ISSN 0975-6299 Vol 3/Issue 2/April – June 2012

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE PHARMACOLOGY

PHARMACOKINETIC AND PHARMACODYNAMIC INTERACTION BETWEEN ACECLOFENAC AND ROSIGLITAZONE IN RATS

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ABSTRACT

The present study was carried out to investigate the pharmacokinetics and pharmacodynamic interaction of Aceclofenac and Rosiglitazone alone and their combination in rats. The animals were maintained on standard laboratory conditions and were divided into 3 and 6 groups for pharmacokinetic and pharmacodynamic studies respectively. Aceclofenac and Rosiglitazone alone produced antiarthritic and marked reduction in blood glucose levels (Antidiabetic) respectively but their combination produced a decreased efficiency of rosiglitazone for antidiabetic activity and increased antiarthritic activity of aceclofenac when co-administered with Rosiglitazone.

KEYWORDS

Drug interaction, Cox-inhibitors, Aceclofenac, Rosiglitazone, Rheumatoid arthritis and Diabetics

INTRODUCTION

The treatment of inflammation and pain is an important area of therapeutics. In the last decade, nonsteroidal anti-inflammatory drugs (NSAIDs) have played a central role in these indications and they are currently considered as the first choice, being one of the most widely prescribed drugs.[1, 2] Prostaglandins are important mediators of inflammation. They are a family of chemicals that are produced by the cells of the body and have several important functions. They promote inflammation, pain and fever; support the blood clotting function of platelets; and protect the lining of the stomach from the damaging effects of acid.[3, 4] The effect of NSAIDs is mediated to large extent by inhibition of prostaglandin synthesis through cyclo-oxygenase (COX). COX has two isoenzymes in humans: COX-1 has cytoprotective function in the gastric mucosa and COX-2 is detected in several tissues when an inflammatory reaction takes place.[5, 6] Aceclofenac is a novel NSAID known to exhibit multifactor mechanism of action. Aceclofenac is developed in order to provide a highly effective pain relieving therapy with a reduced site profile. It is known for directly blocking PG2 secretion of the site of inflammatory cells (intracellular action) it is Cox inhibitor. Aceclofenac is rapidly absorbed after oral administration and the bio availability almost 100% peak plasma concentrations are reached approx 1.25-3.00h following ingestion. Aceclofenac is highly protein bound (<99.7%). This metabolized via CYP2c9 to the main metabolite hydroxyl aceclofenac and the mean plasma elimination half-life is 4 - 4.3h. About two -third of administered dose is excreted via urine. Aceclofenac (2-[(2, 6-dichlorophenyl) amine] phenylacetoxyacetic acid), a non-steroidal anti-inflammatory drug (NSAID), is one of the promising molecules for arthritis treatment. It is indicated for pains of various etiologies, such as musculoskeletal pain, dental pain or post-surgical pain. [7] Aceclofenac is an orally effective and well-tolerated drug among the NSAIDs, with a lower incidence of gastrointestinal adverse effects. [8] Aceclofenac in rat plasma was analyzed by RP- HPLC method. [9, 10]

Rosiglitazone is an oral hypoglycemic agent used for the treatment of type – II diabetes mellitus. It is chemically 5-[4-[2-(methyl-2-pyridinelamino)ethoxy]phenyl]methyl]-2-4-thiazolidine. It belongs to thiazolidinedione class. By oral administration in fasting state, rosiglitazone is first measurable in serum within 30min with peak concentrations observed within 2hours. Rosiglitazone is extensively protein bound (>99%) in human serum principally to serum albumin. Rosiglitazone Maleate is an antidiabetic drug that can precisely control the blood glucose level in diabetic patients. It requires control release owing to its short biological half-life of 3-4 hr.[11, 12] CYP450 enzymes play a central role in the biotransformation of a great number of drugs. Among the several CYP enzyme families, the first three, CYP1, CYP2 and CYP3 are involved in human drug metabolism.[13, 14]

MATERIALS AND METHODS

(i) Animals

Male wistar rats weighing about 200-220gm were selected kept under standard animal house conditions of 12/12h day/night cycle at temperature of 25±2°c, humidity 60±2%. The animals were allowed water *ad libitum* and free access to standard food. The blood samples were drawn after application of

topical lignocaine anesthesia to minimize pain to the animals. This study protocol was approved by the Institutional Animal Ethics Committee (IAEC).

(ii) Study design

The male wistar rats (200-250g) were randomly divided into three and six groups consisting of six animals for pharmacokinetic and pharmacodynamics study respectively.

For pharmacokinetic study, animals were treated as follows:

Group I: 10mg / kg / p.o. Aceclofenac alone dispersed in 0.25% Na CMC once a day, Group II: 2mg/kg/ p.o. Rosiglitazone alone dispersed in 0.25% Na CMC once a day, Group III: Aceclofenac 10mg/kg/p.o. and Rosiglitazone 2mg/kg/p.o. concomitantly once a day for seven days.

For pharmacodynamic study, animals were treated as follows:

Group I: 10mg / kg / p.o. Aceclofenac alone dispersed in 0.25% Na CMC once a day, Group II: 0.5mg/kg/ p.o. Rosiglitazone alone dispersed in 0.25% Na CMC once a day in diabetic rats, Group III: Aceclofenac 10mg/kg/p.o. and Rosiglitazone 0.5mg/kg/p.o. concomitantly in adjuvant induced arthritic rats, Group IV: Rosiglitazone 0.5mg/kg/ p.o. and Aceclofenac 10mg/kg/p.o. concomitantly in diabetic rats, Group V: Diabetic rats served as control group with. (10ml/kg i.p) normal saline solution, Group VI: Adjuvant induced arthritic rats served as control group with (10ml/kg i.p) normal saline solution.

(iii) Collection of blood sample

On 1st and 8th day, blood samples of 0.5mL were drawn at 0, 0.5,1,2,4,6,8 and 24hrs and equal amount saline was administered to replace the blood volume for every blood withdrawal. [15] Blood samples were drawn through retro orbital sinus into the eppendorff tube. Serum was obtained by immediate centrifugation of blood samples. Centrifugation was performed by using REMIULTRA cooling centrifuge at 2500-3000rpm for 5min. All samples were stored at -4°c until pharmacokinetic and pharmacodynamics measurements were carried out.

(iv) Method of analysis

The chromatographic analysis were performed on a shimadzu LC10 HPLC system with ODS UV detector using Phenomenex C18 column 250 × 4.60mm at 258 nm accomplished with CLASS VP data station. The mobile phase consisted of buffer and Actonitrile (50:50 v/v) with pH-6.2. The flow rate was fixed to 1ml/min with sample volume 20 µl and the mobile phase was filtered through a 0.22µ membrane and degassed using ultrasonicator injected into HPLC system using rheodyne injector. The column temperature was maintained at 29±2^{0}C. The HPLC analysis was carried out at room temperature of about 20^{0}C. Different standard solutions containing of 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 30, 40 and 50 µg/ml of rosiglitazone using water: acetonitrile (1:1 v/v). The standard plasma solutions of different concentrations prepared were used for constructing the calibration curve. Further, 0.5ml of rosiglitazone standard solution and 0.5ml of precipitating agent (perchloric acid) were vortexed and centrifuged at 3000 rpm for 5minutes and the supernatant was separated and used for analysis. The same type of procedure was employed for Aceclofenac also.

(v) Statistical analysis

Data were expressed as mean ±SEM (n=6) for statistical evaluation of data. One-way ANOVA and student t-test were performed by using PRISM PAD statistical software program.

RESULTS AND DISCUSSION

Pharmacokinetic Parameters
Chromatography

The HPLC method for simultaneous estimation of Aceclofenac and Rosiglitazone was developed with better reproducibility, sensitivity, and accuracy. The calibration curves were constructed for both Rosiglitazone and Aceclofenac by using the

different graded drug concentration and ratio of chromatogram area of drug. The retention time of rosiglitazone and Aceclofenac under the above mentioned chromatographic conditions was 3.8 and 5.4 minutes respectively (Fig.1-3).

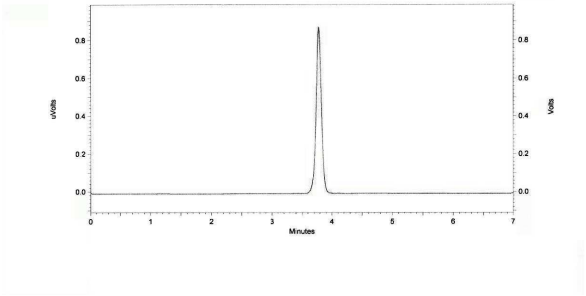

Figure 1
Chromatogram of Aceclofenac

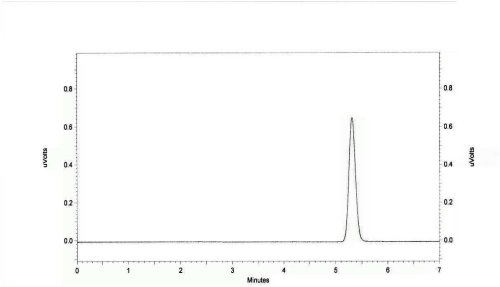

Figure 2
Chromatogram of Rosiglitazone

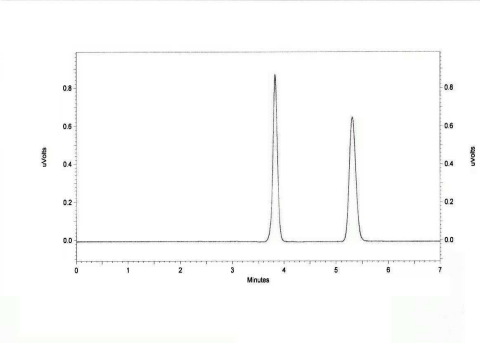

Figure 3
Chromatogram of Aceclofenac and Rosiglitazone

The correlation co efficient for rosiglitazone was found to be 0.9995 with slope 4473.8x and intercept of 211.5, whereas for Aceclofenac it was 0.9998, slope 4523.6 x and intercept of 498.86 (Fig.4 and 5).

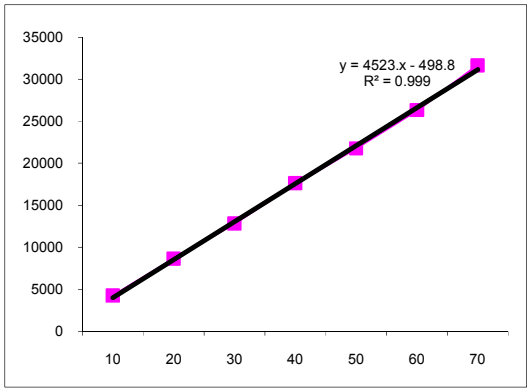

Figure 4
Standard Calibration curve of Aceclofenac

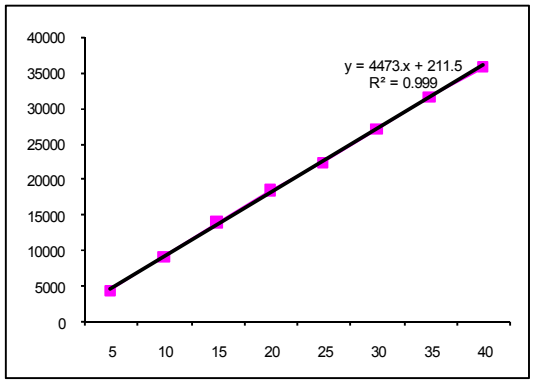

Figure 5
Standard calibration curve of Rosiglitazone

The % of drug recovery in the rat plasma samples collected at different concentrations was ranged as 78.4 to 94.18% and 87.9-99.2% for Aceclofenac and Rosiglitazone respectively (Table-1).

Table – 1
Absolute Recovery of Aceclofenac and Rosiglitazone

Concentration (μg / ml)		Absolute Recovery (%)			
		Aceclofenac		Rosiglitazone	
Aceclofenac	Rosiglitazone	Mean ± S.D	Range (min-max)	Mean ± S.D	Range (min-max)
100	5	82.00 ± 2.62	78.4 – 83.6	85.1 ± 2.88	83.8 – 86.4
200	10	92.71 ± 3.00	88.19 – 93.68	88.3 ± 2.27	87.9 – 92.2
400	15	86.17 ± 3.10	83.06 –90.19	86.6 ± 2.68	85.3 – 92.0
800	20	91.48 ± 3.10	87.46 –94.18	95.9 ± 2.46	83.0 – 91.6
1200	25	87.98 ± 2.79	85.63 – 91.49	97.4 ± 2.63	82.4 - 91.0
2000	50	92.14 ± 2.00	86.78 – 94.10	99.2 ± 2.48	83.7 - 90.0

Values are expressed as Mean ± S.E.M.

Pharmacokinetic analysis

Based on the data from the HPLC analysis the mean plasma concentrations and pharmacokinetic parameters were calculated and presented in Table-2. The mean concentration of Aceclofenac and rosiglitazone alone after one-week treatment was increased slightly after half an hour of administration compared to day-1 concentration in plasma, but it was not statistically significant (P>0.05). Concurrent administration of these two drugs in rats for 7 days showed that there was a significant enhancement in the plasma concentration of aceclofenac after 0.5h of last dose

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Table - 2.

Mean changes in concentration (µg/ml) of Aceclofenac (10mg/kg/p.o.) and Rosiglitazone (2mg/kg/p.o.) alone and in combination in rats.

Time (hrs)	Aceclofenac alone once daily		Rosiglitazone alone once daily		Aceclofenac with Rosiglitazone once daily after concomitant treatment			
					Aceclofenac		Rosiglitazone	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
0	0.00±0.00	0.00±0.00	0.00±0.00	0.14±0.00*	0.00±0.00	2.42±0.14*	0.00±0.00	0.10±0.01*
0.5	8.82±1.16	9.34±1.10	0.21±0.02	0.36±0.01*	6.98±0.82	11.07±1.00*	0.16±0.01	0.19±0.01*
1	36.41±2.49	37.85±2.36	0.88±0.02	0.72±0.02*	32.19±2.35	43.10±3.22*	0.74±0.02	0.56±0.02*
2	38.78±3.76	39.10±4.75	0.45±0.03	0.46±0.02	38.44±3.72	46.37±3.18	0.48±0.02	0.39±0.01*
4	29.60±3.51	32.20±2.45	0.38±0.03	0.41±0.02	26.21±2.89	37.18±2.71*	0.41±0.02	0.34±0.01*
6	21.48±3.00	23.88±2.98	0.29±0.01	0.30±0.01	18.00±2.17	31.46±2.48*	0.33±0.01	0.26±0.01*
8	10.26±1.43	12.69±1.46	0.21±0.01	0.20±0.01	13.86±2.00	24.28±2.15*	0.26±0.01	0.14±0.01*
24	7.25±1.00	8.53±1.13	0.16±0.01	0.18±0.01	6.10±1.12	13.66±1.54*	0.18±0.01	0.09±0.00*

Values are expressed as Mean ± S.E.M.; (n=6); (*P<0.05); Comparison made between alone and in combination on day1 and 7.

The pharmacokinetic study of these drugs on simultaneous treatment showed a statistically significant raise in Cmax, AUC_{0-t}, $AUC_{0-\infty}$ and $T_{1/2}$ were observed (P<0.05). All the other parameters were decreased after 7 days of treatment. The increased alterations in AUC_{0-24} and Cmax of Aceclofenac on day 1 & 7 were 1084.37 ± 76.18 to 1682.30 ± 91.06ng. h. ml^{-1} and 429.98 ± 37.61 to 587.8 ± 43.80ng/ ml (P<0.05) respectively (Table-3). It is believed that by blocking the Cox-1 enzyme preferentially, the adverse effects typically seen with traditional NSAIDs treatment. Aceclofenac is highly protein bound (~98%) primarily to albumin, and metabolized predominantly through the CYP2C9 pathway. So, the common metabolic pathway for both simultaneously administered drug may result in a possibility of *in vivo* drug interaction. With this background the present investigation was initiated in animal models.

Table-3.
Pharmacokinetic changes of Aceclofenac (10mg/kg/p.o.) and Rosiglitazone (2mg/kg/p.o.) alone and after concomitant administration in rats.

Pharmacokinetic Parameter	Aceclofenac alone once daily		Rosiglitazone alone once daily		Aceclofenac with Rosiglitazone once daily after concomitant treatment			
					Aceclofenac		Rosiglitazone	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
C_{max} (ng/ml)	468.4± 56.11	522.79± 48.68*	0.22± 0.04	0.32± 0.1*	429.98± 37.61	587.8± 43.80*	0.28± 0.02	0.17± 0.01*
T_{max} (h)	0.5± 0.1	0.6± 0.1	0.6± 0.12	0.6± 0.12	0.5± 0.1	0.5± 0.1	6.0± 0.1	6.0± 0.2
$AUC_{(0-t)}$ (ng.h/ml)	968.12± 36.56	1134.51± 85.34*	1.72± 0.12	2.10± 0.34	1084.37± 76.18	1682.30± 91.06*	1.88± 0.28	1.21± 0.21
$AUC_{(0-\infty)}$ (ng.h/ml)	893.28± 41.21	1415.23± 98.63*	228± 0.17	123± 0.22*	902.56± 63.49	1889.74± 104.26*	198.45± 0.92	108.23± 0.31*
$T_{½}$ (h)	2.36± 0.54	2.47± 0.68	3.11± 6.42	2.0± 0.12*	2.68± 0.48	4.42± 0.37*	2.66± 0.24	2.00± 0.12
Cl/f (L/h)	2.54± 0.26	2.83± 0.29	12.49± 3.8	8.2± 1.02*	2.41± 0.24	3.66± 0.62*	2.10± 0.35	1.35± 0.40*
Vd/f (L/kg)	9.86± 2.10	10.2± 2.00	20.25± 2.86	12.8± 1.22*	10.7± 2.06	14.82± 2.11*	13.44± 1.56	8.12± 1.00*

Values are expressed as Mean ± S.E.M.; (n=6); (*P<0.05); Comparison made between day1 and 7.

Pharmacodynamic interaction study

From the time of adjuvant challenge, the control group showed the gradual increase in the paw volume indicates the development of arthritis in the experimental animal models. The degree of arthritic progression was significantly reversed by Aceclofenac by 24.37% (P<0.01) and 46.65% (P<0.01) after 5 and 10days of treatment respectively. Similarly, the animals treated with the combination of Aceclofenac and Rosiglitazone simultaneously showed the percentage reduction in foot volume by 28.13% on 5th day of treatment (P<0.05) and 36.12% after 10days of treatment (P<0.01) compared to control. From the results it was clearly identified that there was a statistically significant difference observed between the drug treated groups on day 5 but not on day10 (Table-4). The efficiency of antiarthritic action of Aceclofenac was remarkably increased by simultaneously administered Rosiglitazone after 10days of treatment in rats but comparatively less in combinational drug treated group.

Table - 4
Antiarthritic efficacy on administration of Aceclofenac alone and in combination with Rosiglitazone in arthritic rats.

Treatment and Dose	Mean % changes in foot volume ± SEM		
	0 day	5th day	10th day
Arthritic Control	125.16±10.16	188±7.95	225.83±8.99
ACF alone (0.5mg/kg)	123.16±8.91^{ns}	142.18±10.62^{b}	156.88±12.96^{b}
ACF (10mg/kg) +RGZ (0.5mg/kg)	121.0±7.41^{ns}	135.11±8.84^{a}	144.24±8.12^{b}

N = 6; Values are expressed as mean ± SEM; ^{a}P<0.05; ^{b}P<0.01 Vs. Tumour control; Data were analyzed by using one way ANOVA followed by dunnet's't' multiple comparison test.

Antidiabetic study

Rosiglitazone alone produced 79.60-81.96% of reduction in blood glucose ($p < 0.01$) at the dose of 0.5mg/kg of body weight during one week of treatment. Rosiglitazone (0.5mg/kg) and Aceclofenac (10mg/kg) when given simultaneously produced a significant ($p < 0.01$) reduction in blood glucose at the range of 51.44-76.66% compared to control (Table-5).

Table – 5

Effect of rosiglitazone alone and in combination with Aceclofenac on Glucose concentration in alloxan-induced diabetic rats

Treatment	Glucose concentration (mg/dl) measured at regular intervals (Days)			
	I	III	V	VII
Normal	68.33 ± 2.40	71.33 ± 3.13	71.83 ± 2.81	73.0 ± 2.39
Diabetic Control	349.66±16.64	357.0 ± 14.6	364.83±12.17	369.66 ± 6.76
RGZ alone (0.5mg/kg)	71.33 ± 3.09[d]	66.0 ± 2.20[d]	64.16 ± 1.24[d]	66.66 ± 2.07[d]
RGZ (0.5mg/kg)+ACF (10mg/kg)	81.60 ± 3.98[d]	122.27 ± 2.43[a,d]	163.68 ± 4.15[a,d]	179.47 ± 6.18[a,d]

n=6; Values are expressed as Mean±S.E.M; [a]P<0.001 Vs Normal; [d]P<0.001 Vs Diabetic Control, (Tukey multiple comparison test)

It was observed that the simultaneous treatment with aceclofenac gradually decreases the efficiency of rosiglitazone during 8hrs in sugar control capability, the antihyperglycemic action of combined drug was very least after 6-8hrs of treatment compared to rosiglitazone alone effect. The percentage reduction in blood glucose in the diabetic condition compared to the normal state was highly significant ($P<0.001$). Since our results showed that Rosiglitazone alone reduced blood glucose levels in hyperglycemic animals better than the combinational dose of ACF+RGZ. The total cholesterol and triglyceride levels were also normalized significantly by rosiglitazone alone and in combination with ACF ($P<0.001$) when compared to diabetic control group. Overall, statistically significant difference among the treated group was observed (Table-6).

Table: 6

Effect of Rosiglitazone alone and in combination with Aceclofenac on Total Cholesterol and Triglyceride levels in alloxan-induced diabetic rats.

Treatment and dose	Parameters (mg/dl)	
	Total Cholesterol	Triglycerides
Normal 10mg/kg of vehicle	83.0 ± 1.0	87.0 ± 2.82
Diabetic control	117.5 ± 3.5	123.5 ± 6.36
RGZ alone (0.5mg/kg)	73.0 ± 1.0[c,d]	66.5 ± 2.12[b,d]
RGZ (0.5mg/kg) + ACF (10mg/kg)	89.56 ± 2.61[d]	76.28 ± 2.76[d]

n=6; Values are expressed as mean ±S.E.M; [b]P<0.01; [c]P<0.05 Vs Normal; [d]P <0.001 Vs Diabetic Control

CONCLUSION

Aceclofenac is a phenyl acetic acid derivative that shows analgesic and antiarthritic activity and good tolerability profile in pain conditions. Concomitant administration of ACF with RGZ significantly increases this tolerability and efficiency after 10 days of treatment in CFA induced arthritic rats by increasing the bioavailability of ACF by CYP2C9 inhibitory

mechanisms but the antiarthritic efficacy was minimized on combined therapy. Similarly anti-hyperglycemic activity also affected on concomitant treatment. Literature reveals that the bioavailability of rosiglitazone following oral administration is ~ 99%. In the present study the antihyperglycemic efficacy of rosiglitazone is statistically not equal after concomitant administration of ACB for one week. There was a statistical difference observed between treated groups in pharmacodynamic activity. Hence the enhancement in kinetic parameters demands for the dose adjustment during long term concomitant therapy of Aceclofenac with Rosiglitazone.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Ishari. K. Ganesh, Chancellor, Vels University for providing the facilities necessary to carry out this research

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