Effect of Saroglitazar on Fatty Acid Metabolism in Zucker Fa/Fa Rats

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Abstract

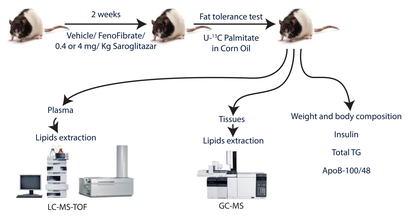
Introduction.
Saroglitazar is a novel dual PPARα/g agonist recently approved in India for the treatment of diabetic hypertriglyceridemia. The mode of action of this drug is unclear and there is a need to understand its underlying molecular mechanism.
Hypothesis.
We tested the hypothesis that Saroglitazar reduces the fatty acid absorption and consquently reduces circulating triglycrides.
Methods.
To understand the mechanisms of action of saroglitazar in vivo, we treated Zucker fa/fa (n=10-12 in each group) with vehicle, fenofibrate (F) (150 mg/kg) or saroglitazar (Saro) (0.4 or 4 mg/kg/day) for 14 days. On day 15, rats were gavaged with 5ml/kg of corn oil which contained [U-^{13}C]Palmitic Acid (PA) (1 gm/5ml). Plasma was obtained hourly for 8 hours. Adiopose and skeletal muscle was collected at 8 hours.
Incorporation of labeled fatty acid was monitored in various lipids using LC-MS and GC-MS.
Results.
Only 4 mg/kg/day Saro increased body weight (p<0.01) and reduced fasting insulin (p=<0.01 vs. vehicle) as well as reducing plasma triglyceride (TG) at 0 and 2 hour post corn oil treatment (p<0.01 and p<0.001, respectively). LC-MS and GC-MS were used to assess the incorporation of 13C-lipids into plasma and tissue lipids (n=5 for tissue metabolomics studies). The major M+16 isotopomers of the major TG species, TG(52:3) and TG(52:4), rose in the first two h following gavage (likely reflecting chylomicron production) and declined over the next 4 hours with a secondary rise at 6-8 h. In contrast, F-treatment caused a greater increase in M+16 TG species; both low and high dose Saro significantly attenuated the appearance of M+16 TG. In all animals, Major M+16 phosphatidyl choline PC(34:1), carried primarily in HDL and VLDL, rose at similar rates, however the % labeling in the F-treated animals was significantly lower, suggesting a reduction in liver derived lipids by F. Low and high dose Saro significantly increased the accumulation of M+16 palmitate in adipose tissue (by 86% and 247%, respectively, p<0.01). F, low and high dose Saro decreased Gastrocnemeous M+16 palmitate labeling, which could be due to induction of lipid oxidation by F and potentially Saro and reduced plasma levels of TG in Saro
Conclusion.
Saro significantly reduces fasting and postprandial TG levels through enhanced clearance of TG into adipose tissue and works by a mechanism distinct from that of F, a 'pure' PPARα activator.

Background

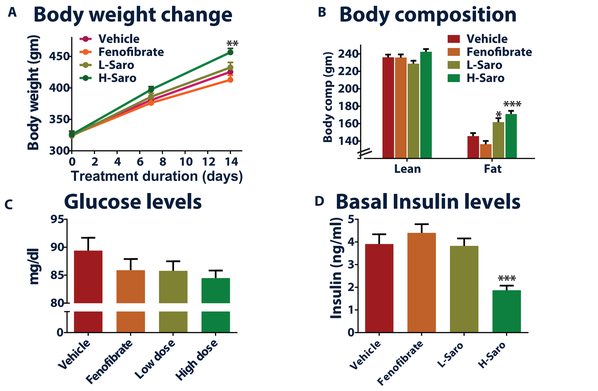
Dyslipidemia in type 2 diabetes (T2DM) is usually characterized by high triglycerides, high proportion of dense LDL-particles and low HDL cholesterol levels (1-2). An ideal therapy for T2DM should target both the glycemic and lipid abnormalities. PPARs are the nuclear receptors that primarily modulate lipid metabolism and that activation has salutary effects on lipid and glucose metabolism. Thiazolidinediones (TZDs) are agonists of PPARγ which improve insulin sensitivity and lower blood glucose levels. TZDs have a variable ability to lower triglyceride and elevated HDL cholesterol levels and can decrease the levels of small, dense LDL particles. PPARα receptors are activated by fibrates and are thought to induce the production of HDL-associated lipoproteins and lower apoC-III production and decreasing VLDL turnover.

Saroglitazar is a potent activator of both PPARγ and PPARα. Clinical studies have shown potent lowering of triglyceride levels in humans and in animal models as well as favorable effects on blood glucose level (3). Chylomicrons are made by the small intestines in the fed state, carry triglycerides from the intestines to the skeletal muscle where they are used as fuel, and to the adipose tissue for storage. The chylomicron remnants are then deposited in the liver. However, in impaired conditions like obesity, insulin resistance and diabetes, approximately 60% of triglycerides in the liver is reported to arise from free fatty acids a smaller fraction derived from de novo lipogenesis (4). Excessive accumulation of triglycerides in the liver can cause serious conditions like NAFLD and NASH. To define the mechanism for modulation of fatty acid metabolism by saroglitazar, we developed a mass-spectrometry based method to determine the dynamics of absorption and redistribution of an orally administered lipid load to fa/fa rats following 14 days of treatment with fenofibrate (F) (150 mg/kg) or saroglitazar (Saro) (0.4 or 4 mg/kg/day).

Experiment outline

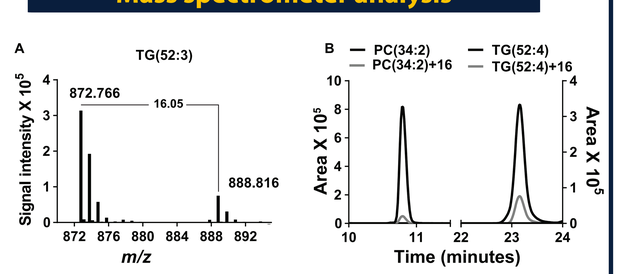


Clinical Characteristics



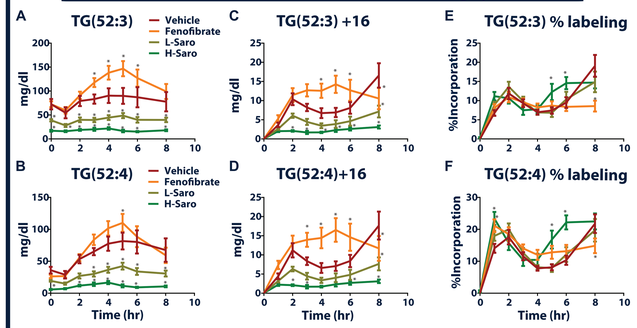
Fa/Fa rats were treated for 2 weeks with either a vehicle, Fenofibrate, low dose of Saroglitazar (L-Saro) or high dose Saroglitazar (H-Saro). Body weight (A) increased approximately 12% only in the H-Saro group while fat mass (B)increased in both the L- and H-saro groups. Glucose was not affected by any treatment (C) while the H-saro group showed a significant reduction in serum insulin (D) and triglyceride (E) levels. Westerblotting for ApoB-100 and Apo-48 showed that Fenofibrate and high dose saroglitazar increased ApoB-100 levels while saroglitazar, but not fenofibrate, markedly reduced Apo-B48 levels (* P ≤ 0.05, ** P≤ 0.01, *** P≤ 0.001, **** P≤0.0001).

Mass spectrometer analysis



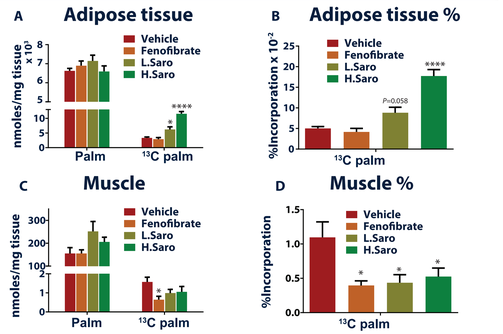
To further understand the lipids lowering effect of Saroglitazar, rats were orally gavaged with U-^{13}C palmitate (1 g/kg) dissolved in 2 ml corn oil (n=9 for each group except fenofibrate where n=8). U-^{13}C palmitate will cause a mass shift of +16 Daltons that can be monitored using mass spectrometer (A).The palmitate label was introduced in many lipids species with different percentage. A representative chromatogram for phosphatidylcholines (PC) and triglycerides (TG) and their corresponding label is shown (B)

Fatty acid incorporation into triglyceride



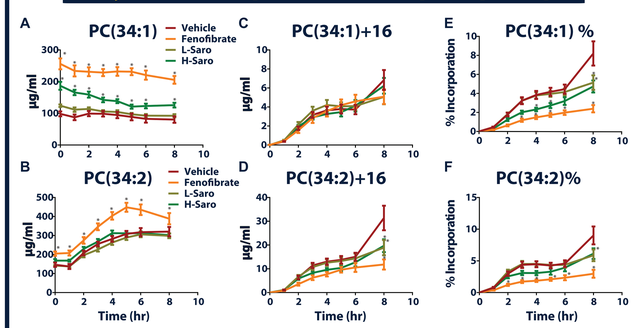
^{13}C-palmitate flux into Triglycerides
The fasting levels of the highly abundant TG (52:3) and TG (52:4) were significantly lower in H-Saro compared to vehicle (A-B). Fenofibrate did not affect fasting TG levels compared to vehicle (A-B). After gavage, the levels of the unlabeled TG increased in both vehicle and fenofibrate while it remained significantly lower with H-Saro and L-Saro compared to vehicle. Fenofibrate caused an elevation in the unlabeled TG levels after ~ 3h of the fat administration while L-Saro and H-Saro significantly blunted the TG rise. The labeled (^{13}C palmitate) was absorbed and esterified into triglycerides which enters the blood stream as chylomicrons. The incorporation of ^{13}C-palmitate into TG (52:4) and TG(52:3) was detected after 1 hour of fat dosage and peaked at ~ 2 hours and started increasing again at 6-8 hours (C-D) in vehicle treated rats. Fenofibrate treated rat showed a rise and remained elevated for the remainder of the sampling, consistent wtih a reduced clearance. Both L-saro and H-Saro showed a diminished peak of of labeled M+16 TG (C-D). The incorporation ratios of U-^{13}C palmitate into triglycerides was similar in all groups with a peak at ~1-2 hours and another increase at 8 hours (E-F) suggesting the roduction of TG-laden VLDL. Fenofibrate did not show this late increase suggesting a reduction in VLDL production.

Fatty acid uptake into fat and muscle



Fatty acid flux into adipose tissue and muscles
To understand the reason for the significant reduction in labeled and unlabeled triglycerides in rats treated with saroglitazar, we measured the uptake of U-^{13}C palmitate into visceral adipose tissue gastrocnemius muscles. Total lipids were extracted from tissues and hydrolyzed to free fatty acid total and U-^{13}C palmitate assessed by MS. Adipose tissues (normalized to tissue weight) showed equal levels of unlabeled palmitate (A). Adipose tissue from both low and high dose of saroglitazar treated animals increased the uptake and the incorporation of U-^{13}C palmitate (A and B). Muscle also showed no differences in the total levels of fatty acids with a reduction in total ^{13}C palmitate in the fenofibrate treated animals (C) with a statistically significant reduction in the muscle lipids in all treatment groups (D).

Fatty acid incorporation into phospholipid



^{13}C-palmitate flux into phosphatidyl choline
We examined the production of phosphatidyl choline (PC), which is contained primarily in HDL and VLDL particles produced in the liver. Both FenoFibrate and H-Saro increased the level of PC (34:1) at baseline with a lesser effect on PC(34:2) (A and B). The corresponding ^{13}C-labeled PC species did not show significant difference between the groups (C-D). Consistent with the production in the liver, the levels of ^{13}C-PC increased Starting at 2h (C-D). The percent labeling was lowest in the fenofibrate and H-Saro groups with fenofibrate having a greater effect (E-F), suggesting a lower turnover of the PC pools by fenofibrate and H-Saro.

Summary

- Fenofibrate has neutral effects on weight, body composition and glucose homeostasis.

- High dose, but not low dose saroglitazar increased body weight and both doses caused a small increase in fat mass. High dose saroglitazar markedly reduced insulin levels and reduced triglyceride levels.

- Both fenofibrate and high dose saroglitazar increase ApoB-100 levels in the blood. The increase appears to be due, in part, to a decrease in clearance of VLDL particles as evidenced by the increase in PC levels in the blood. An increase in HDL likely contributes to the increase.

- The blunting of the post-meal rise in ^{13}C-labeled triglyceride species by saroglitazar is likely due to enhanced clearance of chylomicron particles by increased uptake into adipose tissue.

Conclusion

- Saroglitazar demonstrates evidence of both PPARα and PPARγ effects in vivo.
- Low dose saroglitazar which achieves in vivo levels equivalent to that seen in humans has potent ability to lower post-prandial triglyeride levels without affecting body wight.
- The clinical effect of saroglitazar may be primarily due to enhanced clearance of chylomicrones into adipose tissue.
- Similar studies in humans will clarify whther there are similar actions in humans following saroglitazar treatment.

References

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