Global Journal of Pharmacology 7 (1): 20-24, 2013
ISSN 1992-0075

DOI: 10.5829/idosi.gjp.2013.7.1.7122

Evaluation of Anti-Inflammatory Effects of Rosiglitazone, a PPAR Gamma Agonist in Models of Acute and Chronic Inflammation and Assessment of its Effect on Gastric Mucosa

[1]Snehalata V. Gajbhiye and [2]Neelam A. Manjrekar

[1]Department of Pharmacology and Therapeutics,
Seth GS Medical College and KEM Hospital Mumbai, India
[2]Department of Pharmacology and Therapeutics,
Topiwala National Medical College and Nair Hospital Mumbai, India

Abstract: Objectives-To evaluate anti-inflammatory activity of rosiglitazone in the acute and chronic models of inflammation. Also to determine ulcerogenic potential on gastric mucosa in Wistar rats in comparison with diclofenac. Methodology-Anti-inflammatory effects of rosiglitazone in the dose of 1, 3 and 10 mg/kg b.wt., i.p were studied in acetic acid-induced peritoneal capillary permeability model in mice and cotton pellet-induced granuloma model in Wistar rats. The dose of rosiglitazone that showed maximum anti-inflammatory effect i.e 10mg/kg b.wt. was chosen to study ulcerogeinc potential. Cotton pellet granuloma model was repeated with the drugs being given orally and effect on inflammation and ulcer index was assessed. Results-All three doses of rosiglitazone did not significantly reduce inflammation in the model of acetic acid-induced peritoneal capillary permeability. However, there was significantly reduced inflammation in a dose dependent manner in the model of cotton pellet granuloma in rats. Rosiglitazone in the dose of 10 mg/kg showed significant reduction in inflammation. Ulcer index with rosiglitazone 10mg/kg was comparable to vehicle. Whereas diclofenac sodium showed statistically significant ulcer index as compared to rosiglitazone. Discussion-In conclusion the results of this study suggest that rosiglitazone has anti-inflammatory effects without ulcerogenic potential and can be useful in inflammatory disorders especially in patients with diabetes mellitus.

Key words: Rosiglitazone · Inflammation · Cotton Pellet Granuloma Model · Ulcerogenic Potential

INTRODUCTION

The thiazolidinedione class of drugs (pioglitazone and rosiglitazone) is specific high-affinity ligands for PPAR gamma. They enhance the expression of a number of genes encoding proteins involved in glucose and lipid metabolism. They can activate genes that regulate fatty acid metabolism. Three different members of the PPAR family have been identified, encoded by separate genes: PPAR alpha, beta and gamma. These three isotypes exhibit distinct patterns of tissue distribution and differ in their ligand-binding domains [1].

PPAR gamma agonists have shown to have antioxidant activity *in vitro* [2]. It has been shown in few studies that PPAR gamma agonists have an anti-inflammatory effect [3]. The possible anti-inflammatory mechanisms postulated are various. PPAR- gamma is expressed in human monocytes and macrophages. PPAR-gamma agonists negatively regulate the expression of pro-inflammatory genes induced during macrophage differentiation and activation [4]. Specifically, PPAR- activation reduces monocyte secretion of interleukin (IL)-1, IL-6 and TNF-alpha [4]. It causes rapid and consistent suppression of intranuclear content of the proinflammatory transcription factor NF kappa B in monocytes [5]. Rosiglitazone, a ligand of PPAR-gamma caused a substantial reduction in acute inflammation in an experimental model [6]. Thus we decided to evaluate the anti-inflammatory effects of PPAR gamma agonist, rosiglitazone in acute and chronic models of inflammation.

Corresponding Author: Snehalata V. Gajbhiye, Department of Pharmacology and Therapeutics,
Seth GS Medical College and KEM Hospital Mumbai, India.

The most common adverse reaction of NSAIDs noted is gastro-intestinal. Based on FDA estimates, approximately 3% of NSAID users develop serious NSAID-induced gastrointestinal complications each year, resulting in 200,000 cases of bleeding or perforated ulcers and at least 10,000 deaths annually [7]. Thus there is a need for anti-inflammatory agents with better safety profile. Although there are few studies on anti-inflammatory effect of rosiglitazone, there is limited data regarding their effect on gastrointestinal tract. However, pioglitazone in one study has shown gastroprotective and hyperemic action on the gastric tissue [8]. It has also been shown in another study that pioglitazone accelerates healing with preexisting gastric ulcer [9]. As there is concern for G.I. tolerability with most anti-inflammatory agents, we decided to determine the ulcerogenic potential of rosiglitazone in experimental model.

The aim of the present study was to evaluate the anti-inflammatory role of various doses of rosiglitazone in experimental models of acute and chronic inflammation and to determine the ulcerogenic potential of rosiglitazone in experimental model.

MATERIALS AND METHODS

Ethics Committee Permission: The study was initiated after obtaining permission from the Animal Ethics Committee of the institution.

Experimental Animals: Experimental animals used in the study were Wistar rats weighing approximately 150-250 grams and Swiss albino mice weighing 15-25 grams of either sex. They were housed under standard laboratory conditions with free access to water and commercial rat feed in the form of pellets. The animals were housed in polypropylene cages with husk paddy as the bedding with stainless steel top grill having facilities for providing food and water *ad libitum*. Twelve hourly light and dark cycles were maintained. The guidelines recommended by the CPCSEA were carefully followed during the entire study.

Acetic Acid-induced Peritoneal Capillary Permeability in Mice Model (N=36): This is the model for acute inflammation [10]. Mice were fasted 12 prior to experiment. Drugs or vehicle were given 30 min prior to intravenous administration of Evans blue. 1% solution of Evan's blue was injected intravenously. Acetic acid 0.4 ml of 0.5% was injected intraperitoneally after 30 min. Mice were then killed by cervical dislocation 20min after the injection of acetic acid. Then the peritoneal cavity was lavaged with saline in a total of 10ml per mouse. The washing solutions were collected and centrifuged for 10min (2000 rpm). The content of Evans blue in the supernatant was determined with a spectrophotometer.

Group 1 received CMC (0.5%) with no acetic acid injection. All the rest of the groups were injected with acetic acid along with drugs. Group 2 received diclofenac sodium (5mg/kg), groups 3, 4 and 5 received rosiglitazone in the doses 1, 3 and 10mg/kg. All the drugs were administered 30 min prior to administration of Evans blue intravenously.

Cotton Pellet Granuloma Model: In this model chronic inflammation was induced [11]. Cotton pellets weighing 30mg ± 1mg were autoclaved and soaked in 0.2 ml of distilled water containing penicillin (0.1 mg) and streptomycin (0.13 mg) and implanted subcutaneously bilaterally in axilla on ventral aspect of each rat. Procedure was carried under stringent aseptic precautions by using autoclaved instruments and carrying the whole procedure in fumigated glass hood on the day of experiment. Rats were fed with the test and control agents for 7 days following the procedure. On 8th day rats were sacrificed and pellets dissected out. When dissecting granulation tissue was identified as firm vascular tissue surrounding/ adherent to inserted pellets, which was generally well defined from the surrounding normal subcutaneous tissue and weighed. It was weighed and recorded as wet weight in mg after subtracting 30mg original weight of cotton pellet. They were dried in hot air oven at 60°C for 24 hrs and weighed again. This was recorded as dry weight after subtracting 50 mg original weight of cotton pellet. The groups were similar to earlier model. The respective treatment was administered daily for 7 days intraperitoneally. The first dose was administered 3 hours after completion of cotton pellet implantation procedure.

Ulcerogenic Potential of Oral Rosiglitazone in Comparison to Diclofenac: Cotton pellet granuloma model was repeated in the above mentioned manner with all the drugs given orally. The dose of rosiglitazone that showed maximum anti-inflammatory effect i.e 10mg/kg was chosen. On the 8th day after removal of pellets gastric tissue was removed. The stomach was then taken out and cut open along the greater curvature. Its inner surface was cleaned with saline and stomach lesions were examined under a magnifying hand lens and ulcer index was determined [12].

Lesion size (mm) was measured along its greatest length and in the case of patches; five such lesions were considered the equivalent of a 1 mm ulcer. The sum of the lesion lengths in each group of animals was divided by its number and expressed as the mean gastric hemorrhagic lesion index. Group 1 received CMC (0.5%), group 2 received diclofenac sodium (5mg/kg) and group 3 received rosiglitazone (10mg/kg)

Statistical Analyses: Results were expressed as mean ± SEM. The results were analyzed for statistically significant difference using one-way analysis of variance (ANOVA) and Tukey's post-hoc test; *p* value less than 0.05 was considered significant.

RESULTS

Acetic Acid-Induced Peritoneal Capillary Permeability Model: The percentage inhibition of the rosiglitazone in the dose of 3 and 10 mg/kg was 10% and 13% respectively. Although none of the doses of rosiglitazone showed statistical significance as compared to vehicle control, there was trend towards reduction in vascular permeability with increasing doses. The inhibition of vascular permeability with diclofenac sodium was statistically significant compared to vehicle control and the percentage inhibition was 36%. However, dose of 10 mg/kg rosiglitazone did not show significant difference when compared to diclofenac sodium. The results are depicted in Table I

Cotton Pellet Induced Granuloma Model: The percentage inhibitions with rosiglitazone 1, 3 and10 mg/kg are 10, 13 and 27% respectively for the wet weight. The percentage inhibitions with rosiglitazone 1, 3 and10 mg/kg are 11, 20 and 27% respectively for the dry weight. Doses 3 and 10 mg/kg of rosiglitazone showed significant reduction in both wet and dry weight in comparison with vehicle. Dose 10mg/kg rosiglitazone showed reduction in wet and dry weight that was not statistically significant to diclofenac 5mg/kg. The results are depicted in Table II.

Ulcerogenic Potential of Oral Rosiglitazone in Comparison to Diclofenac: Rosiglitazone oral 10 mg/kg and diclofenac oral 5mg/kg showed significant reduction in both wet and dry weight in comparison with vehicle. Rosiglitazone showed similar reduction in wet and dry weight to diclofenac 5mg/kg. Rosiglitazone in the dose of 10 mg/kg showed gastric ulcer index of 7.51. Ulcer index with rosiglitazone 10mg/kg were comparable to vehicle. Diclofenac sodium 5mg/kg showed statistically significant ulcer index as compared to vehicle. The results are depicted in Table III.

Table 1: Effect Of *Rosiglitazone* I.P In Three Doses On Acetic Acid Induced Vascular Permeability In Mice

Groups	Treatment Dose (mg/kg)	After 1 hour	Absorbance read at 610 nm	Percentage inhibition
Group A	Normal control (CMC 0.5%) i.p	Normal saline i.p	0.157± 0.046	
Group B	Vehicle Control (CMC 0.5%) i.p	0.6% acetic acid i.p	0.403±0.060*	-
Group C	Diclofenac 5mg/kg i.p		0.257±0.064$	36.22%
Group D	Rosigltazone 1mg/kg i.p		0.402±0.062##	0.24%
Group E	Rosigltazone 3mg/kg i.p		0.362 ±0.058#	10.17%
Group F	Rosigltazone 10mg/kg i.p		0.350±0.032	13.15%

Values are mean ± SEM, n=6 *p<0.001 Vs normal control, $ p<0.01Vs vehicle control, # p <0.05, # # p <0.01 Vs diclofenac

Table II: Effect Of *Rosigltazone* I.P In Three Doses In Cotton Pellet Granuloma Model In Comparison With Diclofenac.

Treatment Dose (mg/kg)	Weight of cotton pellets (mg) {Wet}	Percentage inhibition {Wet}	Weight of cotton pellets (mg) {Dry}	Percentage inhibition {Dry}
Vehicle Control (CMC 0.5%)	763.75±55.36	-	178.66±15.29	-
Diclofenac 5mg/kg	539.91±48.35*	29.30%	110.58±6.57*	38.10%
Rosigltazone 1mg/kg	683.41±42.35 ns	10.51%	158.41±27.58 ns	11.33%
Rosigltazone 3mg/kg	661.33±44.83*	13.41%	142.58±21.85*	20.19
Rosigltazone 10mg/kg	556.25±46.02*	27.16%	129.33±15.08*	27.61%

Values are mean ± SEM, n=6 * p <0.05 as compared to vehicle control, ns not significant as compared to vehicle control

Table III: Effect Of *Rosigltazone* Oral In Cotton Pellet Granuloma Model In Comparison With Diclofenac

Treatment Dose (mg/kg)	Weight of cotton pellets (mg) {Wet}	Weight of cotton pellets (mg) {Dry}	Gastric ulcer index
Vehicle Control (CMC 0.5%)	793.66±12.42	186±10.63	6.86±0.68
Rosigltazone 10mg/kg	596.5±29.79*	140.5±10.59*	7.51±0.71 ns
Diclofenac 5mg/kg	571.83±33.89*	123.5±12.32*	16.98±1.02*

Values are mean ± SEM, n=6, *p <0.05 as compared to vehicle control, ns not significant as compared to vehicle control

DISCUSSION

Various PPAR isoforms have been implicated in inflammation [13]. Both PPAR-alpha and PPAR-gamma receptor have been reported to regulate the inflammatory response [14]. The acute inflammation was induced in the present study using acetic acid which leads to increase in vascular permeability. Increased vascular permeability is a major feature of acute inflammation and results from contraction and separation of endothelial cells at their boundaries to expose the basement membrane which is freely permeable to plasma proteins and fluid [15]. Acetic acid causes an immediate sustained reaction that is prolonged over 24 hrs and the inhibition caused by the extract suggests it may suppress exudation and its consequences. The inhibition of vascular permeability with the vehicle (CMC 0.5%) was similar to the control used in other studies [16]. Though there was a trend towards reduction of inflammation with higher doses of rosiglitazone, there was no statistically significant difference between the test drugs and the vehicle control. This could be due to the slow onset of action owing to binding of rosiglitazone to nuclear receptors. However, there are studies in the literature where rosiglitazone has shown anti-inflammatory effect in models of acute inflammation [17-19].

Model of cotton pellet induced granuloma was chosen as rosiglitazone has not been evaluated using models of chronic inflammation. The cotton pellet method is widely used to evaluate the transudative and proliferative components of the chronic inflammation. The wet weight of the cotton pellets correlates with the exudate; the dry weight of the pellets correlates with the amount of the granulomatous tissue [20]. It was found that all three doses 1, 3 and 10mg/kg exhibited anti-inflammatory effects in dose dependent fashion. As we got the results in accordance with previous studies [21], it was decided to compare its ulcerogenic potential with diclofenac sodium. So in the same model, that is cotton pellet induced granuloma model, anti-inflammatory effects of rosiglitazone given orally were evaluated. Ulcer index with diclofenac showed significant increase as compared to the vehicle control and rosiglitazone. It was found that group receiving vehicle also had gastric ulcers as procedure of cotton pellet insertion is surgical event and resulted in stress. This finding suggests that rosiglitazone does not have ulcerogenic potential as compared to diclofenac suggesting mechanisms other than COX inhibition being involved in its anti-inflammatory effect.

The probable mechanism of action anti-inflammatory effects of rosiglitazone could be due to PPAR activation that may regulate inflammatory responses and cellular proliferation and differentiation as well as apoptosis [22]. PPAR-gamma decreases the expression of pro-inflammatory mediators at the transcriptional level by inhibiting NF-kappa B, STAT-1 and activation protein-1 (AP-1) signaling. Inflammation is emerging as an important mechanism for micro and macro vascular complication of diabetes. Thus glucose-induced macrophage generation of pro-inflammatory cytokines could be involved in both short term and long term vascular complications of diabetes [23]. Rosiglitazone which is used as an antidiabetic drug may have a role to play in prevention of complications of diabetes. Specific studies in this direction are required.

To conclude present study indicates that rosiglitazone has anti-inflammatory effects in acute and chronic models of inflammation with minimal ulcerogenic potential as compared to diclofenac.

ACKNOWLEDGEMENTS

- Glenmark Pharmaceuticals-For providing the drug rosiglitazone as the gift sample for the study.
- Diamond Jubilee Society Trust-For providing research grant for the study.

REFERENCES

1. Clark, R.B., 2002. The role of PPARs in inflammation and immunity. Journal of Leukocyte Biology, 71: 388-400.
2. Jiang, C., A.T. Ting and B. Seed, 1998. PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. Nature, 391: 82-86.
3. Paola, R.D., E. Mazzon D. Maiere, D. Zito, D. Britti, M.D. Majo, *et al*., 2006. Rosiglitazone reduces the evolution of experimental periodontitis in the rat. Journal of Dental Research, 85(2): 156-161.
4. Ricote, M., A.C. Li, T.M. Willson, C.J. Kelly and C.K. Glass, 1998. The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. Nature, 391: 79-82.
5. Delerive, P., K.D. Bosscher, S. Besnard, W.V. Berghe, J.M. Peters, F.J. Gonzalez, J.C. Fruchart, A. Tedgui, G. Haegeman and B. Staels, 1999. Peroxisome proliferator-activated receptor alpha negatively regulates the vascular inflammatory gene response by negative cross-talk with transcription factors NF-kappa B and AP-1. J. Biol. Chem., 274: 32048-32054.

6. Cuzzocrea, S., B. Pisano, L. Dugo, A. Ianaro, P. Maffia, N.A. Patel, *et al*., 2004. Rosiglitazone, a ligand of the peroxisome proliferator-activated receptor-gamma, reduces acute inflammation. European Journal of Pharmacology, 483: 79-93.
7. Jones, A.C., P. Berman and M. Doherty, 1992. Nonsteroidal anti-inflammatory drug usageand requirement in elderly acute hospital admissions. Br J. Rheum, 3: 45-48.
8. Brzozowski, T., P.C. Konturek, R. Pajdo, S.N. Kwiecień, S. Konturek, A. Targosz*, et al*., 2005. Agonist of peroxisome proliferator-activated receptor gamma (PPAR-gamma): a new compound with potent gastroprotective and ulcer healing properties. Inflammopharmacology, 13(1-3): 317-30.
9. Konturek, P.C., T. Brzozowski, J. Kania, S.J. Konturek, S. Kwiecien, R. Pajdo and E.G. Hahn, 2003. Pioglitazone, a specific ligand of peroxisome proliferator-activated receptor-gamma, accelerates gastric ulcer healing in rat. Eur J. Pharmacol., 472(3): 213-20.
10. Whittle, B.A., 1964. The use of changes in capillary permeability in mice to distinguish between narcotic and non-narcotic analgesics. Brit. J. Pharmacol., 22: 246-253.
11. Winter, C.A. and C.C. Porter, 1957. Effect of alteration in side chain upon anti-inflammatory and liver glycogen activities of hydrocortisone ester. J. Am. Pharma Ass. Science, 46: 515-9.
12. Qiu, B.S., C.H. Cho and C.W. Ogle, 1991. Chronic nicotine treatment intensifies gastric ulceration by cold-restraint stress in rats. Agents Actions., 33: 367-370.
13. Delerive, P., J.C. Fruchart and B. Staels, 2001. Peroxisome proliferator-activated receptors in inflammation control. J. Endocrinol, 169: 453-459.
14. Chinetti, G., J.C. Fruchart and B. Staels, 2000. Peroxisome proliferator-activated receptors (PPARs): nuclear receptors at the crossroads between lipid metabolism and inflammation. Inflamm. Res., 49: 497-505.
15. Brown, J.N. and J. Roberts, 2007. Histamine, bradykinin and their antagonists. In Goodman and Gilman's The Pharmacological Basis of Therapeutics 11th edition. Edited by: Brunton LL, Parker KL, New Delhi: McGraw Hill Co, pp: 403.
16. Okoli, C.O., P.A. Akah, N.J. Onuoha and T.C. Okoye, 2008. Nwoye AC, Nworu CS. Acanthus montanus: An experimental evaluation of the antimicrobial, anti-inflammatory and immunological properties of a traditional remedy for furuncles BMC Complementary and Alternative Medicine, 8: 27.
17. Celiński, K., A. Madro, B. Prozorow-Król, A. Korolczuk, H. Cichoz-Lach, M. Słomka and E. Korobowicz, 2009. Rosiglitazone, a peroxisome proliferator-activated receptor gamma (PPARgamma)-specific agonist, as a modulator in experimental acute pancreatitis. Med. Sci. Monit., 15(1): BR21-9.
18. Cuzzocrea, S., B. Pisano, L. Dugo, A. Ianaro, D. Britti, N.S. Patel, R.D. Paola, T. Genovese, M.D. Rosa and A.P. Caputi, 2004. Thiemermann C. Rosiglitazone, a ligand of the peroxisome proliferator-activated receptor-gamma, reduces acute pancreatitis induced by cerulein. Intensive Care Med., 30(5): 951-6.
19. Liu, D., B.X. Zeng, S.H. Zhang, Y.L. Wang, L. Zeng, Z.L. Geng and S.F. Zhang, 2005. Rosiglitazone, a peroxisome proliferator-activated receptor-gamma agonist, reduces acute lung injury in endotoxemic rats. Crit Care Med., 33(10): 2309-16.
20. Gupta, M., U.K. Mazumder, P. Gomathi and V.T. Selvan, 2006. Anti-inflammatory evaluation of leaves of Plumeria acuminate. BMC Complementary and Alternative Medicine, 6: 36.
21. Taylor, B.K., N. Dadia, C.B. Yang, S. Krishnan and M. Badr, 2002. Peroxisome Proliferator-Activated Receptor Agonists Inhibit Inflammatory Edema and Hyperalgesia. Inflammation, 26: 121-127.
22. Escher, P. and W. Wahli, 2000. Peroxisome proliferator-activated receptors: insight into multiple cellular functions. Mutat. Res., 448: 121-138.
23. Wen, Y., G. Jiali, L. Shu-Lian, M.A. Reddy, R. Natarajan and J.L. Nadler, 2006. Elevated Glucose and Diabetes promote Interleukin 12 Cytokine Gene Expression in Mouse Macrophages. Endocrinology, 147(5): 2518-25.