Saroglitazar Treatment Prevents NASH, Eliminates Hepatocyte Ballooning, and Significantly Improves Serum LFTs, Lipids and Insulin Resistance in DIAMOND™ Mice Compared to Pioglitazone Benchmark

R. CAFFREY[1], J. MARIONEAUX [1] , P. SANTHEKADUR[2], P. BEDOSSA[3], B. PHILIP[4], M. JAIN[4], S. GIRI[4], and A. SANYAL[2]

1 Sanyal Biotechnology, Norfolk, VA USA
2 Virginia Commonwealth University, Richmond VA USA
3 Liverpat, Paris, France
4 Zydus Cadila, Gujarat, India

EASL The Home of Hepatology
THE INTERNATIONAL LIVER CONGRESS™ 2018
APRIL 11-15 PARIS FRANCE

INTRODUCTION

In this study the efficacy of the dual PPAR-α/γ agonist Saroglitazar in preventing progression of early NASH F0 to more advanced NASH with fibrosis was investigated in Sanyal Biotechnology's DIAMOND™ mouse model. It has previously been demonstrated that the pure PPAR-α agonist, Pioglitazone, attenuates some measures of NASH and metabolic syndrome in this mouse model, which develops all the symptoms of human metabolic syndrome and NASH when fed a Western Diet[1,2,3]. Saroglitazar has previously improved liver function and fibrosis in other rodent models of NASH such as CCL4-induced fibrosis model and the choline-deficient high-fat diet model[4]. We hypothesized that administration of Saroglitazar may also prevent progression of NASH in the DIAMOND™ model.

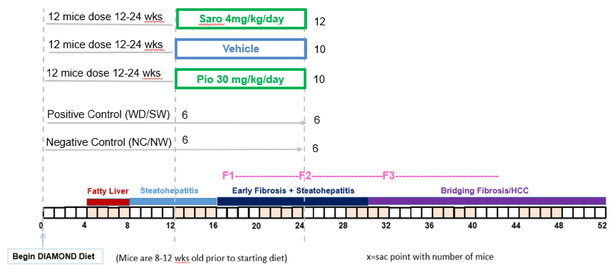
AIMS

(1) To determine if Saroglitazar administration could prevent progression of NASH,
(2) To compare the efficacy of Saroglitazar with benchmark Pioglitazone and positive and negative natural history controls.

METHOD

8 week old DIAMOND™ mice (10-12 per group) were weight randomized and placed on either normal chow/normal water (NC/NW) or Western Diet/sugar water (WD/SW) for 12 weeks. At 12 weeks on diet the WD/SW groups to progress to full metabolic syndrome with NASH F0 while NC/NW negative natural history controls remain healthy. Daily oral gavage of Saroglitazar (4 mg/kg/day), pioglitazone (30 mg/kg/day) and vehicle (water) began at 12 weeks and both dosing and diet continued for 3 months. At 24 weeks mice were necropsied and liver tissue and serum were collected. Sirius Red and H&E stained sections were made from each mouse, and scored for measures of NASH pathology including fibrosis, steatosis grade and percentage, inflammation, ballooning, NAS, SAF activity, Fibrosis (NASH CRN), Perisinusoidal Fibrosis, and NASH category. Oil Red) staining of frozen sections for neutral lipids was also performed. Serum LFTs, lipids, fasting insulin and glucose were measured and HOMA IR score was calculated. The Saroglitazar treated group was compared to pioglitazone benchmark, vehicle control, and both Western Diet/Sugar Water (WD/SW) positive and normal chow/normal water (NC/NW) negative natural history controls. Statistical significance was calculated with Student's 2-tailed T-Test.

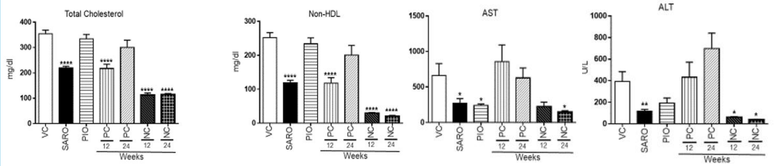
EXPERIMENTAL DESIGN



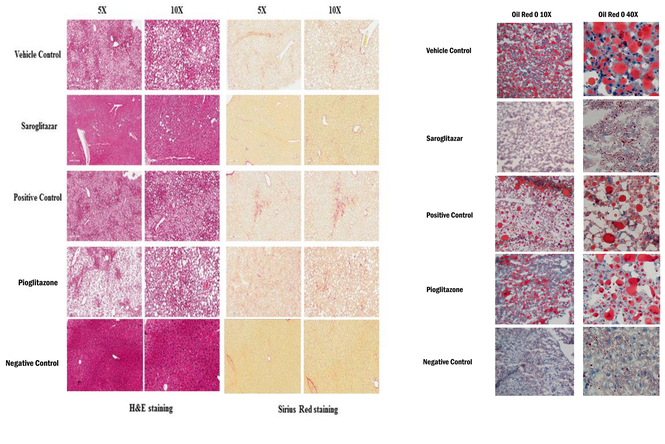
RESULTS

- Compared to pioglitazone, Saroglitazar significantly reduced steatosis (both percent and grade), NAS score, and SAF activity score.
- Hepatocyte ballooning was completely eliminated in the Saroglitazar-treated group.
- There was significantly less liver fibrosis as measured by NASH CRN score and perisinusoidal fibrosis score in the Saroglitazar-treated group compared to the positive control group.
- No Saroglitazar treated mice progressed past steatosis to NASH whereas most pioglitazone and positive WD/SW controls did progress.
- The body weight of the Saroglitazar treatment group at end of study was significantly less than pioglitazone, vehicle control, and positive controls.
- Saroglitazar lowered fasting blood glucose more than pioglitazone and lowered fasting insulin more than all other groups.
- HOMA IR was improved and total serum cholesterol and triglycerides were significantly decreased in the Saroglitazar treated mice.
- Saroglitazar treatment successfully attenuated and even reversed some serological and pathological measures of NASH.

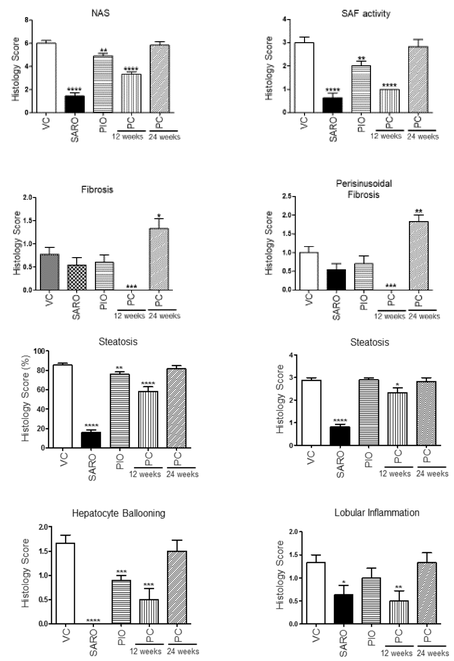
Serum Lipids and LFT Measurements



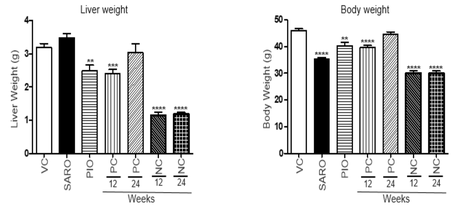
Left Panel: H&E and Sirius Red staining of PPFE liver sections. Right Panel: Oil Red O staining of neutral lipids in frozen liver sections.



Comparative Histology Scores (liver)



Liver weight (left) and body weight at necropsy (right).



CONCLUSIONS

The pathogenesis of NASH and metabolic syndrome/diabetes have mechanistic drivers in common. This study demonstrated that Saroglitazar inhibits steatosis, inflammation, ballooning, and fibrosis in addition to lowering body weight, serum LFTs and lipids. Saroglitazar ameliorated NASH development and progression in addition to improving measures of insulin resistance and diabetes. Saroglitazar met the primary study endpoint of preventing NASH progression in the DIAMOND™ mouse model, and the secondary endpoint of outperforming the efficacy of benchmark Pioglitazone in the DIAMOND™ mouse model.

ACKNOWLEDGEMENTS

We thank Maryann Clements of EVMS for her histology services, and Ryan Huyck of EVMS for his technical assistance.

REFERENCES

1. **A. Asgharpour, et al**. A diet-induced animal model of non-alcoholic fatty liver disease and hepatocellular cancer. J Hepatol. 2016 Sep;65(3):579-88.

2. S. Cazanave et al. The Transcriptomic Signature Of Disease Development And Progression Of Nonalcoholic Fatty Liver Disease. Scientific Reports, 08 Dec 2017.

3. A. Oseini, et al. The Physiological, Pathological and Molecular Basis of Nonalcoholic Steatohepatitis Associated Cardiomyopathy. Abstract 2057, AASLD 2017.

4. **Jain, M., et al**. Dual PPARα/γ agonist saroglitazar improves liver histopathology and biochemistry in experimental NASH models.. *Liver Int. 2017 Nov 21*.

CONTACT INFORMATION

Dr. Rebecca Caffrey, Sanyal Biotechnology, rebecca@sanyalbio.com