CONTENTS

Editorial

- Pharmacologists of India: Shiv Prakash 259

Review Article

- Therapeutic alternatives from venoms and toxins: Shivaji P. Gawade 260

Research Articles

- Genotoxic evaluation of morphine, buprenorphine, pentazocine, and noscapine by micronucleus and comet assay in albino mice: Lakshman Kumar Puli, P. A. Patil 265

- Age-related susceptibility to chronic haloperidol-induced orofacial dyskinesia: Biochemical and neurochemical evidence: Mahendra Bishnoi, Kanwaljit Chopra, Shrinivas K. Kulkarni 269

- Effect of amlodipine on blood and aortic tissue concentration of endothelin in male rabbits receiving atherogenic diet: M. Mohammadi, F. Mirzaei, Reza Badalzadeh 276

- Free radical scavenging activity of gossypin and nevadensin: An *in-vitro* evaluation: S. Ganapaty, V.M. Chandrashekhar, H.R. Chitme, M. Lakashmi Narsu 281

- Gastrointestinal permeability studies using combinations of rifampicin and nucleoside analogue reverse transcriptase inhibitors in rats: T.T. Mariappan, Saranjit Singh 284

- Effects of meloxicam and rofecoxib on psychomotor performance: A randomized, double-blind, placebo-controlled cross-over study: Marwan S.M. Al-Nimer 291

- Non-invasive evaluation of arterial stiffness in patients with increased risk of cardiovascular morbidity: A cross-sectional study: Yashmaina Sridhar, M.U.R. Naidu, P. Usharani, Y.S.N. Raju 294

- Serum glucose and triglyceride lowering activity of some novel glitazones against dexamethasone-induced hyperlipidemia and insulin resistance: B.R. Prashantha Kumar, T.K. Praveen, M.J. Nanjan, M.D. Karvekar, B. Suresh 299

Workshop Report

- The basic concepts of scientific research and communication: (A Report on Preconference Workshop Held in Conjunction with the 40th Annual Conference of the Indian Pharmacological Society-2007): Pitchai Balakumar, Sreekant Murthy, Gowraganahalli Jagadeesh 303

- Author Index, 2007** 307

- Title Index, 2007** 310

Serum glucose and triglyceride lowering activity of some novel glitazones against dexamethasone-induced hyperlipidemia and insulin resistance

B.R. Prashantha Kumar, T.K. Praveen, M.J. Nanjan, M.D. Karvekar¹, B. Suresh

ABSTRACT

Objectives: To study the serum glucose and triglyceride lowering activity of some novel glitazones against dexamethasone-induced hyperlipidemia and insulin resistance in rats.

Materials and Methods: Serum glucose and triglyceride lowering activity of the test compounds and a standard, rosiglitazone, was tested at a dose of 10 mg/kg p.o. against dexamethasone-induced hyperlipidemia and insulin resistance in Sprague-Dawley (SD) rats. On day 11 of treatment, blood was collected for the estimation of serum glucose and triglyceride levels.

Results: All the 14 compounds and rosiglitazone significantly ($P < 0.05$) decreased dexamethasone-induced elevation of serum glucose when compared to dexamethasone-alone-treated group. Among these compounds, compound 10 showed better antihyperglycemic activity than rosiglitazone. Compounds 7, 8 and 9 have shown significant ($P < 0.05$) serum triglyceride lowering activity than did rosiglitazone. Out of the 14 compounds tested, only compounds 7, 8 and 9 have shown both serum glucose and triglyceride lowering activity.

Conclusion: The present study indicates that some of the newer glitazones show significant glucose and triglyceride lowering activity. In comparison to rosiglitazone, these glitazones show comparable serum glucose lowering and better triglyceride lowering activity.

KEY WORDS: Dexamethasone, glitazones, hyperlipidemia, insulin resistance, peroxisome proliferator-activated receptor

TIFAC CORE JSS College of
Pharmacy, Rocklands, Ootacamund
- 643 001, Tamil Nadu,
¹Government College of Pharmacy,
P Kalingarao Road, Bangalore - 560
027, Karnataka, India

Received: 06.09.2007

Revised: 05.01.2008

Accepted: 18.01.2008

Correspondence to:

Dr. M.J. Nanjan

E-mail: mjnanjan@gmail.com

Diabetes is a global disease with a huge adverse impact on health and mortality, particularly from cardiovascular disorders. It occurs at any time of life from infancy to old age. Type-2 diabetes is primarily a lifestyle disorder, which accounts for around 90% of diabetes cases and increasing at an astonishing rate, particularly in developing countries like India. In 1995, it has been estimated that around 135 million people had this condition, and this may increase to as many as 300 million by the year 2025.^[1]

Most commonly employed oral hypoglycemic agents are sulfonylureas and biguanides. These drugs, however, have disadvantages such as primary and secondary failure of efficacy as well as the potential for induction of severe hypoglycemia.^[2] There is a need, therefore, for new candidate molecules that may effectively reduce insulin resistance or potentiate insulin action in genetically diabetic or obese individuals. New drugs that reverse insulin resistance without stimulating insulin release from β -cells also fulfill a major medical need in the treatment of non-insulin-dependent diabetes mellitus (NIDDM). The search for such drugs with a potential to reduce long-term

complications of NIDDM is, therefore, of current interest. Between 1997 and 1999, a new class of drugs called 'glitazones' was approved by the FDA for the treatment of type-2 diabetes.^[3] These agents share a common chemical structure, namely thiazolidine-2,4-dione (TZD).

Glitazones correct hyperglycemia by enhancing tissue sensitivity to insulin,^[4-6] as a result of which glitazone treatment is not associated with dangerous hypoglycemic incidents that are normally observed with conventional sulfonylurea agents and insulin therapy.^[7] In the mid-1990s, the molecular target of glitazones was reported to be peroxisome proliferator-activated receptor gamma (PPAR- γ).^[8-11] Undesirable side-effects associated with existing glitazones, however, have been observed in animal and human studies, including cardiac hypertrophy, haemodilution and severe liver toxicity.^[12-17]

Furthermore, it is known that PPAR- α , another nuclear receptor belonging to PPAR family, regulates genes that are important for lipid metabolism. As people with diabetes often have adverse changes to their plasma lipid profile,^[18] it would be worthwhile to find an agent that could activate PPAR- α . An existing class of medicines that act on PPAR- α and being prescribed to control dyslipidaemia are fibrates. A single agent that could stimulate both PPAR- α and PPAR- γ , however, would be more valuable.^[19] The focus today is, therefore, on the development of newer glitazone PPAR agonists, which might surmount the problem associated with the known TZDs, and thus offer advantage as anti-diabetic agents. The objective of the present study was, therefore, to evaluate some newer glitazone molecules for their serum glucose and triglyceride lowering potential.

Materials and Methods

Animals

Male Sprague-Dawley rats weighing between 200 and 250 g were procured from in-house animal facility of J.S.S. College of Pharmacy, Ootacamund. The animals were housed under standard conditions of temperature ($22 \pm 3^\circ\text{C}$) and relative humidity (30–70%) with a 12:12 light-dark cycle. The animals were fed with standard pellet diet (Amrit Feeds Ltd., Bangalore) and water *ad libitum*. The Institutional Animal Ethics Committee (IAEC) of J.S.S. College of Pharmacy approved the proposal.

Chemicals

Dexamethasone sodium phosphate was obtained as a gift sample from M/s. Strides Arcolabs, Bangalore, India. Triglyceride and glucose estimation kits were from Ecoline, Merck Ltd., Mumbai, India. All the other reagents and chemicals used in the study were of analytical grade.

Synthesis of newer glitazones

Novel glitazones were synthesized with an appropriate synthetic scheme using both conventional and microwave methods of synthesis. The synthesized glitazones were characterized for their structures by IR, NMR, mass and elemental analysis. The details of synthesis and characterization of these glitazones are reported elsewhere.^[20] The structures of synthesized glitazones, which are selected for pharmacological screening based on quantitative structure activity relationship (QSAR) studies, are given in Figure 1.

Preparation of the test item for oral gavage

All the 14 compounds and rosiglitazone were suspended in 0.5% w/v sodium carboxymethyl cellulose (CMC) at the concentration of 1 mg/ml. These suspensions were administered at a dose volume of 10 ml/kg body weight.

Dose selection

All the newly synthesized glitazones were structural analogs of rosiglitazone. All these compounds were, therefore, tested at an effective anti-diabetic dose of rosiglitazone in rats, i.e., 10 mg/kg, p.o. As a preliminary study, all these synthesized compounds and rosiglitazone were tested at a single dose level.

Dexamethasone-induced insulin resistance and hyperlipidemia^[21]

Animals were divided into 17 groups, each consisting of six rats. Rats in the first group received vehicle (10 ml/kg

Figure 1: Structures of some novel glitazones synthesized and screened

p.o.) and served as control group, while the second group of rats received vehicle (10 ml/kg p.o.) plus dexamethasone (10 mg/kg s.c.) and served as positive control group. Rats in experimental groups 3–16 were treated with compounds 1–14 (10 mg/kg p.o.) plus dexamethasone (10 mg/kg s.c.), whereas rats in the 17th group were treated with rosiglitazone (10 mg/kg p.o.) plus dexamethasone (10 mg/kg s.c.). All the animals received their respective assigned treatment daily for a period of 10 days. Rats of group 2–17 were daily fasted overnight before dexamethasone treatment.^[21] On day 11, the animals were anesthetized with ether, and blood was collected from retro-orbital plexus. Serum was then separated for the estimation of glucose and triglyceride using Ecoline triglyceride and glucose estimation kits.

Statistics

All the results were expressed as mean $\pm$ SEM, and the data were analyzed using one-way ANOVA followed by Dunnett's multiple comparison post-test using GraphPad Prism 4 software. *P*-values <0.05 were considered significant.

Results

Effect on serum glucose level

All the 14 compounds and the standard, rosiglitazone, at a dose of 10 mg/kg p.o. significantly ($P < 0.05$) decreased dexamethasone-induced elevation of serum glucose. Among the 14 compounds tested, compound 10 (group 12) showed maximum anti-hyperglycemic activity that was superior to rosiglitazone (group 17) [Table 1].

Table 1**Effect of glitazones on dexamethasone-induced elevation in serum glucose levels**

Group	Treatment	Serum glucose (mg/dl)	Serum triglyceride (mg/dl)
1 (Normal)	Vehicle (10 ml/kg, p.o.)	61.5 ± 1.5	58.3 ± 1.9
2 (Positive control)	Vehicle (10 ml/kg, p.o.)	377.5 ± 39.5*	146.3 ± 12.3*
3	Compound-1 (10 mg/kg, p.o.)	140.3 ± 19.2*	119.8 ± 18.9
4	Compound-2 (10 mg/kg, p.o.)	102.0 ± 2.4*	219.0 ± 14.6
5	Compound-3 (10 mg/kg, p.o.)	101.0 ± 10.8*	229.3 ± 16.5
6	Compound-4 (10 mg/kg, p.o.)	87.0 ± 5.05*	139.3 ± 14.4
7	Compound-5 (10 mg/kg, p.o.)	102.5 ± 10.2*	121.3 ± 13.8
8	Compound-6 (10 mg/kg, p.o.)	118.3 ± 16.1*	120.0 ± 10.7
9	Compound-7 (10 mg/kg, p.o.)	81.5 ± 10.6*	86.8 ± 15.8*
10	Compound-8 (10 mg/kg, p.o.)	88.0 ± 4.9*	96.8 ± 13.3*
11	Compound-9 (10 mg/kg, p.o.)	83.2 ± 11.2*	99.5 ± 11.6*
12	Compound-10 (10 mg/kg, p.o.)	61.5 ± 10.3*	125.0 ± 13.8
13	Compound-11 (10 mg/kg, p.o.)	77.7 ± 12.7*	120.2 ± 13.5
14	Compound-12 (10 mg/kg, p.o.)	72.5 ± 11.3*	144.5 ± 13.9
15	Compound-13 (10 mg/kg, p.o.)	67.7 ± 8.5*	123.5 ± 14.9
16	Compound-14 (10 mg/kg, p.o.)	79.2 ± 5.2*	126.3 ± 19.6
17	Rosiglitazone (10 mg/kg, p.o.)	69.2 ± 2.1*	106.8 ± 8.2*

Values mean ± SEM, *n* = 6, **P* < 0.05. Animals of groups 2 to 17 also received dexamethasone (10 mg/kg, s.c.)

Effect on serum triglyceride level

Out of the 14 compounds, compounds 7 (group 9), 8 (group 10) and 9 (group 11) showed significant triglyceride lowering activity as compared to dexamethasone-alone-treated group. Among these, compound 7 showed better activity when compared to the other two. Compounds 7, 8 and 9, however, showed better activity when compared to the standard, rosiglitazone [Table 1].

Discussion

Insulin resistance in type-2 diabetes is not only associated with hyperglycemia but also with hyperlipidemia and atherosclerosis.^[18,22] A drug that simultaneously ameliorates insulin resistance and hyperlipidemia, therefore, facilitates better management of type-2 diabetes. The present study aims towards the development of such a drug. Out of the

14 compounds screened, only three compounds, namely compounds 7, 8 and 9, showed both serum glucose and triglyceride lowering activity. Compound 10, which was similar in structure to compound 7 except an oxo (=O) substitution instead of thione (=S) at position 2 in the thiazolidine ring, however, showed only glucose lowering effect, which was better than rosiglitazone. The absence of triglyceride lowering effect of this compound may be attributed to the presence of oxo substitution in the thiazolidine ring instead of thio. The same, however, was not observed with compounds 8 and 9, where both share similar structure except oxo and thione substitutions at position 2 of the thiazolidine ring, respectively. Compound 8, unlike compound 10, showed a significant triglyceride lowering potential; this may be attributed to the presence of an entirely different side chain at position 5 in the thiazolidine ring when compared to compound 10.

Compounds 7, 8 and 9 significantly lowered dexamethasone-induced serum glucose and triglyceride levels. In comparison to rosiglitazone, all these compounds have shown relatively less anti-diabetic activity, but better anti-hyperlipidemic activity. Among compounds 7, 8 and 9, compound 7 exhibited better anti-hyperlipidemic activity.

The better anti-hyperlipidemic activity of these three compounds when compared to rosiglitazone suggests that these compounds, in addition to PPAR- γ , may activate PPAR- α receptors, thereby resulting in better anti-hyperlipidemic activity. Further studies, however, are needed to confirm the same.

Conclusion

The results of the present study indicate that some of the newer glitazones synthesized show significant glucose and triglyceride lowering activities. In comparison to rosiglitazone, all these compounds show comparable serum glucose lowering and better triglyceride lowering activity.

References

- King H, Aubert RE, Herman WH. Global burden of diabetes 1995-2025: Prevalence, numerical estimates and projection. *Diabetes Care* 1998;21:1414-31.
- Dow RL, Bechle BM, Chou TT, Clark DA, Hulin B, Stevenson RW. Benzyloxazolidine-2,4-diones as potent hypoglycemic agents. *J Med Chem* 1991;34:1538-44.
- Hulin B, McCarthy PA, Gibbs EM. The glitazone family of antidiabetic agents. *Curr Pharm Des* 1996;2:85-102.
- Wang YX, Lee CH, Tiep S, Yu RT, Ham J, Kang H, *et al.* Peroxisome-proliferator-activated receptor δ activates fat metabolism to prevent obesity. *Cell* 2003;113:159-70.
- Lowell BB. PPAR γ : An essential regulator of adipogenesis and modulator of fat cell function. *Cell* 1999;99:239-42.
- Lehrke M, Lazar MA. The many Faces of PPAR γ . *Cell* 2005;123:993-9.
- Oguchi M, Wada K, Honma H, Tanaka A, Kaneko T, Sakakibara S, *et al.* Molecular design, synthesis and hypoglycemic activity of a series of thiazolidine-2,4-diones. *J Med Chem* 2000;43:3052-66.
- Kletzien RF, Clarke SD, Ulrich RG. Enhancement of adipocyte differentiation by an insulin-sensitizing agent. *Mol Pharmacol* 1992;41:393-8.
- Ibrahimi A, Teboul L, Gaillard D, Amri EZ, Ailhaud G, Young P, *et al.* Evidence for a common mechanism of action for fatty acids and thiazolidinedione antidiabetic agents on gene expression in preadipose cells. *Mol Pharmacol* 1994;46:1070-6.
- Lehmann JM, Moore LB, Smith-Oliver TA, Wilkison WO, Willson TM, Kliewer SA. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor γ (PPAR γ). *J Biol Chem* 1995;270:12953-6.
- Geneviève M, Kristina S, Bart S, Johan A. PPAR γ activators improve glucose homeostasis by stimulating fatty acid uptake in the adipocytes. *Atherosclerosis* 1998;137:75-80.

12. Staels B. Fluid retention mediated by renal PPAR γ . *Cell Metab* 2005;2:77-8.
13. Lindenfeld J, Masoudi FA. Fluid retention with Thiazolidinediones. *J Am Coll Cardiol* 2007;49:1705-7.
14. Nesto RW, Bell D, Bonow RO, Fonseca V, Grundy SM, Horton ES, *et al.* Thiazolidinedione use, fluid retention and congestive heart failure: A consensus statement from the american heart association and American diabetes association. *Circulation* 2003;108:2941-8.
15. Delea TE, Edelsberg JS, Hagiwara M, Oster G, Phillips LS. Use of thiazolidinediones and risk of heart failure in people with type 2 diabetes: A retrospective cohort study. *Diabetes Care* 2003;26:2983-9.
16. Dormandy JA, Charbonnel B, Eckland DJ, Erdmann E, Massi-Benedetti M, Moules IK, *et al.* Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study (Prospective Pioglitazone Clinical Trial In macroVascular Events): A randomised controlled trial. *Lancet* 2005;366:1279-89.
17. Marceille JR, Goins JA, Soni R, Biery JC, Lee TA. Chronic heart failure-related interventions after starting rosiglitazone in patients receiving insulin. *Pharmacotherapy* 2004;24:1317-22.
18. DeFronzo RA, Bonadonna RC, Ferrannini E. Pathogenesis of NIDDM: A balanced overview. *Diabetes Care* 1992;15:318-68.
19. Ye JM, Doyle PJ, Iglesias MA, Watson DG, Cooney GJ, Kraegen EW. Peroxisome Proliferator-Activated Receptor (PPAR)- α Activation Lowers Muscle Lipids and Improves insulin Sensitivity in High Fat-Fed Rats: Comparison With PPAR- γ Activation. *Diabetes* 2001;50:411-7.
20. Prashantha Kumar BR, Karvekar MD, Adhikary L, Nanjan MJ, Suresh B. Microwave assisted synthesis of thiazolidine-2,4-dione motif and microwave induced efficient synthesis of 5-Benzylidene-thiazolidine-2,4-dione and 5-Benzylidene-2-thioxo-thiazolidine-4-one compounds. *J Heterocycl Chem* 2006;43:897-903.
21. Mahendran P, Devi CS. Effect of *Garcinia cambogia* extract on lipids and lipoprotein composition in dexamethasone administered rats. *Indian J Physiol Pharmacol* 2001;45:345-50.
22. Reaven GM. The role of insulin resistance in human disease. *Diabetes Care* 1988;37:1495-507.