PROTECTIVE EFFECT OF PIOGLITAZONE ON GENTAMICIN INDUCED NEPHROTOXICITY IN RABBITS

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ABSTRACT

Objective: To evaluate potential role of pioglitazone in protecting kidneys from nephrotoxic insult produced by Gentamicin.

Study design: Comparative study on animal model.

Place and Duration of Study: Department of Pharmacology Army Medical College, duration of study was six months.

Material and Methods: Twenty four rabbits were randomly divided into four groups (n=6). Group (Gp)-1 received 1 milliliter (ml) isotonic saline intraperitoneally (IP) daily for 13 days. Gp-2 received gentamicin 40 miligram/kilogram/day (mg/kg/day) IP daily for 13 days. Gp-3 received pioglitazone salt 10 mg/kg/day dissolved in drinking water via feeding tube for 13 days. Gp-4 received pioglitazone salt 10 mg/kg/day via feeding tube plus gentamicin 40 mg/kg/day IP for 13 days. Blood was collected on days 0 and 14 for estimation of serum urea and creatinine. All animals were sacrificed and kidneys were removed for renal histological examination.

Results: Pioglitazone did not show any nephroprotective effect against gentamicin induced nephrotoxicity.

Conclusion: Pioglitazone fails to exhibit nephroprotective potential when administered along with nephrotoxic dose of gentamicin.

Keywords: Gentamicin, Nephroprotection, Nephrotoxicity, Pioglitazone.

INTRODUCTION

The prototype aminoglycoside gentamicin is among the most frequently used antibiotics against infections by gram negative bacillary microorganisms[1], but regrettably its use generates nephrotoxicity in almost 10-20% of the therapeutic regimes[2]. Gentamicin induced oxidative stress appears to play the pivotal role in the development of this nephrotoxicity[3]. The drug augments renal mitochondrial synthesis of reactive oxygen species that leads to progressive renal structural and functional deterioration. Characteristic changes include cellular desquamation, glomerular atrophy, tubular necrosis and fibrosis, proximal tubular epithelial edema, perivascular edema and inflammation and glomerular hypertrophy and congestion. The worsening renal function is marked by an elevation of serum urea and creatinine above normal levels, albuminuria and urinary losses of carnitine, decrease in glomerular filtration rate, and renal dysfunction[4].

Pioglitazone is peroxisome proliferator-activated receptor-gamma (PPAR-γ) agonist. The agonists of PPARs have been shown to possess anti-hyperlipidemic, anti-hyperglycemic, anti-inflammatory, anti-oxidative, anti-fibrotic and anti-proliferative properties[5]. Pioglitazone selectively stimulates the nuclear PPAR-γ, and to a lesser extent PPAR-α, which results into augmented genes transcription involved in regulation of glucose and lipid metabolism. The overall effect is improved glycaemic control with no increase in the endogenous insulin secretion[6]. Pioglitazone has also been shown to possess anti-inflammatory, anti-oxidative and anti-fibrotic properties in addition to its anti-diabetic effects[7]. It has been shown to attenuate oxidant injury by reversing the increase in monocyte

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chemoattractant protein-1 in renal tubular cells and reduction in C-reactive protein in patients with overt diabetic nephropathy[8].

Thus studies in various animal models suggest that it can act as a potent general antioxidant[9] and reduce oxidative stress at the kidney level[10]. Such data strongly suggests a potential benefit of pioglitazone in diabetic and non-diabetic nephropathies, such as induced by drugs.

MATERIAL AND METHODS

The study was conducted in the Pharmacology Department, Army Medical College, Rawalpindi. Sampling technique was 'Simple Random Sampling'. Twenty four healthy rabbits were used as they are an established animal model of nephrotoxicity. Animals were 7–10 months of age, non-pregnant and weighed between 1 and 1.9 kg at the beginning of study. They were kept in animal house. Their diet consisted of grass, grain, seasonal vegetables and water adlibitum. Twelve hours light and dark cycle was maintained.

The drugs used include

Gentamicin injection (80 mg of gentamicin sulfate in 2 ml aqueous solution) by Reckitt and Coleman Pharmaceuticals, Karachi, Pakistan purchased from local market.

Pure salt of pioglitazone donated by Werrick Pharmaceuticals, Islamabad, Pakistan.

It was a randomized controlled trial. Twenty four rabbits were randomly divided into 4 groups, with six rabbits in each. Gentamicin was administered by intraperitoneal (IP) route. Pioglitazone was given by mouth mixed in drinking water via feeding tube.

The drugs were given over a period of 13 days according to the following regimen.

Table-1: Comparative analysis of serum parameters of different groups of rabbits on day 0 and day 14.

Comparative Groups	Serum Urea (mmol/l)				Within groups *p* value	Serum Creatinine (µmol/l)				Within Groups *p* value
	Day 0	SD	Day 14	SD		Day 0	SD	Day 14	SD	
G-1(normal saline)	6.96	0.41	6.38	1.30	0.298	104.33	6.08	104	8.85	0.910
G-2(gentamicin 40mg/kg/day)	6.38	0.99	12.71	3.22	0.002	107	10.17	140.33	13.96	0.006
Between Groups *p*value	0.001					< 0.001*				
G-1(normal saline)	6.96	0.41	6.38	1.30	0.298	104.33	6.08	104	8.85	0.910
G-3 (pioglitazone10mg/kg/day)	6.61	0.47	6.75	0.41	0.175	100.5	6.92	102.6	6.71	0.071
Between Groups *p* value	0.526^{NS}					0.775^{NS}				
G-2(gentamicin 40 mg/kg/day)	6.38	0.99	12.71	3.22	0.002	107	10.17	140.33	13.96	0.006
G-4(pioglitazone 10 mg/kg/day+ gentamicin 40 mg/kg/day)	6.21	0.85	12.81	1.58	<0.001	114.6	4.88	141.33	11.18	0.001
Between Groups *p* value	0.947^{NS}					0.894^{NS}				

p value < 0.05 = Significant(*)
p value > 0.05 = Not significant (NS)

Table-2: Comparison of histopathological findings of different groups (*p* value=1.00 by applying Fisher's Exact Test).

Comparative groups	Grades of necrosis		
	Mild	Moderate	Severe
G-2 (gentamicin 40mg/kg/day)	1	5	0
G-4 (pioglitazone 10 mg/kg/day+ gentamicin 40 mg/kg/day	1	5	0
p-value	1.00		

Group (gp)-1 (control group) received 1 ml of isotonic saline intraperitoneally once daily[11] for 13 days.

Gp-2 received a daily gentamicin injection IP in nephrotoxic dose of 40 mg/kg[12] for 13 days.

Gp-3 received pioglitazone salt(10 mg/kg/day) dissolved in drinking water via feeding tube for 13 days[13].

Gp-4 received gentamicin (40 mg/kg/day) IP[12] plus pioglitazone salt(10 mg/kg/day) dissolved in drinking water via feeding tube for 13 days [13].

All animals survived the 14 days experimental period.

Collection of samples: Blood samples of rabbits were collected on day 0 and 14. About 2 ml of blood was withdrawn from marginal ear vein and stored in a centrifuge tube. It was allowed to clot at room temperature and then centrifuged at 3000 rpm for 15 minutes. Serum was separated with the help of an automatic micropipette and stored in a vial at -20°C for urea and creatinine estimation. Biochemical analysis was carried out using automatic chemistry analyser SELECTRA E. Urea was estimated by Urease/kinetic method[16] and serum creatinine by Jaffe reaction[17].

Rabbits were sacrificed twenty four hours after the last dose of drug[14]. Kidneys were taken out; sections were made and further processed for histopathologic examination under light microscope with special attention to proximal tubules[15]

Histopathology

Grading criteria for tubular injury on microscopic examination of specimens were graded as follows[18]:

Grade-0-no cell necrosis; grade 1- mild, only single cell necrosis in sparse tubules; grade 2-moderate, more than one cell involved in sparse tubules; grade 3-marked, tubules exhibiting total necrosis in almost every power field; and grade 4-massive total necrosis.

Statistical analysis: Results were assessed by Independent T test and paired T test. $p < 0.05$ was taken to indicate statistical significance. The histopathology results were analyzed by 'Fisher's Exact Test'. A *pvalue* < 0.05 was considered significant.

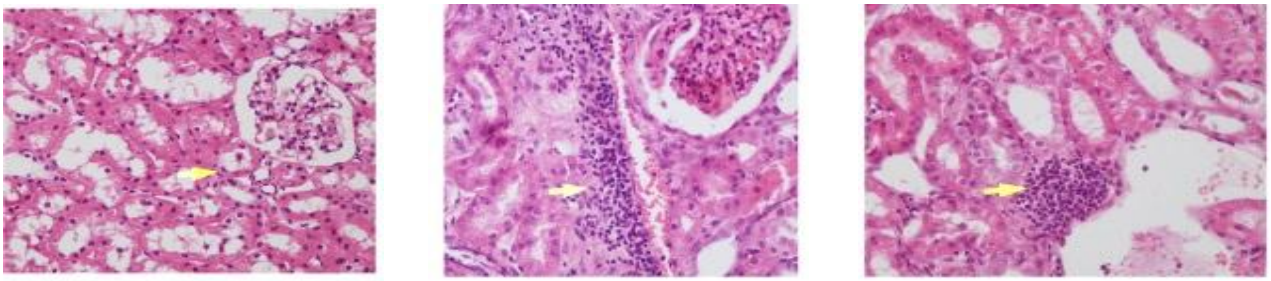
RESULTS

Observation of Individual Parameters

Serum urea and creatinie: Serum urea and creatinine remained within normal range over 14 day period in gp-1 and gp-3. *p* value was found statistically insignificant when gp-3 was compared with gp-1 ($p > 0.05$).

Serum urea and creatinine increased markedly in gp-2. *p* value was significant when compared with gp-1 ($p < 0.05$).

The nephroprotective effect of pioglitazone against gentamicin induced renal insult was assessed in group 4. In gp-4, serum urea and creatinine were found to be deranged and when compared with gp-2 it appeared that pioglitazone failed to mitigate the rise of urea and creatinine and the difference between gp-2



Light micrographs showing normal histology of proximal renal tubules in Gp-1 saline treated rabbits (400X).

40 mg/kg/day gentamicin treated Gp-2 showing inflammatory cells infiltration, congestion and tubular necrosis in renal tubules (400X)

Pioglitazone 10 mg/kg/day and gentamicin 40 mg/kg/day treated Gp-4 showing similar degree of inflammatory cells infiltration and tubular necrosis in renal tubules as in Gp-2 (400 X)

Figure-1: Light micrographs showing histology of proximal renal tubules in Gp-1, Gp-2 and Gp-4.

and gp-4 was found to be statistically insignificant ($p > 0.05$) (table-1).

Histological Examination

Histological examination of the renal sections of Gp-1was normal (grade 0 necrosis).

A total of 67% of rabbits in gp-2 exhibited moderate grade 2 necrosis. The remainder 33% animals showed mild grade 1 necrosis marked by single cell necrosis in rare proximal tubules. Histological examination of gp-3 showed normal finding without any evidence of necrosis. gp-4 showed moderate necrosis in 67% and mild grade 1 necrosis in 33% of the animals (fig-1). When we applied the Fisher's Exact test, '*p*' value was $p=1.00$ showing statistical insignificance (table-2).

DISCUSSION

The present research project was carried out to study the nephrotoxicant insult induced by gentamicin, the prototypical and the most widely used drug among aminoglycoside group of antibiotics, and to determine the renoprotective potential of antidiabetic drug pioglitazone when it was used concurrently with gentamicin.

The nephrotoxic group that received 40 mg/kg/day of gentamicin showed raised serum urea and creatinine values on day- 14 as compared to day-1. The difference is statistically significant for serum urea ($p < 0.002$) as well as for serum creatinine ($p< 0.001$). The renal histology revealed grade- 2 necrosis.

These findings are consistent with the results of Chaware et al[19] and Yasin et al[12].

The gp-4 which received gentamicin and pioglitazone did not show any nephroprotective effect and the serum urea and creatinine values were found to be equally raised as that of gp-2. The difference was statistically insignificant for serum urea (*p* value- 0.996) as well as for serum creatinine (*p* value- 0.989) when gp-4 was compared with gp-2. Renal histology also revealed grade-2 necrosis.

These findings negate the hypothesis that pioglitazone, being the member of PPAR- γ, could counter the gentamicin induced oxidative stress by its antioxidant potential. Results comparable to this study were also observed by Boulanger and the co-researchers concluding PPAR- γ agonist failure to demonstrate renoprotective potential[20].

These findings, however, are in contrast with the studies which suggested pioglitazone to possess antioxidant[21] and nephroprotective effect[22]. In research work by Petrica[23], pioglitazone was shown to delay proximal tubular dysfunction in type 2 diabetics through its antioxidant effect. Omasu[24] reported the protective effect of pioglitazone on renal fibrosis in rats. Another study showed blockade of angiotensin II- mediated oxidative stress by pioglitazone[25].

It can be concluded from this study that pioglitazone does not provide any nephroprotection with regards to gentamicin.

CONCLUSIONS

Gentamicin, a highly efficient antibiotic against gram-negative infections, is a potentially nephrotoxic drug.

Pioglitazone, an antidiabetic drug of the thiazolidinedione class, fails to exhibit any renoprotective potential when administered alongwith toxic dose of gentamicin.

CONFLICT OF INTEREST

The authors of this study reported no conflict of interest.

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