan PPAR agonist) have been tested in randomized trials for treatment of NAFLD or NASH.[18-21] Weight gain and peripheral edema were reported commonly with pioglitazone and lanifibranor—side effects attributed to the PPAR-γ effects of these agents. Increased risk of cardiovascular events and bladder cancer were reported with pioglitazone, whereas reversible increase in serum creatinine was reported with elafibranor.

Saroglitazar magnesium, a dual PPAR-α/γ agonist, was designed to have a weaker PPAR-γ effect to reduce untoward side effects related to PPAR-γ agonism.[22] Through its PPAR-α agonism, saroglitazar increases

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hepatic oxidation of fatty acids, lowers synthesis and secretion of triglycerides (TGs), and produces favorable changes in circulating lipoproteins. Through its PPAR-γ agonism, saroglitazar regulates transcription of insulin-responsive genes, increases insulin sensitivity, and reduces blood glucose and glycosylated hemoglobin A1c (HbA1c) levels.[23] Saroglitazar was granted marketing authorization in India in 2013 for management of diabetic dyslipidemia and hypertriglyceridemia in those with T2DM not controlled by a statin alone.

In mice with choline-deficient high-fat diet or Western diet–induced NASH,[24,25] saroglitazar significantly decreased alanine aminotransferase (ALT) and improved hepatic steatosis, hepatocellular ballooning, and lobular inflammation. These changes were accompanied by a decrease in expression of fibrosis and inflammation biomarkers. Saroglitazar was also associated with reduction in homeostasis model assessment–insulin resistance (HOMA-IR), TGs, total cholesterol, and metabolically active lipid species, including diglycerides, ceramides, and sphingomyelins, in mice with Western diet–induced NASH.[25]

In this study, we evaluate the efficacy and safety of saroglitazar in patients with NAFLD/NASH.

Experimental Procedures

STUDY DESIGN AND PARTICIPANTS

The EVIDENCES IV study was a multicenter, randomized, double-blind, placebo-controlled phase 2 study to evaluate the safety and efficacy of saroglitazar (1 mg, 2 mg, and 4 mg) compared with placebo in patients with NAFLD/NASH treated for 16 weeks. The study enrolled 106 eligible patients at 23 participating medical centers in the USA between June 2017 and August 2019 (ClinicalTrials.gov identifier: NCT03061721). The study protocol was reviewed and approved by the Western Internal Review Board (IRB), which served as the central IRB for this study. The study was conducted in accordance with the International Conference on Harmonization Good Clinical Practice guidelines and the ethical principles of the Declaration of Helsinki.

Key inclusion criteria were age 18 to 75 years, body mass index of at least 25 kg/m^2, a diagnosis of NAFLD established either by imaging (ultrasound, CT, or MRI) or liver biopsy showing NASH or simple steatosis in the last 24 months before screening, and ALT ≥ 50 U/L at both screening visits 1 and 2 with <30% variance between the two screening visits. The imaging-based diagnosis of fatty liver was based on local reading of the imaging study. Key exclusion criteria included history of other chronic liver disease, established diagnosis of cirrhosis, HbA1c > 9%, use of thiazolidinediones (pioglitazone, rosiglitazone), CYP2C8 inhibitors/substrate, fibrates (clofibrate, fenofibrate), vitamin E >100 IU/day, or multivitamins containing >100 IU/day of vitamin E in the 3 months before the screening visit. Unstable weight (>5% change) or glucose or lipid lowering agents' doses in the 3 months before screening visit were also exclusionary. The detailed inclusion and exclusion criteria are provided in the Supporting Information. All patients provided written, informed consent before the study participation.

PROCEDURES

Eligible patients were randomly assigned in a 1:1:1:1 ratio to receive daily dose of saroglitazar 1 mg, saroglitazar 2 mg, saroglitazar 4 mg, or placebo for 16 weeks. A block randomization schedule was generated using SAS software (version: 9.4; SAS Institute Inc., Cary, NC). Identical tablets in containers labeled with code numbers were provided to each site before randomization. Patients, investigators, clinical staff, and pathologists were blinded to the treatment assignment.

This study was conducted over a period of 22 weeks, which included a 5-week screening period, a 16-week treatment period, and a safety follow-up visit 1 week after the last dose of study drug. Patients were followed every 4 weeks for clinical and biochemistry assessments. The caspase-cleaved cytokeratin-18 (CK18) fragment levels were determined using the M30 Apoptosense enzyme-linked immunosorbent assay (Peviva AB, Bromma, Sweden). Liver fat content (LFC) was assessed by MRI proton density fat fraction (MRI-PDFF) at baseline and at week 16. Liver stiffness measurement (LSM) and controlled attenuation parameter (CAP) measurement were performed by vibration-controlled transient elastography (VCTE) using FibroScan at baseline and week 16. For the duration of the study, patients were advised to maintain the same lifestyle, including diet and exercise, that they had before enrollment.

Lipoprotein analysis was performed at baseline and week 16 using the Liposcale test, a two-dimensional proton nuclear magnetic resonance spectroscopy. Lipidomic profiling was studied at baseline and week 16 using two separate ultrahigh-performance liquid chromatography coupled with mass spectrometry–based platforms for the analysis of glycerolipids (TGs and diglycerides), glycerophospholipids (phosphatidylcholines, phosphatidylethanolamines and phosphatidylinositols), sphingolipids (ceramides, sphingomyelins, sphingoid bases, and monohexosylceramides), sterol lipids, acylcarnitines, and fatty acids (OWL Metabolomics, Derio, Spain). Quality of life (QoL) assessments were conducted using the Short-Form 36 Health Survey Version 2.0. Pharmacokinetics analysis was conducted following the first and last dose of the study drug.

OUTCOMES

The primary efficacy endpoint was the percentage change from baseline in serum ALT levels at week 16. Secondary efficacy endpoints included proportions of patients with reduction of at least 25% and 50% in ALT at week 16, percent change from baseline in LFC at week 16, change from baseline in enhanced liver fibrosis (ELF) score at week 16, and change from baseline in CK18. Exploratory efficacy endpoints included change from baseline in LSM and CAP, glycemic control parameters, lipoprotein and lipidomic parameters, and QoL total scores at week 16.

Pharmacokinetics parameters include peak plasma concentration (C_{max}), time to reach peak plasma concentration (T_{max}), area under plasma concentration versus time curve in a 24-hour dosing interval (AUC_{tau}), elimination half-life (t1/2), apparent volume of distribution, and apparent clearance. The safety was assessed by analysis of adverse events, physical examination, vital signs, body weight, clinical laboratory evaluations, and 12-lead electrocardiogram.

All laboratory tests were conducted by a central laboratory (Eurofins Central Laboratory, Lancaster, PA, USA and Breda, The Netherland).

STATISTICAL ANALYSIS

All efficacy endpoints were analyzed using full analysis set, which included all randomized patients who received at least one dose of study drug and had at least one post-baseline efficacy assessment. The last observation carried forward method was used to impute the missing values. Baseline assessment values were not used for the imputation of missing post-baseline values. All safety parameters were analyzed using the safety analysis set, which included all randomized patients who received at least one dose of study drug. Baseline value was defined as the last assessment before the administration of the first dose.

Comparison of change from baseline to week 16 in liver biochemistries, LFC, ELF, CK18, LSM, CAP, glycemic control parameters, and lipid profile parameters between saroglitazar (1 mg, 2 mg, and 4 mg) and placebo were performed using the analysis of covariance (ANCOVA) model, using treatment as the fixed effect and baseline value as a covariate. Least-square (LS) means for each treatment group and associated standard errors were provided. In addition, treatment differences in LS means (saroglitazar – placebo) and 95% CIs derived from the ANCOVA model were also provided. Comparison of percentage of patients with at least 25% and 50% reduction in ALT and percentage of patients with normal ALT at week 16 between saroglitazar (1 mg, 2 mg, and 4 mg) and placebo were performed using Fisher's exact test. Comparison of effect of dyslipidemic profiles of patients with abnormal baseline lipoprotein measurements between saroglitazar (1 mg, 2 mg, and 4 mg) and placebo were performed using a paired Student *t* test.

Adverse events were summarized according to the Medical Dictionary for Regulatory Activities (MedDRA) System Organ Class, the MedDRA preferred term, severity (as defined in the protocol), and causal relationship (as assessed by the individual investigators). All statistical testing was two-sided and was performed at a 5% level of significance. Statistical analyses were conducted using SAS software (version 9.4; SAS Institute Inc.).

ROLE OF THE FUNDING SOURCE

The study was funded by Zydus Discovery DMCC, which, with the collaboration of the authors, was involved in the study design, data collection, and analysis. The authors of the study were responsible for the data analysis, data interpretation, and

manuscript preparation. All authors had full access to the study data and approved the manuscript prior to submission.

Results

STUDY POPULATION

A total of 361 patients were screened from April 2017 to April 2019, and 106 patients were randomized; 26 patients in the saroglitazar 1 mg group, 25 patients in the saroglitazar 2 mg group, 27 patients in the saroglitazar 4 mg group, and 28 patients in the placebo group (Fig. 1). Imaging-based diagnosis of NAFLD was made in 87 participants and biopsy-based diagnosis of NASH in the remaining 19 participants. Overall, 98 (92.4%) patients completed the study and 8 (7.6%) patients discontinued the study.

The demographic and baseline characteristics of the patients were similar across the four treatment groups (Table 1).

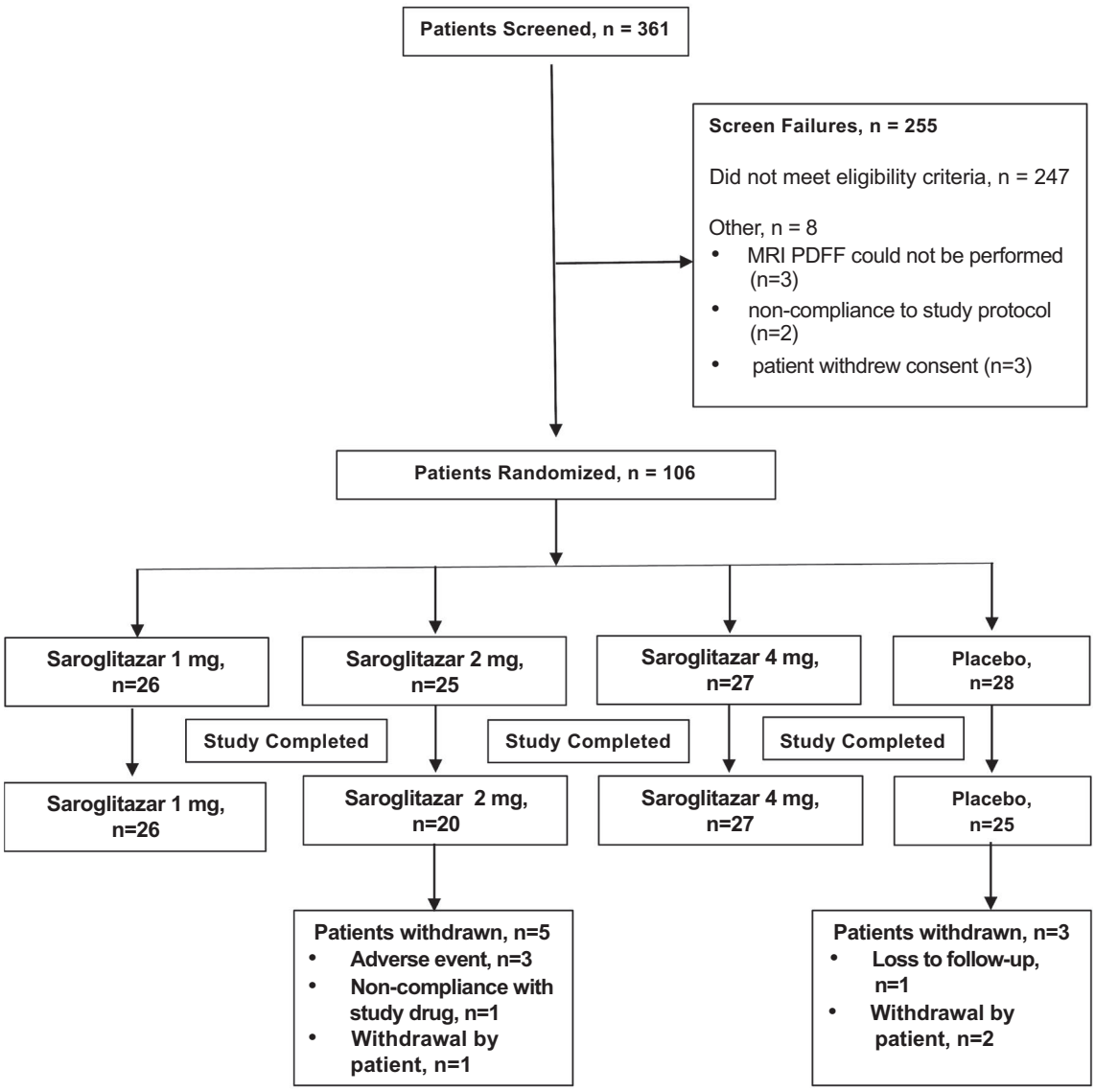

FIG. 1. Patient disposition.

TABLE 1. Baseline Characteristics

	Saroglitazar 1 mg (n = 26)	Saroglitazar 2 mg (n = 25)	Saroglitazar 4 mg (n = 27)	Placebo (n = 28)
Age (in years)	51.1 (12.9)	47.9 (10.4)	49.0 (11.0)	48.7 (10.5)
Female, n (%)	13 (50.0)	12 (48.0)	12 (44.4)	13 (46.4)
Race, n (%)				
White	22 (84.6)	21 (84.0)	24 (88.9)	24 (85.7)
Asian	3 (11.5)	3 (12.0)	3 (11.1)	3 (10.7)
American Indian or Alaska Native	1 (3.8)	0	0	0
Other	0	1 (4.0)	0	1 (3.6)
Body mass index (kg/m^2)	33.6 (4.2)	35.7 (8.6)	32.5 (5.2)	33.8 (4.5)
Diabetes, n (%)	11 (42.3)	17 (68.0)	12 (44.4)	16 (57.1)
ALT (U/L)	93.7 (32.5)	84.8 (29.3)	83.4 (27.9)	93.4 (42.1)
AST (U/L)	58.0 (20.6)	55.0 (22.1)	53.3 (15.5)	54.5 (29.5)
ALP (U/L)	82.0 (26.3)	84.2 (31.6)	93.1 (35.2)	81.1 (24.0)
GGT (U/L)	59.4 (42.0)	68.8 (57.9)	75.6 (64.7)	66.7 (49.8)
TG (mg/dL)	173.2 (103.0)	201.9 (116.6)	190.9 (98.5)	181.1 (62.2)
LDL (mg/dL)	116.4 (38.5)	124.3 (36.9)	132.7 (56.1)	121.6 (38.1)
VLDL (mg/dL)	24.2 (20.1)	26.5 (17.6)	25.3 (17.7)	23.4 (10.3)
HDL (mg/dL)	47.1 (12.6)	44.5 (7.3)	46.8 (15.8)	46.9 (12.0)
Total cholesterol (mg/dL)	187.7 (45.1)	194.0 (44.0)	204.8 (62.3)	191.7 (39.7)
HbA1c (%)	5.9 (0.7)	6.8 (1.5)	6.1 (0.9)	6.2 (1.0)
FPG (mg/dL)	107.7 (24.5)	142.8 (68.8)	110.7 (23.0)	120.5 (23.8)
HOMA-IR	10.7 (17.5)	12.5 (9.1)	11.1 (14.2)	13.6 (12.1)
Insulin (mU/L)	35.3 (41.9)	37.4 (24.7)	36.9 (38.9)	43.0 (32.6)
Creatinine (mg/dL)	0.8 (0.2)	0.8 (0.2)	0.8 (0.2)	0.8 (0.2)
Adiponectin (ug/mL)	3.7 (1.7)	2.7 (1.1)	3.6 (1.5)	3.4 (1.4)
LFC (%)	20.2 (8.9)	23.9 (8.9)	22.8 (8.5)	24.8 (10.4)
CK18 (U/L)	490.5 (234.8)	757.3 (788.4)	434.3 (221.4)	482.2 (268.9)
ELF score	9.5 (0.8)	9.4 (0.8)	9.4 (0.8)	9.1 (1.0)
APRI	0.7 (0.5)	0.4 (0.5)	0.4 (0.5)	0.5 (0.6)
Liver stiffness (kPa)	9.2 (4.1)	9.1 (4.6)	8.4 (5.7)	8.0 (5.3)
CAP (dB/m)	327.1 (32.1)	326.1 (64.8)	332.9 (42.6)	324.9 (51.0)

Note: All continuous variables are presented as mean (SD).
Abbreviations: FPG, fasting plasma glucose; GGT, gamma-glutamyltransferase.

EFFICACY ANALYSIS

Effects on ALT

A significant dose-dependent reduction in the primary efficacy endpoint, percent reduction of ALT level at week 16 relative to baseline, was observed in each saroglitazar dose level compared with placebo (Table 2). The LS mean percent change from baseline in ALT at week 16 was −25.5% (SEM = 5.8, $P < 0.001$) in the saroglitazar 1 mg group, −27.7% (SEM = 5.9, $P < 0.001$) in the saroglitazar 2 mg group, and −45.8% (SEM = 5.7, $P < 0.001$) in the saroglitazar 4 mg group compared with the 3.4% (SEM = 5.6) increase in the placebo group. Treatment with saroglitazar resulted in rapid reduction of ALT level at week 4 at all dose levels and sustained throughout the duration of treatment (Fig. 2A). Similar to the percentage reduction in ALT levels, a significant dose-dependent reduction was also observed in alkaline phosphatase (ALP) and AST levels when compared with placebo (Table 2 and Fig. 2).

ALT reduction of at least 25% at week 16 relative to baseline was observed in 69.2%, 64.0%, 70.4%, and 17.9% of patients treated with saroglitazar dose of 1 mg, 2 mg, 4 mg or placebo, respectively. ALT reduction of at least 50% at week 16 was observed in 15.4%,

TABLE 2. Percent Change From Baseline in Liver Enzymes at Week 16

Liver Enzyme Parameter	Saroglitazar 1 mg (n = 26)	Saroglitazar 2 mg (n = 25)	Saroglitazar 4 mg (n = 27)	Placebo (n = 28)
Percent change from BL in ALT				
LS mean (SEM)	−25.5 (5.8)	−27.7 (5.9)	−45.8 (5.7)	3.4 (5.6)
Treatment difference (95% CI)	−28.8 (−44.7, −12.9)	−31.0 (−47.2, −14.9)	−49.1 (−65.0, −33.3)	
P value	<0.001	<0.001	<0.001	
Percent change from BL in AST				
LS mean (SEM)	−15.7 (5.8)	−19.2 (5.9)	−25.1 (5.7)	9.8 (5.6)
Treatment difference (95% CI)	−25.4 (−41.4, −9.4)	−29.0 (−45.1, −12.8)	−34.9 (−50.8, −19.1)	
P value	0.002	<0.001	<0.001	
Percent change from BL in ALP				
LS mean (SEM)	−17.0 (2.7)	−22.5 (2.8)	−35.7 (2.7)	3.3 (2.6)
Treatment difference (95% CI)	−20.3 (−27.8, −12.8)	−25.8 (−33.3, −18.2)	−38.9 (−46.4, −31.5)	
P value	<0.001	<0.001	<0.001	
Percent change from BL in GGT				
LS mean (SEM)	−31.8 (6.2)	−29.4 (6.3)	−45.7 (6.1)	10.9 (5.9)
Treatment difference (95% CI)	−42.7 (−59.7, −25.7)	−40.3 (−57.5, −23.2)	−56.6 (−73.4, −39.8)	
P value	<0.001	<0.001	<0.001	

Note: *P* values related to the treatment difference compared with placebo using the analysis covariance model.
Abbreviation: GGT, gamma-glutamyltransferase.

16.0%, 51.9%, and 3.6%, respectively. Reduction of at least 17 U/L in ALT at week 16 was observed in 25 (96.2%), 24 (96.0%), 27 (100%), and 21 (75.0%) in the saroglitazar 1 mg, 2 mg, 4 mg, and placebo groups, respectively. At week 16, normal ALT level per central laboratory reference (<45 U/L) was observed in 14 (51.9%, *P* = 0.001) patients in the saroglitazar 4 mg group, 7 (28.0%, *P* = 0.16 vs. placebo) patients in the saroglitazar 2 mg group, 7 (26.9%, *P* = 0.17 vs. placebo) patients in the saroglitazar 1 mg group, and 3 (10.7%) patients in the placebo group. In addition, normal level of ALT defined as ALT level of <30 U/L in males or <19 U/L in females[16] was observed in 6 (22.2%, *P* = 0.01 vs. placebo) patients in the saroglitazar 4 mg group at week 16 compared with none in the other treatment groups.

At week 16, female patients had a higher percentage reduction in ALT levels when compared with male patients. The LS mean percentage reduction from baseline at week 16 was −14.2% (SEM = 9.1), −27.2% (SEM = 9.1), −36.8% (SEM = 8.4), and 5.5% (SEM = 8.4) for saroglitazar 1 mg, 2 mg, 4 mg, and placebo, respectively, for male patients and −37.2% (SEM = 6.9), −27.7% (SEM = 7.3), −56.4% (SEM = 7.2), and 0.4% (SEM = 7.0), respectively, for female patients. Although a higher percent reduction of ALT was observed in female patients, the gender difference was not statistically significant (*P* = 0.07) in the overall model, adjusting for both gender and baseline ALT values. After adjusting for gender and baseline ALT values, the LS mean percent reduction in ALT at week 16 from baseline was −25.6% (SEM = 5.7), −27.8% (5.8), −46.2% (SEM = 5.6), and 2.9% (SEM = 5.5), respectively, for saroglitazar 1 mg, 2 mg, 4 mg, and placebo groups.

Effects on Liver Fat Content

LFC data from MRI-PDFF were available on all participants at baseline and on 99 (93.4%) participants at week 16. A significantly higher percentage reduction in LFC at week 16 relative to baseline was observed in the saroglitazar 4 mg group (LS mean = −19.7%, SEM = 5.6, *P* = 0.004) when compared with placebo (LS mean = 4.1%, SEM = 5.9). The LS mean difference between saroglitazar and placebo (95% CI) in LFC at week 16 was −0.3 % (−16.8, 16.2; *P* = 0.97), −3.6% (−20.8, 13.5; *P* = 0.67), and −23.8% (−39.9, −7.7; *P* = 0.004) for the saroglitazar 1 mg, 2 mg, and 4 mg groups, respectively (Table 3).

At week 16, 9 (34.6%, *P* = 0.92 vs. placebo) patients in the saroglitazar 1 mg group, 9 (42.9%, *P* = 0.64 vs. placebo) patients in the saroglitazar 2 mg group, and 17 (63%, *P* = 0.052 vs. placebo) in the saroglitazar

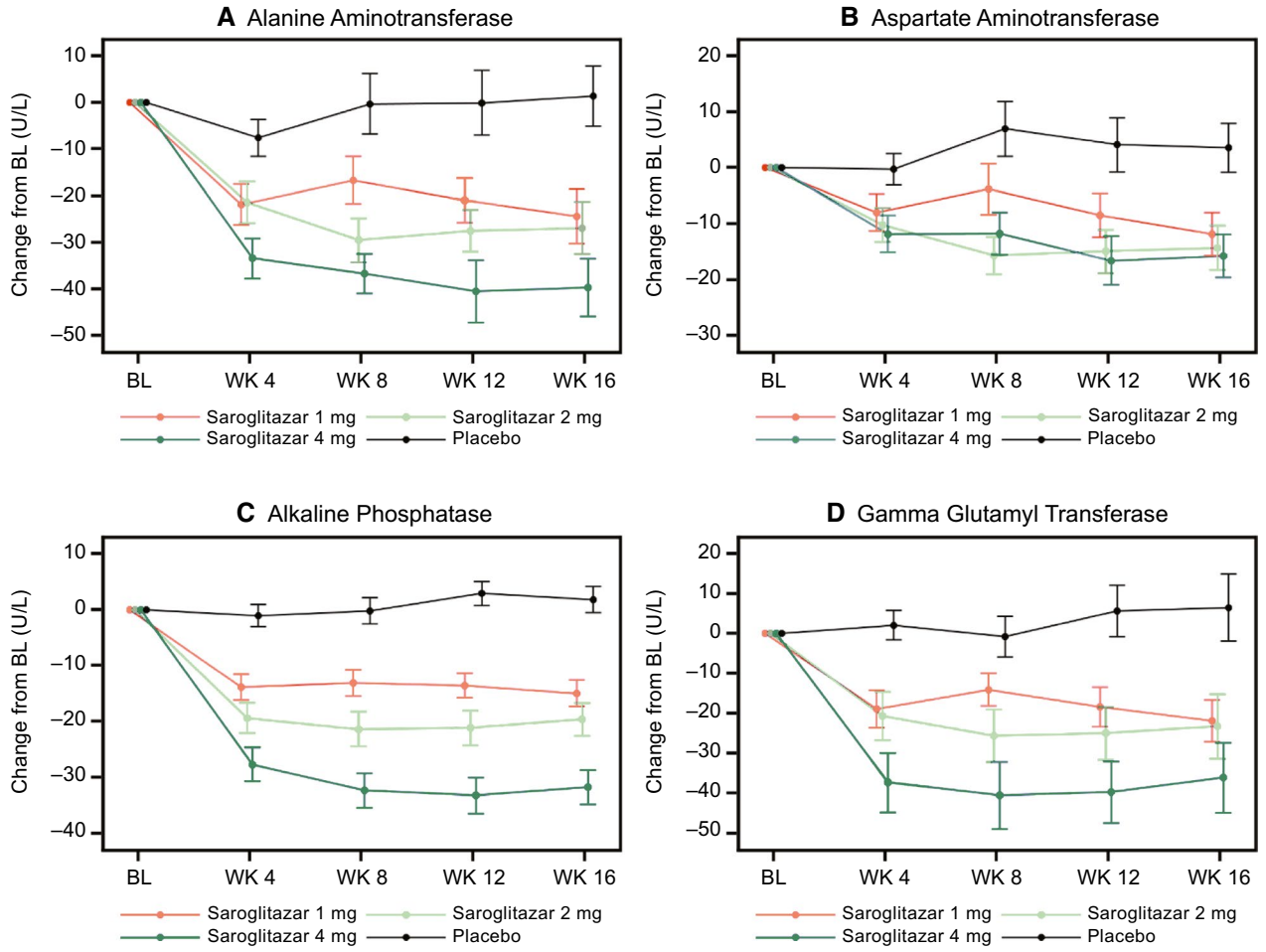

FIG. 2. Change from baseline in liver enzymes. Abbreviations: BL, baseline; WK, week.

4 mg group had a reduction of >5% LFC, compared with 9 (36%) in the placebo group.

At week 16, 11 (40.7%) patients in the saroglitazar 4 mg group had a reduction of at least 30% in LFC when compared with only 2 (8.0%) patients in the placebo group (P = 0.01) (Fig. 3). In comparison, 3 (11.5%) patients in the saroglitazar 1 mg group and 1 (4.8%) patient in the saroglitazar 2 mg group had a reduction of at least 30% in LFC at week 16.

Effects on Markers of Necroinflammation and Fibrosis

An insignificant reduction of CK18 was observed in the saroglitazar 1 mg (LS mean = −32.8 U/L, SEM = 89.1), saroglitazar 2 mg (LS mean = −184.8 U/L, SEM = 94.9), and saroglitazar 4 mg (LS mean = −129.6 U/L, SEM = 85.6) groups compared with placebo (LS mean = 63.2 U/L, SEM = 88.4) (Table 3).

A significant but small reduction in ELF at week 16 relative to baseline was observed in both the saroglitazar 2 mg (LS mean = −0.17, SEM = 0.13, P = 0.027) and saroglitazar 4 mg (LS mean = −0.22, SEM = 0.12, P = 0.011) groups when compared with placebo (LS mean = 0.23, SEM = 0.12).

A significant reduction from baseline in aspartate aminotransferase (AST)–to–platelet ratio index (APRI) at week 16 was observed in all three dose levels of saroglitazar when compared with placebo (all P < 0.01; Table 3).

LSM and CAP data from VCTE were available on 73 (69%) participants at baseline and on 71 (67.0%) participants at week 16. No significant changes in

TABLE 3. Change From Baseline in Secondary and Exploratory Endpoints

	Saroglitazar 1 mg (n = 26)	Saroglitazar 2 mg (n = 25)	Saroglitazar 4 mg (n = 27)	Placebo (n = 28)
Percent change from BL in LFC				
LS mean (SEM)	3.8 (5.7)	0.5 (6.3)	−19.7 (5.6)	4.1 (5.9)
Treatment difference (95% CI)	−0.3 (−16.8, 16.2)	−3.6 (−20.8, 13.5)	−23.8 (−39.9, −7.7)	
P value	0.974	0.675	0.004	
Change from BL in CK18 (U/L)				
LS mean (SEM)	−32.8 (89.1)	−183.9 (95.6)	−131.0 (86.3)	69.3 (92.9)
Treatment difference (95% CI)	−102.1 (−357.3, 153.1)	−253.3 (−520.0, 13.5)	−200.3 (−451.1, 50.5)	
P value	0.429	0.062	0.116	
Change from BL in ELF score				
LS mean (SEM)	−0.07 (0.12)	−0.17 (0.13)	−0.22 (0.12)	0.23 (0.12)
Treatment difference (95% CI)	−0.30 (−0.65, 0.05)	−0.40 (−0.75, −0.05)	−0.45 (−0.79, −0.10)	
P value	0.089	0.027	0.011	
Change from BL in APRI				
LS mean (SEM)	−0.24 (0.10)	−0.28 (0.10)	−0.35 (0.10)	0.13 (0.10)
Treatment difference (95% CI)	−0.37 (−0.65, −0.09)	−0.41 (−0.69, −0.12)	−0.48 (−0.75, −0.20)	
P value	0.009	0.005	<0.001	
Change from BL in liver stiffness (kPa)				
LS mean (SEM)	−1.1 (0.6)	−1.4 (0.6)	−1.9 (0.6)	−0.6 (0.6)
Treatment difference (95% CI)	−0.5 (−2.3, 1.2)	−0.8 (−2.5, 1.0)	−1.3 (−3.1, 0.4)	
P value	0.531	0.375	0.132	
Change from BL in CAP (dB/m)				
LS mean (SEM)	1.0 (10.3)	−4.2 (10.6)	−25.4 (10.7)	4.5 (10.7)
Treatment difference (95% CI)	−3.4 (−33.2, 26.3)	−8.6 (−38.8, 21.5)	−29.9 (−60.3, 0.4)	
P value	0.819	0.570	0.053	

Note: *P* values related to the treatment difference compared with placebo using the analysis of covariance model.

LSM were observed in the saroglitazar 4 mg (LS mean = −1.9 kPa, SEM = 0.6), saroglitazar 2 mg (LS mean = −1.4 kPa, SEM = 0.6), and saroglitazar 1 mg (LS mean = −1.1 kPa, SEM = 0.6) when compared with placebo (LS mean = −0.6, SEM = 0.6). Similarly, observed changes (LS mean [SEM]) in CAP in the saroglitazar 1 mg, 2 mg, 4 mg, and placebo groups were 1.0 dB/M (10.3), −4.2 (10.6), −25.4 dB/M (10.7), and 4.5 dB/M (10.7), respectively (all $P > 0.05$ vs. placebo) (Table 3).

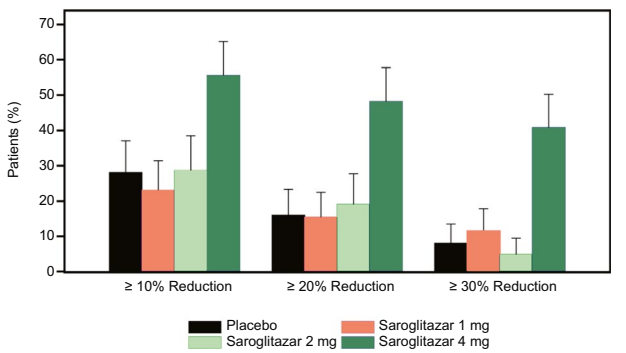

FIG. 3. Percent reduction in liver fat content at week 16.

Effects on Lipid Profiles

A significant improvement in TG levels at week 16 relative to baseline was observed in both the saroglitazar 4 mg (LS mean = −68.7, SEM = 10.3, $P < 0.001$) and saroglitazar 1 mg (LS mean = −56.0, SEM = 10.5, $P = 0.001$) groups when compared with placebo (LS mean = −5.3, SEM = 10.7) (Table 4 and Fig. 4). A similar trend was also observed in patients treated with the saroglitazar 2 mg group, but this difference was not statistically significant (LS mean = −25.1, SEM = 10.7, $P = 0.194$).

Patients treated with saroglitazar 4 mg had a significant improvement in VLDL levels (LS mean = −7.4, SEM = 2.0, $P = 0.017$) at week 16 when compared

TABLE 4. Change From Baseline in Metabolic Parameters at Week 16

Parameter	Saroglitazar 1 mg (n = 26)	Saroglitazar 2 mg (n = 25)	Saroglitazar 4 mg (n = 27)	Placebo (n = 25)
Body weight (kg)				
LS mean (SEM)	0.0 (0.8)	1.3 (0.8)	1.5 (0.8)	0.3 (0.8)
Treatment difference (95% CI)	–0.3 (–2.5, 1.9)	1.0 (–1.3, 3.2)	1.2 (–1.0, 3.4)	
P value	0.804	0.398	0.277	
Total cholesterol (mg/dL)				
LS mean (SEM)	–14.2 (5.3)	–3.3 (5.4)	–21.0 (5.2)	–7.4 (5.4)
Treatment difference (95% CI)	–6.8 (–21.8, 8.3)	4.1 (–11.1, 19.3)	–13.6 (–28.6, 1.4)	
P value	0.374	0.593	0.075	
TGs (mg/dL)				
LS mean (SEM)	–56.0 (10.5)	–25.1 (10.7)	–68.7 (10.3)	–5.3 (10.7)
Treatment difference (95% CI)	–50.7 (–80.4, –21.0)	–19.8 (–49.8, 10.2)	–63.4 (–92.8, –34.0)	
P value	0.001	0.194	<0.001	
HDL (mg/dL)				
LS mean (SEM)	2.3 (1.5)	1.6 (1.6)	3.7 (1.5)	–0.3 (1.6)
Treatment difference (95% CI)	2.6 (–1.8, 7.0)	1.9 (–2.5, 6.4)	4.0 (–0.3, 8.4)	
P value	0.247	0.392	0.070	
LDL (mg/dL)				
LS mean (SEM)	–9.1 (5.1)	–2.2 (5.3)	–17.3 (5.0)	–7.3 (5.2)
Treatment difference (95% CI)	–1.8 (–16.2, 12.7)	5.1 (–9.6, 19.9)	–10.0 (–24.4, 4.5)	
P value	0.809	0.493	0.173	
VLDL (mg/dL)				
LS mean (SEM)	–8.1 (2.0)	–1.5 (2.1)	–7.4 (2.0)	–0.5 (2.0)
Treatment difference (95% CI)	–7.6 (–13.2, –1.9)	–0.9 (–6.7, 4.8)	–6.9 (–12.4, –1.3)	
P value	0.009	0.750	0.017	
Creatinine (mg/dL)				
LS mean (SEM)	0.01 (0.02)	0.01 (0.02)	0.04 (0.02)	–0.02 (0.02)
Treatment difference (95% CI)	0.03 (–0.02, 0.08)	0.03 (–0.02, 0.08)	0.05 (0.00, 0.10)	
P value	0.223	0.208	0.032	
HbA1c (%)				
LS mean (SEM)	–0.08 (0.10)	0.13 (0.11)	–0.22 (0.10)	–0.07 (0.10)
LS mean difference (95% CI)	–0.01 (–0.30, 0.28)	0.20 (–0.10, 0.50)	–0.15 (–0.44, 0.13)	
P value	0.945	0.181	0.290	
Fasting plasma glucose (mg/dL)				
LS mean (SEM)	–18.2 (7.2)	8.6 (7.6)	–16.3 (7.1)	–4.0 (7.3)
Treatment difference (95% CI)	–14.3 (–34.6, 6.1)	12.6 (–8.2, 33.4)	–12.3 (–32.4, 7.9)	
P value	0.168	0.234	0.229	
HOMA-IR				
LS mean (SEM)	–5.8 (1.7)	0.4 (1.8)	–6.3 (1.7)	–1.3 (1.8)
Treatment difference (95% CI)	–4.5 (–9.5, 0.4)	1.7 (–3.5, 6.8)	–5.0 (–9.9, –0.1)	
P value	0.072	0.515	0.047	
Insulin (mU/L)				
LS mean (SEM)	–13.2 (4.5)	–0.3 (4.9)	–15.9 (4.4)	–3.1 (4.8)
Treatment difference (95% CI)	–10.1 (–23.1, 2.9)	2.8 (–10.7, 16.4)	–12.8 (–25.7, 0.1)	
P value	0.128	0.677	0.052	
Adiponectin (ug/mL)				
LS mean (SEM)	0.8 (0.3)	0.5 (0.3)	1.3 (0.3)	–0.3 (0.3)
Treatment difference (95% CI)	1.1 (0.3, 1.9)	0.8 (–0.0, 1.6)	1.6 (0.8, 2.4)	
P value	0.007	0.057	<0.001	

Note: *P* values related to the treatment difference were compared with placebo.

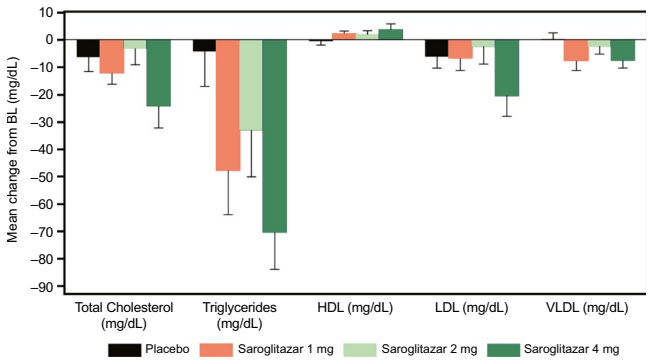

FIG. 4. Change from baseline in lipid profile parameters at week 16 (data presented in mean ± SEM).

with placebo (LS mean = −0.5, SEM = 2.0). Similar results were also observed in patients treated with the saroglitazar 1 mg group (Table 4). Improvement in total cholesterol, LDL, and HDL were also observed in patients treated with saroglitazar 4 mg when compared with placebo, but the differences were not statistically significant (Table 4 and Fig. 4). No significant differences in reduction in other lipid profile parameters were observed in the saroglitazar 2 mg and 1 mg groups.

In patients with abnormal baseline lipoprotein levels, a dose-dependent reduction of at least 20% at week 16 relative to baseline was observed in VLDL-TG, LDL-cholesterol (LDL-C), the ratio HDL-TG/HDL-cholesterol (HDL-C) and remnant cholesterol (Supporting Table S1). A significantly higher proportion of patients treated with saroglitazar 4 mg had at least a 20% reduction of HDL-TG/HDL-C (50% vs. 10%, P = 0.048) and remnant cholesterol (76% vs. 17%, P = 0.035) at week 16 compared with placebo. A similar trend was also observed in VLDL-TG and LDL-C, but these differences were not statistically significant.

Metabolic Parameters

No significant changes to the body weight at week 16 relative to baseline was observed in patients treated with saroglitazar when compared with placebo (Table 4). The LS mean differences in weight (95% CI) at week 16 between saroglitazar and placebo were −0.3 kg [−2.5, 1.9), 1.0 kg (−1.3, 3.2), and 1.2 kg (−1.0, 3.4) for the 1 mg, 2 mg, and 4 mg saroglitazar arms (P > 0.05). At week 16, 2 (7.7%) patients in the saroglitazar 1 mg group, 5 (20.8%) patients in the saroglitazar 2 mg group, and 4 (14.8%) patients in the saroglitazar 4 mg group had a weight gain of 3 kg or more compared with 3 (12.0%) patients in the placebo group. Weight loss of >2 kg occurred in 3 (11.5%) patients in the saroglitazar 1 mg group, 3 (12.5%) in the saroglitazar 2 mg group, and 3 (14.8%) patients in the saroglitazar 4 mg group compared with 2 (8%) patients in the placebo group.

Even though a significant increase in adiponectin was observed in the 1 and 4 saroglitazar arms (Table 4), the favorable changes observed in glucose, HbA1c, and insulin levels at week 16 were not statistically significant, except for HOMA-IR (P = 0.047) in the saroglitazar 4 mg group.

Effects on Lipoprotein Particle Compositions and Size

Saroglitazar decreased the cholesterol content of VLDL and IDL, TG content of VLDL, IDL, LDL and HDL, number of VLDL particles (small, medium, and large), number of medium and large HDL particles, diameter of HDL particles, ratio HDL-TG/HDL-C, non-HDL cholesterol, and remnant cholesterol (Supporting Table S2). Although differences in comparison to placebo were clear in the three saroglitazar arms, the greatest effects were observed with the 1-mg and 4-mg doses.

Lipidomic Analysis

Dose-dependent changes in metabolically active and lipotoxic lipid species were observed in patients treated with saroglitazar (Supporting Tables S3-S6). These changes were most pronounced with saroglitazar 4 mg (Supporting Fig. S1), which had higher reduction in TGs, diglycerides, diacylglycerophosphatidylcholines, lysoglycerophosphatidylcholines, and ceramides from baseline to week 16. At week 16, saroglitazar 1 mg reduced diglycerides and lysoglycerophosphatidylcholines, and saroglitazar 2 mg reduced TGs, ceramides, and lysoglycerophosphatidylcholines.

Saroglitazar 4 mg also significantly reduced the levels of several bile acids, including the primary bile acid chenodeoxycholic acid, glycochenodeoxycholic acid, and glycoursodeoxycholic acid.

Although there were inconclusive patterns of reduction in ceramides, diglycerides, and TGs in association with ALT reduction responses in the saroglitazar 1 mg and 2 mg groups, all of these lipid species were reduced, regardless of the ALT response with saroglitazar 4 mg, but were more pronounced in patients with ALT reduction to <50 U/L (Supporting Fig. S2).

Effects on QoL

At week 16, the change from baseline in patient-reported QoL mean total score was −1.7 (SD = 7.2) in the saroglitazar 1 mg group, −2.2 (SD = 7.6) in the saroglitazar 2 mg group, and −3.8 (SD = 10.8) in the saroglitazar 4 mg group, compared with −1.0 (SD = 6.2) in the placebo group ($P > 0.05$).

Pharmacokinetic Analysis

Saroglitazar was rapidly well absorbed across all saroglitazar dose levels at both day 1 and last dose (Week 16), with a median time to the peak plasma concentration (T_{max}) of less than 1.67 hours (range: 0.78-1.67 hours) under fasting conditions (Supporting Table S7). The mean peak plasma concentration (C_{max}) ranged from 54.32 to 219.48 ng/mL across the dose levels. The area under plasma concentration versus time curve in a 24-hour dosing interval (AUC_{tau}) increased in a dose-related manner. The elimination half-life ($t_{1/2}$) of saroglitazar ranged from 4.59 to 6.59 hours.

SAFETY PROFILE

Overall, 112 treatment-emergent adverse events were reported in 59 patients: 13 (50.0%) patients in the saroglitazar 1 mg group, 13 (52.0%) patients in the saroglitazar 2 mg group, 14 (51.9%) patients in the saroglitazar 4 mg group, and 19 (67.9%) patients in the placebo group. Adverse events related to the study drug were reported in 4 (15.4%) patients in the saroglitazar 1 mg group, 2 (8.0%) patients in the saroglitazar 2 mg group, and 9 (32.1%) patients in the placebo group. The most frequently reported treatment-emergent adverse events in the saroglitazar treatment group were diarrhea (3 patients), cough (3 patients), abdominal pain (2 patients) and bronchitis (2 patients). Edema was reported only in 1 patient in the placebo group.

Severe adverse events were reported in 2 patients, 1 patient reported cough in the saroglitazar 1 mg group, and another patient in the placebo group had a decrease in neutrophil counts; both were deemed unrelated to study drug and resolved during the study.

One patient in the saroglitazar 4 mg group had a serious adverse event of altered mental status. This patient developed confusion and headache 11 days after starting the study drug. The event was thought possibly due to new onset seizures and unrelated to the study drug. The patient resumed the study drug on discharge from the hospital through the end of study.

Adverse events leading to withdrawing the participant occurred in 3 participants in the 2-mg arm and included rash, foot fracture, and T2DM. The event rash was reported as related to the study drug. The remaining two events were deemed as not related to study drug.

Discussion

In this randomized placebo-controlled double-blind trial, saroglitazar 4 mg resulted in significant reduction in ALT and LFC. Importantly, saroglitazar use was associated with improvement in insulin resistance and atherogenic dyslipidemia. Saroglitazar use was safe and well tolerated.

Insulin resistance and altered lipid and glucose metabolism play key roles in NAFLD pathogenesis.[4,6,26] Saroglitazar, a dual PPAR-α/γ agonist, is an attractive agent for the treatment of NAFLD and NASH because its mechanism of action targets many of these pathogenic processes.[14,27] Saroglitazar has predominant PPAR-α and modest PPAR-γ agonistic activities.[28] Its PPAR-α effects increase hepatic mitochondrial and peroxisomal oxidation of fatty acids and improve lipid profile, and its PPAR-γ effects improve glucose homeostasis and insulin resistance.

Patients treated with saroglitazar experienced a significant dose-dependent percent reduction in ALT levels at week 16 relative to baseline. Treatment with saroglitazar resulted in early reduction of ALT level at week 4 at all dose levels that was sustained throughout the duration of treatment. A significantly higher proportion of patients in the saroglitazar 4 mg group (51.9%) achieved at least 50% reduction in ALT level at week 16 relative to baseline compared with

placebo (3.6%). A recent analysis of the FLINT trial data showed that ALT reduction of at least 17 IU/L at 72 weeks was associated with a higher histological response rate compared with ALT reduction of <17 IU/L.[29] Although liver histology was not a planned endpoint (and thus not collected in this study), we observed ALT reduction of at least 17 U/L in 25 (96.2%), 24 (96.0%), 27 (100%), and 21 (75.0%) participants in the saroglitazar 1 mg, 2 mg, 4 mg, and placebo groups, respectively.

Treatment with saroglitazar 4 mg resulted in a significantly higher percentage reduction in LFC at week 16 (−19.7%) relative to baseline when compared with an increase in LFC of 4.1% in the placebo group. A dose-dependent percent reduction of LFC compared with placebo was observed in patients treated with saroglitazar compared to placebo; however, these differences were small in the saroglitazar 1 mg and 2 mg arms (LS mean difference between saroglitazar and placebo was −0.3% and −3.6%, respectively) compared with the effect seen in the 4-mg arm (LS mean difference between saroglitazar and placebo was −23.8%). ALT level had been previously shown to correlate with LFC.[30] The decrease in ALT in the 4-mg arm was nearly double that observed in the 1-mg and 2-mg arms. Similarly, the favorable effects of saroglitazar on insulin, HOMA-IR, and adiponectin were larger in magnitude with the 4-mg arm. Therefore, the physiological effects of the 1-mg and 2-mg doses may not be sufficient to significantly improve LFC.

A 30% reduction in LFC by MRI-PDFF is associated with 2-point improvement in NAFLD activity score.[29,31] In this study, 11 (40.7%) patients in the saroglitazar 4 mg group had a reduction of at least 30% in LFC compared with only 2 (8.0%) patients in the placebo group.

Markers of hepatocellular injury and fibrosis including CK18, ELF, LSM, and APRI showed encouraging trends following 16 weeks of saroglitazar use. However, these changes, even when statistically significant, should be interpreted with caution, given the small magnitude of change. Furthermore, improvement in inflammation as reflected by improving AST could reduce APRI.

The association among NAFLD, atherogenic dyslipidemia, and CVD is well established.[9,32] Indeed, CVD is the leading cause of death in patients with NAFLD,[12,33] who are enriched with CVD risk factors such as T2DM and atherogenic dyslipidemia.[34] Therefore, any therapy for NAFLD or NASH that is projected to be needed on a long-term basis should ideally exert a favorable or neutral impact on cardio-metabolic comorbidities in these patients.[35] In this regard, saroglitazar, designed to have stronger PPAR-α and weaker PPAR--γ effects, exhibited modest PPAR-γ effects on insulin sensitivity that were detected mostly in the 4-mg group. There was a significant increase in adiponectin with the 1-mg and 4-mg saroglitazar arms, but only the 4-mg arm experienced a significant decrease in TGs, VLDL-C, and HOMA-IR, and an insignificant improvement in total cholesterol, LDL, HDL, HbA1c, fasting plasma glucose, and insulin at week 16 compared with placebo. The lack of statistically significant effects on these metabolic parameters despite favorable trends in the 1-mg and 2-mg saroglitazar arms may be due to dose effect and relatively small numbers in these arms.

In patients with abnormal baseline lipoprotein levels, saroglitazar in a dose-dependent fashion resulted in >20% reduction in VLDL-TG, LDL-TG, HDL-TG/HDL-C ratio, and remnant cholesterol—a reduction that could reduce CVD risk in these participants. When we examined the saroglitazar effect on lipoprotein particle compositions and size, similar beneficial and dose-dependent effects were observed. These changes reflect improved atherogenic profile and lower CVD risk in patients with NAFLD/NASH and abnormal baseline lipoprotein levels, who are at increased risk of CVD.

The abundance of free fatty acids in the setting of NAFLD results in lipotoxicity after overwhelming safer lipid storage in hepatocytes in the form of TGs.[7] Increased lipotoxic species such as lysophosphatidylcholines and ceramides can trigger signaling and apoptosis pathways, endoplasmic reticulum stress, alterations in mitochondrial function, and oxidative stress, and have been shown to be increased in animal and human NASH.[36-39] Increased diglyceride levels have been also linked to hepatic lipotoxicity and insulin resistance.[40,41] The detailed lipoprotein and lipidomics analyses allowed deeper understanding of the effects of saroglitazar on lipoproteins and lipotoxic lipid species. Saroglitazar 4 mg reduced diacylglycerophosphatidylcholines, ceramides, and diglyceride levels. The effects of saroglitazar 4 mg on lipotoxic species were observed regardless of the ALT response.

Dysregulation of bile acid metabolism in patients with NASH has been previously reported and is characterized primarily by up-regulation of conjugated bile acids, such as glycochenodeoxycholic acid and glycoursodeoxycholic acid.[42-44] Saroglitazar 4 mg reduced the level of several bile acids, including glycochenodeoxycholic acid and glycoursodeoxycholic acid. Taken together, these changes induced by saroglitazar on lipotoxic species may improve drivers of NAFLD/NASH progression.

Saroglitazar was well-tolerated, and the number of subjects with at least one adverse event was similar across the treatment groups. Weight gain is a common side effect with PPAR-γ agonists. Even though saroglitazar has dominant PPAR-α and weaker PPAR-γ effects, we still observed a non-statistically significant mild and dose-dependent weight gain with saroglitazar compared with placebo (LS mean weight difference with placebo = 0 kg, 1 kg, and 1.2 kg for the 1-mg, 2-mg, and 4-mg saroglitazar arms; $P > 0.05$).

We recognize that liver histology was not available on most patients and that ALT level, used as a primary endpoint in this study, is not a sensitive marker for detecting NASH or advanced fibrosis in patients with NAFLD.[45] However, 19 (18%) of the patients enrolled had biopsy-proven NASH. The noninvasive markers of fibrosis used also offer some insights on the severity of fibrosis in this cohort. LSM-estimated significant and advanced fibrosis were common: 35.6% per Siddiqui et al.[14] (Youden's index cutoff for ≥F2 or ≥F3 [8.6 kPa]), and 39.7% and 26.0% per Eddowes et al.[15] (Youden's index cutoffs for ≥F2 [8.2 kPa] or ≥F3 [9.7 kPa], respectively). Furthermore, when using ELF cutoffs for significant (7.7 to <9.8) or severe (≥9.8) fibrosis per manufacturer's recommendations,[13] 67.0% had significant and 29.2% of the participants had advanced fibrosis. Thus, there is justification for testing the efficacy of saroglitazar in fibrosing NASH in the next phase clinical trial.

In summary, in this randomized placebo-controlled double-blind study, saroglitazar 4 mg significantly improved ALT, LFC, insulin resistance, and atherogenic dyslipidemia in participants with NAFLD/NASH. Based on these encouraging findings, saroglitazar has the potential to reduce the risk of CVD along with improving liver parameters and histology in patients with NASH. This will be tested in a next-phase randomized, placebo-controlled trial comparing saroglitazar 4 mg versus placebo in patients with biopsy-proven NASH with histological endpoints.

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Author Contributions: Substantial contributions to conception and design: S.G., D.P., PP., N.C. Acquisition of data: S.G., M.N., N.L., V.A., K.C., K.V.K., M.L., E.S., N.C. Analysis and interpretation of data: All authors. Drafting the original article: S.G., N.C. Revising article critically for important intellectual content: S.G., M.N., N.L., K.C., K.V.K., M.L., N.C. Final approval of the version to be published: S.G., D.P., N.C.

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