Saroglitazar Shows Therapeutic Benefits in Mouse Model of Non-alcoholic Fatty Liver Disease (NAFLD) and Non-alcoholic Steatohepatitis (NASH)

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Abstract

NAFLD and NASH are common clinico-pathological conditions affecting millions of patients worldwide. Although numbers of therapeutic options have been explored for management of NAFLD/NASH, no pharmacological treatment is yet approved. Saroglitazar is a novel PPARα/γ agonist that shows anti-hyperlipidemic, anti-hyperglycemic and insulin sensitizing effects.

C57BL/6 mice fed with choline-deficient amino acid-defined, high-fat diet (CDAHFD) containing 60 kcal% fat is known to develop a condition similar to human NASH. Following eight-weeks of CDAHFD feeding, animals were treated with saroglitazar (1 and 3 mg/kg) or fenofibrate (300 mg/kg) for 8 weeks and maintained on CDAHFD. Saroglitazar (1 and 3 mg/kg) showed dose-dependent and significant (p<0.001) reduction in serum ALT (38 and 57 %), AST (33 and 56%) and MCP-1 (41 and 42%) levels when compared with untreated (CDAHFD-fed) disease control animals. Liver lipid accumulation was also significantly attenuated (60-70%, p<0.001) by saroglitazar treatment. Fenofibrate (300 mg/kg) also showed reduction in serum ALT (44%), AST (51%) and MCP-1(37%), however, the elevated liver lipids were not affected by fenofibrate treatment. The expression of pro-inflammatory genes such as MMP-9, TNF-α and pro-fibrotic marker genes such as α-SMA were also suppressed in saroglitazar-treated animals. Histological investigation of liver revealed significant reduction of steatosis, ballooning, inflammation and fibrosis in animals treated with saroglitazar. The NASH score of saroglitazar (3 mg/kg) group was 4.7 Vs 17.8 for untreated control animals and 16.4 for fenofibrate treated animals. The results indicate that saroglitazar appears to be a promising drug for the management of NAFLD/NASH

Background

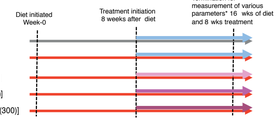
Saroglitazar is a dual peroxisome proliferator activated receptor-alpha (PPARα) and gamma (PPARγ) agonist with a predominant PPARα agonism. In various preclinical and clinical studies, saroglitazar showed significant lipid lowering and insulin sensitizing effects. Saroglitazar has been approved in India for the treatment of "diabetic dyslipidemia" and "hypertriglyceridemia in patients with type 2 diabetes not adequately controlled by statins". Previously we have demonstrated (NAFLD-Keystone Symposia Conference , March-2015) the prevention of NAFLD/NASH by treatment of saroglitazar. Hence, this study was designed to evaluate effects of saroglitazar on prevention and reversal of NAFLD/NASH in a mouse model of this disease.

Methods

Animals

- Male C57BL/6 mice of 6-8 wks of age from Zydus Research Centre, Cadila Healthcare Ltd. India.

Study design



- Normal Diet control (N-C)
- CDAHFD+ Vehicle (D-C)
- CDAHFD+ Saroglitazar 1 mg/kg [D-Saro (1)]
- CDAHFD+ Saroglitazar 3 mg/kg [D-Saro (3)]
- CDAHFD+ Fenofibrate 300 mg/kg [D-Feno (300)]

- CDAHFD is Choline-deficient, L-amino acid-defined, high-fat diet containing 60 kcal% fat with 0.1% methionine and no added choline (Product # A06071302, Research Diet, USA) and Normal Control diet is diet containing 10 kcal% fat and crystalline Amino acids (Product # A013012807; Research Diet, New Brunswick, NJ,USA).

*Parameters Evaluated

- Serum Chemistry : ALT, AST, MCP-1
- Liver Biochemistry : Triglyceride, Total cholesterol, Hydroxyproline (OH-pro)
- Liver Gene Expression : MMP-9, TNF-α, TIMP-1,
- Liver Histochemistry : H&E Staining, Oil-Red-O, Mason's Trichome and PAS staining

Scoring of liver histology slides

- Liver histology slides were evaluated for NAFLD activity scoring (NAS) as per the scoring method given by Kleiner et al, 2005 (Hepatology 41:1313-1321) as described below-

For hepatocellular steatosis-

- Grade 1: steatosis occupying <33% of the hepatic parenchyma
- Grade 2: 34-66% of the hepatic parenchyma
- Grade 3: more than 66 % of the hepatic parenchyma)

For inflammatory cell infiltration:

- Grade 0 : none
- Grade 1: 1-2 foci per 200X field
- Grade 2: 3-4 foci per 200X field
- Grade 3: more than 4 foci per 200X field

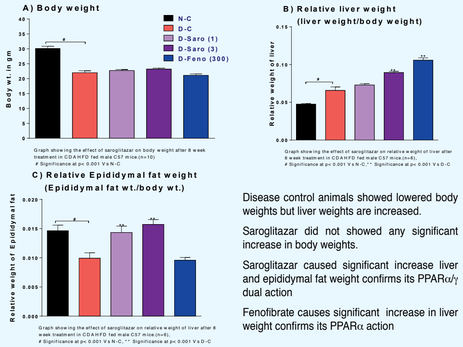
For hepatocellular ballooning;

- Grade 0: none
- Grade 1: few balloon cells
- Grade 2: many cells ballooning

The staging of hepatic fibrosis classified into stages 0-4

- Stage 0: None
- Stage 1: mild perisinusoidal or periportal
- Stage 2: moderate perisinusoidal or periportal
- Stage 3: bridging fibrosis
- Stage 4: cirrhosis

Figure 1. Effect of Saroglitazar on body weights and organ weights



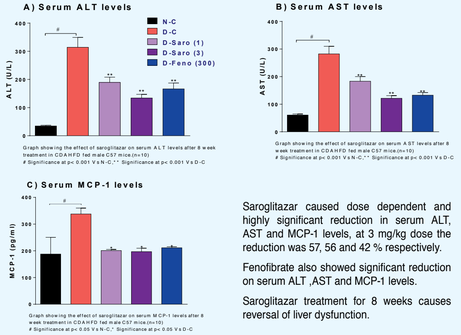
Disease control animals showed lowered body weights but liver weights are increased.

Saroglitazar did not showed any significant increase in body weights.

Saroglitazar caused significant increase liver and epididymal fat weight confirms its PPARα/γ dual action

Fenofibrate causes significant increase in liver weight confirms its PPARα action

Figure 2. Effect of Saroglitazar on serum biomarkers of NASH

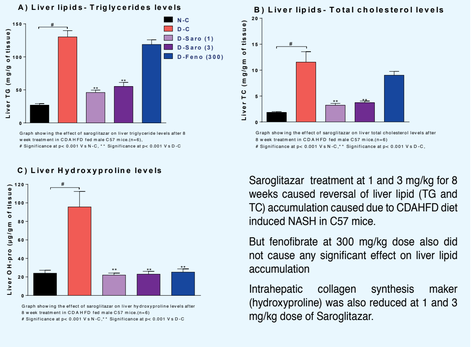


Saroglitazar caused dose dependent and highly significant reduction in serum ALT, AST and MCP-1 levels, at 3 mg/kg dose the reduction was 57, 56 and 42 % respectively.

Fenofibrate also showed significant reduction on serum ALT ,AST and MCP-1 levels.

Saroglitazar treatment for 8 weeks causes reversal of liver dysfunction.

Figure 3. Effect of Saroglitazar on liver biomarkers of NASH

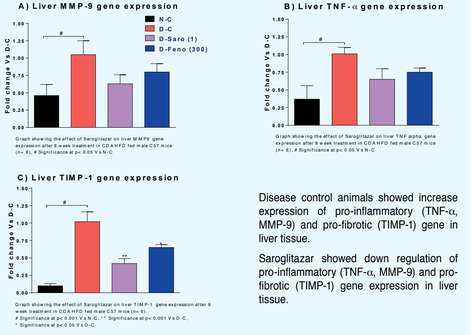


Saroglitazar treatment at 1 and 3 mg/kg for 8 weeks caused reversal of liver lipid (TG and TC) accumulation caused due to CDAHFD diet induced NASH in C57 mice.

But fenofibrate at 300 mg/kg dose also did not cause any significant effect on liver lipid accumulation

Intrahepatic collagen synthesis maker (hydroxyproline) was also reduced at 1 and 3 mg/kg dose of Saroglitazar.

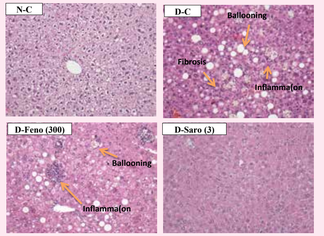
Figure 4. Effect of Saroglitazar on molecular biomarkers in liver

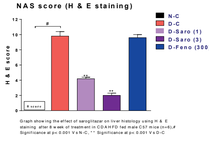


Disease control animals showed increase expression of pro-inflammatory (TNF-α, MMP-9) and pro-fibrotic (TIMP-1) gene in liver tissue.

Saroglitazar showed down regulation of pro-inflammatory (TNF-α, MMP-9) and pro-fibrotic (TIMP-1) gene expression in liver tissue.

Figure 5. Effect of Saroglitazar on hepatocellular steatosis, balloning, inflammation and fibosis (Hematoxylin-Eosin staining, 20X)

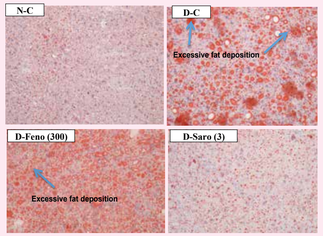


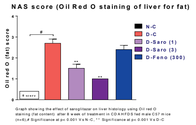


Microscopic Observations

- **N-C:** Normal histological appearance of liver section,
- **D-C:** Grade 3 hepatocellular steatosis, ballooning (grade 2), inflammation (grade 3) & fibrosis (stage-1)
  D-Feno (300): Grade 3 hepatocellular steatosis, ballooning (grade 1-2), inflammation (grade 3) & fibrosis (stage-1)
- **D-Saro (3):** Histological changes reversal to normal

Figure 6. Effect of Saroglitazar on hepatocellular steatosis (lipid accumulation) (Oil Red-O staining for fat deposition, 20X)

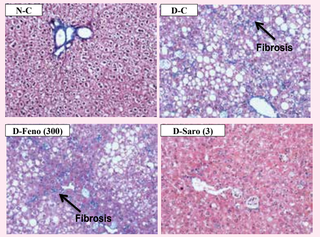


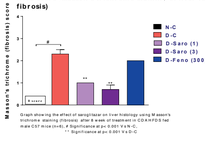


Microscopic Observations

- **N-C:** Normal Control liver section after oil red-o staining
- **D-C:** Grade 2-3 for presence of fat
- **D-Feno (300):** Grade 2-3 for presence of fat
- **D-Saro (3):** Grade 1 presence of fat

Figure 7. Effect of Saroglitazar on hepatic fibrosis (Masson's Trichrome staining, 20X)

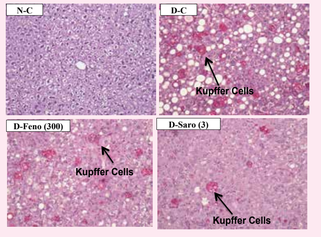


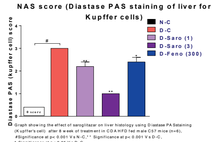


Microscopic Observations

- **N-C:** Control-Negative Masson's Trichrome,
- **D-C:** Stage 1 for presence of fibrosis,
- **D-Feno (300):** Stage 1 for presence of fibrosis,
- **D-Saro (3):** Resolution of fibrosis

Figure 8. Effect of Saroglitazar on hepatic inflammation and fibrosis (diastase periodic acid-schiff staining for Kupffer cells, 20X)





Microscopic Observations

- **N-C:** Normal
- **D-C:** Increased number of Kupffer cells, grade 3
- **D-Feno (300):** Increased number of Kupffer cells, grade 2-3
- **D-Saro (3):** Presence of Kupffer cells of grade 1

Summary and conclusions

Saroglitazar (1 and 3 mg/kg) treatment for 8 weeks, significantly reduced the levels of liver dysfunction markers such as serum ALT, AST and MCP-1.

Saroglitazar reduced the accumulation of lipid in hepatocytes. Furthermore, it also causes down regulation of various pro-inflammatory (TNF-α, MMP-9) and pro-fibrotic (TIMP-1) gene expression.

Histologically saroglitazar caused reversal of hepatocellular steatosis, ballooning, inflammation and fibrosis and overall saroglitazar (3 mg/kg) causes 74% reduction in total NASH score.

Saroglitazar caused reversal of hepatocellular steatosis (lipid accumulation) whereas fenofibrate did not show any effect which was also evident in liver biochemical analysis.

Furthermore Saroglitazar caused reversal of hepatic fibrosis which was not evident in fenofibrate treatment group.

The results indicate that Saroglitazar shows beneficial effects in an established mouse model of non-alcoholic steatohepatitis. Saroglitazar appears to be a promising drug for the management of NAFLD/NASH.

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