International Journal of Basic and Applied Medical Sciences ISSN: 2277-2103 (Online)
An Online International Journal Available at http://www.cibtech.org/jms.htm
2012 Vol. 2 (1) January-April, pp.1-7/Kamble et al.
Research Article

COMPARATIVE STUDY OF ANTI-INFLAMMATORY EFFECTS OF ROSIGLITAZONE AND PIOGLITAZONE WITH DICLOFENAC SODIUM IN CARAGEENAN INDUCED RAT HIND PAW OEDEMA

Pratibha S. Kamble[1], Princy Louis Palatty[2], Sanjay Kamble[3]
[1]Pharmacology, Father Muller Medical College (FMMC)
[2]Pharmacology, FMMC
[3]Radiotherapy, FMMC
**Author for Correspondence*

ABTRACT
Thiazolidinedione ligands for the gamma subtype of peroxisome proliferator-activated receptors (PPARγ), widely used to treat type 2 diabetes mellitus, have been shown to have anti-inflammatory action in some animal models. This study is carried out to evaluate the anti-inflammatory activity of rosiglitazone & Pioglitazone with the standard drug Diclofenac sodium (NSAID). Carageenan is used for inducing inflammation in rats hind paw. Paw volume is measured before and after 0 & 3 hrs by using simple mercury plethysmograph. The percentage inhibition with Diclofenac sodium 5mg/kg is 24.50%, 10mg/kg is 36.76% & 15mg/kg is showing the highest inhibition of 49.01%. Rosiglitazone is showing the percentage inhibition of 20.34%, 30.63% & 40.80% with the respective doses of 0.12mg/kg, 0.24mg/kg & 0.36mg/kg. Pioglitazone shows the least percentage inhibition of 16.29%, 28.55% &38.72% at a concentration of 0.30mg/kg, 0.45mg/kg & 0.60mg/kg respectively. Our study has shown that TZDs have definitive anti-inflammatory effect which was significant with Rosiglitazone. As TZDs act on PPAR- γ, it has been shown to have anti-inflammatory effect by PPAR dependent and independent mechanisms. They particularly enhance COX-2 expression.

***Key Words*:** *Inflammation, Thiazolidinediones, Rosiglitazone, Pioglitazone, Diclofenac Sodium*

INTRODUCTION
With the unifying theory of pain that states “Inflammation is the biochemical cause of all pain” (Omoigui 2007) **e**very patient seeks medical relief especially when in pain. As John Milton (Ricks 1968) has said “Greatest happiness mankind can gain is not from pleasure, but relief of pain.” The quest for analgesics has been going on yet the ultimate pain remedy still illusive.

The causes of pain are many and distinct. Inflammation is veritable cause of pain. Prostaglandin inhibitors, TNF alpha inhibitors have been shown to have remarkable analgesic effect. A wide variety of diverse chemical agents have analgesic effect. Many drugs that are in use have varied therapeutic uses which has been shown to have a potential anti—inflammatory/analgesic effect- Carbamazepine, antidepressants, macrolide antibiotics etc (Bianchi et al., 1995, Hajhashemi *et al.,* 2008 & Munic *et al.,* 2011).

Diabetes is usually associated with inflammation (Mark & Steven 2011). Inflammation contributes to the development of diabetes. Traditional Chinese medicines (TCM) have been effective in the treatment of diabetes mellitus. Researches have propounded the astonishing theory that diabetes is an inflammatory disease. Many of the traditional Chinese medicine used for diabetes mellitus have been found to have additional anti-inflammatory effect as well as hypoglycaemic effect (Xie & Du 2011). The increasing evidence of inflammatory basis for diabetes mellitus necessitates the critical evaluation of anti-inflammatory drugs. It is also advocated to look for putative targets that need validation.

Thiazolidinediones (TZDs) are a unique class of antidiabetic medications that exert multiple effects beyond glycemic control. Thiazolidinediones were shown to have antiarthritic and anti inflammatory

effect on animal model (Koufany *et al.,* 2008) . This new found activity of TZDs could be put to use in therapeutically varied situations. TZDs have potent peroxisome proliferator-activated receptor (PPAR)-γ agonists and a novel PPAR-α activity (Delerive *et al.,* 2001).(PPAR)-γ has an important role in the process of inflammation. Two commonly used TZDs which were selected for the study – Rosiglitazone and Pioglitazone. Diclofenac was used as the standard drug.

Rosiglitazone, an agonist for the nuclear receptor peroxisome proliferator-activated receptor gamma (PPARγ), is a widely used drug for the treatment of type 2 diabetes mellitus. It increases insulin sensitivity of peripheral tissues. In addition, there is evidence that rosiglitazone has anti-inflammatory effects (Delerive *et al.,* 2001 & Haffner *et al.,* 2002). It has been postulated that rosiglitazone exerts its anti-inflammatory effect through inhibition of the transcription factor nuclear factor κB (NFκB) pathway (Mohanty *et al.,* 2004).

The drug is controversial in the U.S. (JD law group 2011). Some reviewers (Graham *et al.,* 2010) have concluded that rosiglitazone caused more deaths than pioglitazone (Actos), and have recommended rosiglitazone be taken off the market, but an FDA Food and Drug Administration panel disagreed, and it remains on the market in the U.S., subject to significant restrictions. From November 18, 2011 the federal government will not allow Avandia to be sold without a prescription from certified doctors (Reinberg 2011). Patients will be required to be informed of the risks associated with the use of Avandia, and the drug will be required to be purchased by mail order through specified pharmacies.

Pioglitazone is a prescription drug of the class thiazolidinedione (TZD) with hypoglycemic (antihyperglycemic, antidiabetic) action. Pioglitazone selectively stimulates the nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR-γ) and to a lesser extent PPAR-α (Gillies, Dunn 2000 & Smith 2001). It modulates the transcription of the insulin-sensitive genes involved in the control of glucose and lipid metabolism in the muscle, adipose tissue, and the liver. Although not clinically significant, pioglitazone decreases the level of triglycerides and increases that of high-density lipoproteins (HDL) without changing low-density lipoproteins (LDL) and total cholesterol in patients with disorders of lipid metabolism.

More recently, pioglitazone and other active TZDs have been shown to bind to the outer mitochondrial membrane protein mitoNEET with affinity comparable to that of pioglitazone for PPARγ (Colca *et al.,* 2004 & Paddock 2007). Pioglitazone has subsequently been found to be associated with bladder tumors and has been withdrawn in some countries.

Diclofen is a non-steroidal anti-inflammatory drug (NSAID)**.** It is widely used for treatment of localized nonarticular rheumatism and inflammations. However, the half-life of diclofenac in human body is 1.8 h after oral administration and the hepatic first pass effect is very extensive (Willis *et al.,* 1979).The primary mechanism responsible for its anti-inflammatory action is inhibition of prostaglandin synthesis by inhibition of cyclooxygenase (COX). It also appears to exhibit bacteriostatic activity by inhibiting bacterial DNA synthesis (Dutta *et al.,* 2000).

Carageenan induced paw edema in animals is the most suitable test procedure to screen anti-inflammatory activity. It is used to study the acute and subacute phases of inflammation in rats.

MATERIALS AND METHODS

Materials

It is a cross sectional study in which three drugs (Diclofenac sodium, Rosiglitazone & Piogliatazone) are used along with normal saline. The different concentration of these drugs have been used of the study. Animals used are male wistar rats (200-250gms). These are divided into four groups.

Table 1: Characterisation of study groups with control, standard and tests drugs

Group	Drug	Concentration	No of rats used
I	Normal Saline	0.2 ml	6
II(a)	Diclofenac	5mg/kg	6
II(b)	Sodium	10mg/kg	6
II(c)		15mg/kg	6
III(a)	Rosiglitazone	o.12mg/kg	6
III(b)		0.24mg/kg	6
III(c)		0.36mg/kg	6
IV(a)	Pioglitazone	o.30mg/kg	6
IV(b)		0.45mg/kg	6
IV(c)		0.60mg/kg	6

Carageenan – 0.1ml of 1% conc.

Methods

The animals were kept fasting overnight and weighed before being taken into the various study groups. Relevant doses were given intraperitonially for the control, standard and test groups. After an hour of drug administration, the carageenan was injected into the hind paw.

In this procedure a mark is made at the ankle joint (tibio-plantar joint) of each rat. Paw volume upto the ankle joint is measured in drug treated and untreated groups before and after 0 & 3 hrs carageenan challenge using a plethysmograph filled with mercury. Oedema is observed and percentage of reduction in oedema is calculated using the following formula-

Percentage inhibition = (1- Vt/ Vc) X 100

Where, Vt = Oedema volume in the drug treated group

Vc = Oedema volume in the control group

The percentage anti-inflammatory effect was noted with various concentrations of different drugs. Results were analysed using SPSS (version 6).

RESULTS AND DISCUSSION

Screening of anti-inflammatory agents have always being going on at a fast pace in response to the ever growing need. Diverse chemical entities have shown promising anti-inflammatory effect. Two of the commonly used oral hypoglycaemic agents from the TZDs have been shown to have an anti-inflammatory effect. In this study we estimated the percentage anti-inflammatory effect in three different doses of Rosiglitazone (test1), Piogliatazone (test2) and Diclofenac sodium (control).

The percentage anti-inflammatory effect noted with Diclofenac (Standard) 5mg was 24.50%, 10mg 36.76% & 15mg 49.01%. The percentage anti-inflammatory effect of Rosiglitazone (Test1) 0.12mg was 20.34%, 0.24mg 30.63% & 0.36mg 40.80%. The percentage anti-inflammatory effect of Pioglitazone (Test2) 0.30mg was 16.29%, 0.45mg 28.55% & 0.60mg 38.72%. The maximal anti-inflmmatory effect with Diclofenac is 49.01% at 15 mg/kg concentration. The maximal anti-inflmmatory effect with Rosiglitazone is 40.80% at 0.36 mg/kg concentration. The maximal anti-inflmmatory effect with Pioglitazone is 38.72% at 0.60 mg/kg concentration. The three varied doses of Diclofenac, Rosiglitazone and Pioglitazone corresponds to their ED25 & ED50. From among the test groups Rosiglitazone showed the maximal anti-inflammatory effect. In the present study, it was clarified that the Rosiglitazone and Pioglitazone markedly inhibited the hind paw edema induced by carageenan.

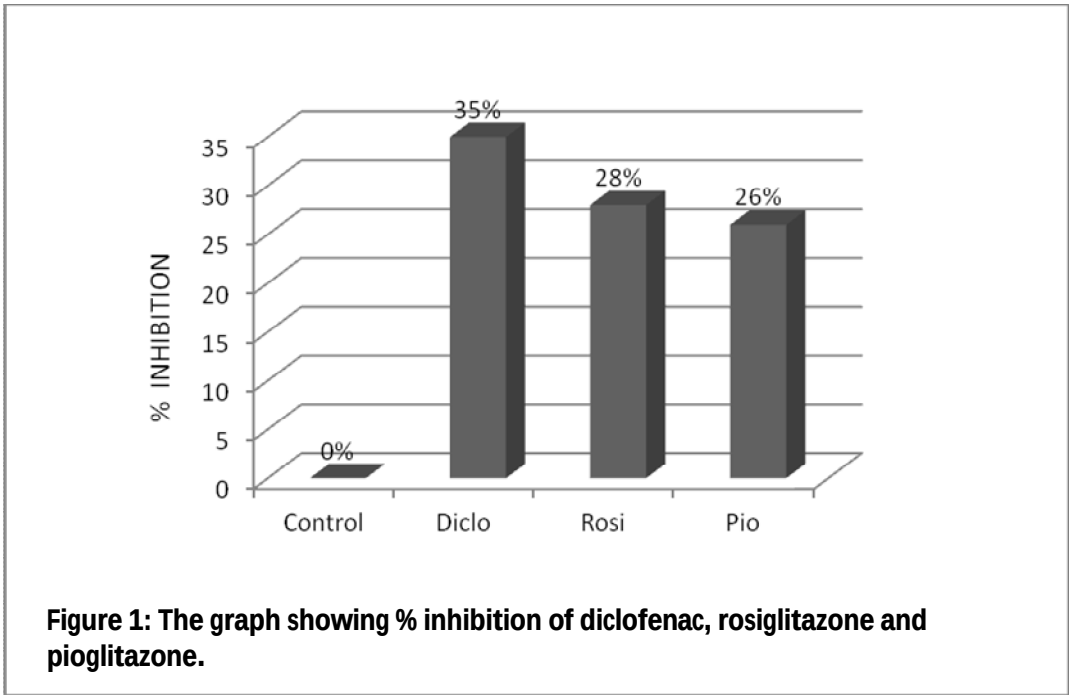

Figure 1: The graph showing % inhibition of diclofenac, rosiglitazone and pioglitazone.

Table 2: Paw Volume of standard & tests drugs in carageenan induced rat hind paw edema

S. No.	ACTUAL PAW VOLUME			
	Normal Saline 0.2 ml (Control)	Diclofenac 15mg/kg (Standard)	Rosiglitazone 0.36 mg/kg (Test 1)	Pioglitazone 0.60 mg/kg (Test 2)
1	0.8	0.5	0.6	0.5
2	0.9	0.4	0.5	0.4
3	0.7	0.4	0.5	0.6
4	0.8	0.3	0.4	0.4
5	0.8	0.4	0.5	0.5
6	0.9	0.5	0.4	0.6
MEAN	0.816	0.416	0.483	0.500
SD	0.435	0.237	0.243	0.260
SE	0.084	0.084	0.075	0.096

Statistical Analysis	Normal Saline Vs Diclofenac	Normal Saline Vs Rosiglitazone	Normal Saline Vs Pioglitazone
t-test	4.598	8.501	2.908
p-value	<0.05 (Statistically highly significant)	<0.05 (Statistically highly significant)	<0.05 (Statistically significant)

PPAR has been identified to have 4 different isotypes- α, β,δ and γ. PPAR- α is expressed in endothelial and vascular smooth muscle cells, as well as in macrophages and foam cells. PPAR-β/δ is expressed ubiquitously and takes part in the reverse transport of cholesterol and the oxidation of fatty acids. PPAR-γ is highly expressed in adipose tissue. PPAR- α contributes to the control of inflammation, the action of PPAR-β/δ has shown anti-obesity and anti-diabetic actions in animal models and promotion of wound healing. PPAR-γ has been linked to insulin-sensitizing properties that have entered the clinics and to the suppression of the release of cytokines, resulting in anti-inflammatory effects (Koufany *et al.*, 2008).

PPARδ may be involved in chronic inflammation (Tan *et al.*, 2001 & Valledor, Ricote 2004). In one study in human monocyte & macrophages & in mouse keratocytes this PPARδ also has a role in stimulating a proinflammatory response.

The highest doses of TZDs decreased the expression of IL-1β and TNF-α in inflamed synovium, which is a primary source for systemic inflammatory cytokines. The major finding of this dose-ranging study showed that the doses of TZDs required to decrease inflammation were equal to or greater than those sufficient to restore insulin sensitivity, as reported separately for troglitazone (Koufany et al., 2008).

The antiinflammatory actions of PPARγ may be responsible for the insulin-sensitizing properties of TZDs; large populations of macrophages reside in adipose tissue where they produce cytokines that mediate obesity-related insulin resistance (Neels,Olefsky 2006 & Neels,Olefsky 2006). TZD-activated PPARγ, via suppression of inflammatory cytokine production from macrophages, increasing the systemic insulin sensitivity (Ricote et al., 1998, Valledor, Ricote 2004 & Zhang, Chawla 2004).

Rosiglitazone and Ciglitazone potentiate COX-2 expression via a PPAR-γ dependent process. TZDs have not only action on PPAR-γ but PPAR independent mechanism (Luna-Medina et al., 2005 & Park et al., 2003). Effect of Rosiglitazone and Ciglitazone have a PPAR independent expression of COX-2 in LPS-stimulated astrocytes. TZDS in the presence of PPAR- β/δ that consequently leads to an increase in COX-2 expression (Stepan et al., 2009).

Pioglitazone has been shown to have effect on streptozotocin induced memory deficit without any significant effect on blood glucose level. This is attributed that anti-dementic effect of Pioglitazone involves its potential anti-inflammatory actions (Rinwa et al., 2008).

In preclinical studies, male rats treated with Pioglitazone developed more bladder tumors but this is not seen with female rats (Nathan et al., 2009 & Rinwa et al., 2008). This could be due to the gene reside in the Y chromosomes.

Hence our study having investigated the significant anti-inflammatory potential brings out multi prounged effects. Not only would TZDs increase peripheral utilization of glucose ,improves insulin sensitivity but also reduce the development of complications that is a fallout of inflammation. The surprisingly significant anti-inflammatory has added to the versatility of this group of drugs.

The limitations of our study could be summarised in having larger animal testing in various animal models and combination of drugs affecting anti-inflammatory as well as anti-diabetic actions.

At the close of our study, the suggestion recommendations are.

TZDs have a definite anti-inflammatory action, the anti-inflammatories could have a potential anti-diabetic action as a corollary. In view of our findings the TZDs have an all encompassing extremely on diabetes due to its comprehensive effect on peripheral utilization, insulin sensitivity and anti-inflammatory actions.

The carageenan induced rat hind paw edema model was taken to validate the suspected anti-inflammatory action of TZDs, in particular, Rosiglitazone and Pioglitazone. Diclofenac sodium was used as a standard. It was indeed surprising to note that TZDs have a peripheral utilization, insulin sensitivity and anti-inflammatory actions. There is corroborative evidence of PPAR- γ having additional PPAR dependent and independent anti-inflammatory activity.

REFERENCES

Bianchi M, Rossoni G, Sacerdote P, Panerai AE, Berti F (1995). Carbamazepine exerts anti-inflammatory effects in the rats. *European Journal of Pharmacology* **294** (1) 71-74

Colca JR, McDonald WG, Waldon DJ, Leone JW, Lull JM, Bannow CA, Lund ET, Mathews WR (2004). Identification of a novel mitochondrial protein ("mitoNEET") cross-linked specifically by a thiazolidinedione photoprobe. *American Journal of Physiology- Endocrinology and Metabolism* **286** (2) E252–60. Avialable.

Delerive P, Fruchart J, Staels B (2001). Peroxisome proliferator-activated receptors in inflammation control. *Journal of Endocrinology* **169** 453-459.

Dutta NK, Annadurai S, Mazumdar K, Dastidar SG, Kristiansen JE, Molnar J, Martins M, Amaral L (2000). The anti-bacterial action of diclofenac shown by inhibition of DNA synthesis. *International Journal of Antimicrobial Agents* **14** (3) 249–51

Graham DJ, Ouellet- Hellstrom R, MaCurdy TE, et al., (2010). Risk of acute myocardial infarction, stroke, heart failure and death in elderly medicare patients treated with rosiglitazone or pioglitazone. *Journal of the American Medical Association* **304** 411-418

Haffner SM, Greenberg AS, Weston WM, Chen H, Williams K, Freed MI (2002). Effect of rosiglitazone treatment on nontraditional markers of cardiovascular disease in patients with type 2 diabetes mellitus. *Circulation* **106** 679-684

Hajhashemi V, Minaiyan M, Eftekhari M (2008). Anti-inflammatory activity of a selection of antidepressant drugs. *Iranian Journal of Pharmaceutical Sciences* **4**(3) 225-300

JD Law group (Nov 19, 2011). Avandia and Glaxosmithkline: The story of a controversial prescription drug. *Society.ezinemark.com*

John Milton: Paradise Lost, II,11.890-910. Alastair Fowler (John Milton, Paradise Lost, edited by Alastair Fowler, second edition (London 1998).

Koufany M et al., (2008). Anti-inflammatory effect of antidiabetic thiazolidinediones prevents bone resorption rather than cartilage changes in experimental polyarthritis. *Arthritis Research And Therapy* **10**(1) R6.

Luna-Medina R, Cortes-Canteli M, Alonso M, Santos A, Martínez A, and Perez-Castillo (2005). A Regulation of inflammatory response in neural cells in vitro by thiadiazolidinones derivatives through peroxisome proliferator-activated receptor γ activation. *The Journal of Biological Chemistry* **280**(22) 21453-21462.

Marc Y Donath & Steven E Shoelson (1 Feb 2011). Type 2 diabetes as an inflammatory disease. *Nature Reviews Immunology* **11** 98-107.

Mohanty P, Aljada A, Ghanim H, Hofmeyer D, Tripathy D, Syed T, Al-Haddad W, Dhindsa S, Dandona P (2004). **Evidence for a potent antiinflammatory effect of rosiglitazone. The** *Journal of Clinical Endocrinology And Metabolism* **89**(6) 2728-35.

Munic V, Banjanac M, Kostrun S, Nujic K, Bosnar M, Marjanoric N, Ralic M, Erakoric Haber V (Sep 2011). Intensity of macrolide anti-inflammatory activity in J774A.1 cells positively correlates with cellular accumulation and phospholipidosis. Pharmcological Research **64**(3) 298-307.

Nathan DM, Buse JB, Davidson MB, et al., (2009). Medical management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy: a consensus statement of the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes Care* **32**(1) 193–203.

Neels JG, Olefsky JM (2006***).*** Inflamed fat: what starts the fire*? The Journal of Clinical Investigation* **116**(1) 33–*35*.

Neels JG, Olefsky JM (2006). Cell signaling. A new way to burn fat*. Science* **312**(5781) 1756–*1758*

Paddock ML, Wiley SE, Axelrod HL, Cohen AE, Roy M, Abresch EC, Capraro D, Murphy AN et al.,(September 2007). MitoNEET is a uniquely folded 2Fe 2S outer mitochondrial membrane protein stabilized by pioglitazone. *Proceeding of the National Academy of Sciences. U.S.A.* **104**(36) 14342-7.

Park EJ, Park SY, Joe EH, and Jou I (2003). 15d-PGJ2 and rosiglitazone suppress Janus kinase-STAT inflammatory signaling through induction of suppressor of cytokine signaling 1 (SOCS1) and SOCS3 in glia. *The Journal of Biological Chemistry* **278**(17) 14747-14752.

Reinberg S (May 19, 2011). FDA to pull diabetes drug Avandia from pharmacy shelves. *U. S. News Health.*

Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK (1998). *The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage activation.* Nature **391** *79–82* **Rinwa P, Kaur B, Singh N, Jaggi AS (oct 2008).** Pioglitazone induced beneficial effects in experimental dementia: An evidence of its antiinflammatory action. *Indian Journal of Pharmacology* **40**(Suppl 2) S112–S142.

Smith U (September 2001). "Pioglitazone: mechanism of action". *International Journal of Clinical Practice* **121** 13–8.

Sota Omoigui (2007). The Biochemical Origin of Pain – Proposing a new law of Pain: The origin of all Pain is Inflammation and the Inflammatory Response PART 1 of 3 – A unifying law of pain. *Medical Hypotheses*. **69**(1) 70-82.

Stepan Aleshin, Sevil Grabeklis, Theodor Hanck, Marina Sergeeva and Georg Reiser (August 2009). Peroxisome Proliferator-Activated Receptor (PPAR)-γ Positively Controls and PPARα Negatively Controls Cyclooxygenase-2 Expression in Rat Brain Astrocytes through a Convergence on PPARβ/δ via Mutual Control of PPAR Expression Levels. *Molecular Pharmacology*. **76**(2) 414-424.

Tan NS, Michalik L, Noy N, Yasmin R, Pacot C, Heim M, Fluhmann B, Desvergne B, Wahli W (2001). Critical roles of PPAR β/δ in keratinocyte response to inflammation. *Genes & Development*. **15**(24) 3263–3277.

Valledor AF (2004). *Ricote MNuclear receptor signaling in macrophages.* Biochemical Pharmacology **67**(2) 201–*212.*

Vosper H, Khoudoli GA, Palmer CN (2003). *The peroxisome proliferator activated receptor δ is required for the differentiation of THP-1 monocytic cells by phorbol ester*. Nuclear Receptor **1** 9

Willis JV, Kendall MJ, Flinn MR, Thornhill DP, Weilling PG (1979). The pharmacokinetics of diclofenac sodium following intravenous and oral administration. *European Journal of Clinical Pharmacology* **16**(6) 405-410.

XieW, Du L (Apr 2011). Diabetes is an inflammatory disease: evidence from traditional Chinese medicines. *Diabetes Obesity & Metabolism* **13**(4) 289-301.

Zhang L, Chawla A (2004). *Role of PPARγ in macrophage biology and atherosclerosis.* Trends in Endocrinology & Metabolism **15**(10) 500–*505.*