Saroglitazar shows Beneficial Effects on Insulin Sensitivity, Dyslipidemia and Blood Pressure in Zucker Fatty Rats

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Abstract

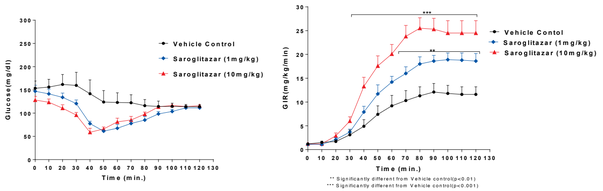
- Saroglitazar is a novel dual PPARαγ agonist that has shown significant lipid-lowering and insulin-sensitizing effects with good safety profile in various preclinical models.
- In present study, we have evaluated insulin sensitizing, lipid lowering and antihypertensive potential of saroglitazar in insulin resistant Zucker fatty rats. Zucker fa/fa showed significantly higher ($P < 0.001$) blood pressure, serum TG and insulin levels than the lean control rats. Oral once-daily treatment of Zucker fa/fa rat with Saroglitazar for 15 days caused a dose-dependent increase in Glucose Infusion Rate (54 % and 127 % increase at 1 and 10 mg/kg respectively) during hyperinsulinemic-euglycemic clamp condition, indicating significant improvement in insulin sensitivity.
- In a separate experiment, once daily treatment of Zucker fa/fa with Saroglitazar at 4 mg/kg dose for 14 days, showed a significant decrease ($P < 0.001$) in SBP (22mmHg), serum TG (81%), serum insulin (76%) and HOMA-IR (79%). These changes were accompanied by up-regulation of serum adiponectin levels & expression of PPAR-related target genes in adipocytes and liver. Pioglitazone (10 mg/kg), showed similar decrease in SBP (21 mmHg), serum insulin (72%) and HOMA-IR (80%), however, the TG-lowering effect of pioglitazone was less pronounced (48% reduction) as compared to Saroglitazar. On the other hand, fenofibrate (100 mg/kg) showed decrease in serum TG (54%), which was similar to Pioglitazone but had no significant effect on SBP.
- Overall, the results suggest that Saroglitazar, a drug approved in India for treatment of diabetic dyslipidemia shows insulin sensitizing, lipid lowering and blood pressure lowering effects in an animal model of metabolic syndrome.

Materials and Methods

- Zucker fatty (fa/fa) rats used in this study were supplied by Animal Research Facility, Zydus Research Centre (ZRC).
- Hyperinsulinemic-euglycemic clamp study was carried out in 11-12 weeks old male Zucker fa/fa rats. Animals were randomized based on their non-fasted serum glucose, body weight and serum triglycerides (TG) levels and treated with vehicle or saroglitazar (1 and 10 mg/kg/day) for 15 days by oral gavage. On day-8 of treatment left carotid artery and right jugular vein were catheterized sing MRE-40 tubing. On Day 15, one-hour post-dose, hyperinsulinemic-euglycemic clamp was performed in 5- hour fasted animals. Briefly 0 min blood collection was performed from the arterial line for insulin measurement and glucose was measured by glucometer (OneTouch Ultra 2) for basal glucose level. Regular Human insulin (Huminsulin, Eli Lilly and Company, India) was infused at 10 mU/kg/min through the jugular vein at a constant rate of 2 μl/min with the help of an automated infusion pump to achieve hyperinsulinemia. To maintain the blood glucose at target euglycemic level (100-120 mg/dL), 50 % glucose was infused at a variable infusion rate by infusion pump. Blood glucose was measured at 10 min interval till the completion of the clamp period (0-120 min). Glucose infusion rate was adjusted if necessary. During the steady state clamp condition (~ 1.5 hours after intiation of clamp experiment) euglycemic levels (100-120 mg/dL) was maintained for at least 20 min.
- In another study, fifteen to sixteen week old Zucker fa/fa rats were randomized and grouped (n=7) based on serum TG and body weights and then for next 15 days each animal was dosed (by oral gavage) daily with vehicle, saroglitazar (Saro) (4 mg/kg/day), pioglitazone (Pio) (10 mg/kg/day) or fenofibrate (Feno) (100 mg/kg/day). On day-14, blood was collected one-hour post-dosing and serum was analyzed for TG and adiponectin (Acrp30) levels. On Day-15, the animals were subjected to non-invasive blood pressure measurement by using 8-channel non-invasive blood pressure monitor (NIBP-8, Columbus Instruments, USA). Animals sacrificed and liver, white adipose tissue (WAT) and skeletal muscle was collected for gene expression study.
- Serum TG was measured by using autoanalyser (Cobas c 311 from Roche Diagnostics) using commercially available kits from Roche Diagnostics,GmbH D-68298 Mannheim, Germany). Serum insulin was adiponectin levels were measured using ELISA kits from Crystal Chem Inc, USA and R& D systems Inc. USA respectively.. Gene expression study was done by Real-time RT-PCR using gene-specific primer pairs and SYBR Green method. Average difference in the Ct value of specific target and internal control genes was determined for calculating the relative gene expression and then fold change Vs control was calculated.

Effect of Saroglitazar on insulin sensitization in Zucker fa/fa rats

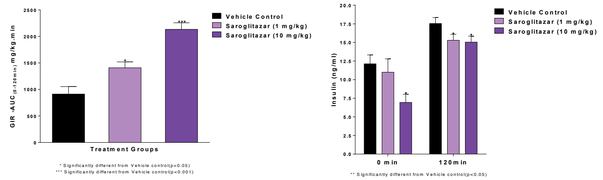
Figure 1. Blood glucose levels and glucose infusion rates (GIR) in hyperinsulinemic-euglycemic clamp study in Zucker fa/fa rats.



Saroglitazar caused a dose-dependent increase in glucose infusion rate (54 % and 127 % increase at 1 and 10 mg/kg respectively)

Effect of Saroglitazar on insulin sensitization in Zucker fa/fa rats

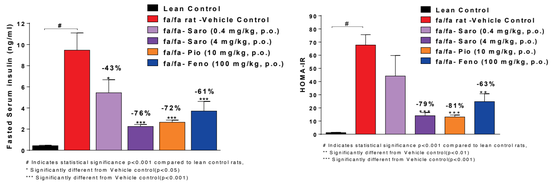
Figure 2. Total area under curve for GIR and insulin levels at basal and clamped stage of hyperinsulinemic-euglycemic clamp study in Zucker fa/fa rats.



Increased GIR with significant decrease in insulin levels in Saroglitazar treatment confirmed its potent insulin sensitization effect.

Effect of Saroglitazar on serum insulin and HOMA-IR levels in Zucker fa/fa rats

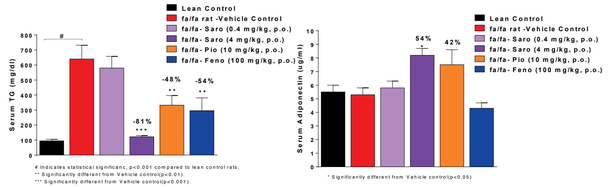
Figure 3. Fasted serum insulin and HOMA-IR after 14 days repeat dose treatment in Zucker fa/fa rats.



All the three comps showed significant reduction in serum insulin and HOMA-IR ratio.

Effect of Saroglitazar on serum triglycerides and adiponectin levels in Zucker fa/fa rats

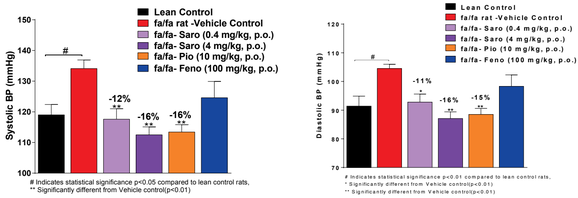
Figure 4. Serum triglycerides and adiponectin levels after 14 days repeat dose treatment in Zucker fa/fa rats.



Saroglitazar showed significant reduction in serum TG and significant increase in serum adiponectin levels.

Effect of Saroglitazar on systolic and diastolic blood pressure in Zucker fa/fa rats

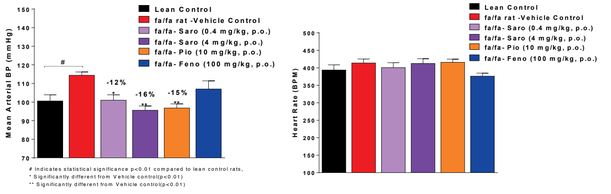
Figure 5. Systolic and diastolic blood pressure after 15 days repeat dose treatment in Zucker fa/fa rats.



Saroglitazar showed significant decrease in systolic blood pressure (22mmHg) and diastolic blood pressure .

Effect of Saroglitazar on mean arterial blood pressure and heart rate in Zucker fa/fa rats

Figure 6. Mean arterial blood pressure and heart rate after 15 days repeat dose treatment in Zucker fa/fa rats.



Saroglitazar showed significant decrease in MABP and no effect on heart rate.

Effect of Saroglitazar on gene expression in liver, WAT and skeletal muscle (fold change Vs control)

	Liver		
Treatment Group	Acox1	FABP4	LPL
Vehicle Control	1.00 ± 0.05	1.07 ± 0.25	1.03 ± 0.15
Saro (0.4 mg/kg, p.o.)	3.27 ± 0.63	0.93 ± 0.22	1.06 ± 0.19
Saro (4 mg/kg, p.o.)	13.02 ± 4.28	3.00 ± 1.37	1.52 ± 0.46
Pio (10 mg/kg, p.o.)	0.74 ± 0.16	1.75 ± 0.77	1.13 ± 0.50
Feno (100 mg/kg, p.o.)	38.68 ± 10.75**	6.84 ± 3.65	2.16 ± 0.35

	White Adipose tissue (WAT)		Skeletal muscle	
Treatment Group	aP2	LPL	CPT	mCAD
Vehicle Control	1.02 ± 0.12	1.05 ± 0.19	1.01 ± 0.08	1.05 ± 0.18
Saro (0.4 mg/kg, p.o.)	1.33 ± 0.10	0.76 ± 0.09	1.0 ± 0.22	1.18 ± 0.18
Saro (4 mg/kg, p.o.)	2.63 ± 0.43*	0.83 ± 0.24	1.39 ± 0.16	1.42 ± 0.20
Pio (10 mg/kg, p.o.)	2.09 ± 0.53	0.97 ± 0.24	1.14 ± 0.21	0.93 ± 0.04
Feno (100 mg/kg, p.o.)	2.66 ± 0.30*	0.95 ± 0.14	1.13 ± 0.12	1.34 ± 0.18

* Indicates statistical significance at p<0.05 and
** indicates statistical significance at p< 0.001 compared to vehicle control

Saroglitazar showed significant up-regulation of PPARα (Acox1 in liver) and PPARγ (aP2 in WAT) related genes.

Conclusions

- **Saroglitazar showed a significant reduction in serum triglycerides, insulin and HOMA-IR along with up-regulation of serum adiponectin levels.**
- **Saroglitazar showed significant improvement in insulin sensitivity as indicated by dose-dependent increase in glucose infusion rate during hyperinsulinemic-euglycemic clamp condition.**
- **Saroglitazar showed a significant decrease in systolic, diastolic and mean arterial blood pressure and did not have any effect on heart rate.**