TO EVALUATE THE ROLE OF DICLOFENAC SODIUM ON RENAL PARENCHYMA OF YOUNG ALBINO RATS

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ABSTRACT
To study the effects of commonly used non steroidal anti-inflammatory drug diclofenac sodium on the kidneys of young albino rats after therapeutic dose. Early clinical and pharmacological studied of oral and parental forms of diclofenac sodium began shortly after the drug was discovered. However there is scanty information about the toxicities of normal dose of diclofenac in young albino rats. The present study is therefore designed to observe the gross and microscopic changes in kidney following administration of this drug in young albino rats. For this experimental study 16 albino rats were used; they were divided into two groups, each comprising of 8 animals. Group-1 received normal saline 10ml/kg body weight while group-2 received diclofenac sodium 2mg/ kg body weight for two weeks. Micrometry was done on kidney tissue for proximal and distal tubular count, their diameter and the no of cells in these tubules. Highly significant changes were observed both in proximal and distal tubules of kidney. Even in therapeutic doses, Diclofenac Sodium causes damage to kidney tubules in young albino rats.

Keywords: Diclofenac sodium, albino rats, kidneys.

INTRODUCTION

Non steroidal anti- inflammatory drugs (NSAIDs) which are often used for the relief of non specific fever (Radwan and Zidovudin 2000) continue to be important for the palliation of pain (Simon, 1994). They are most frequently used medication for the treatment of variety of common chronic and acute inflammatory conditions. In recent years the number of anti-inflammatory and analgesic drugs in use has increased by many folds. Toxic effects are usually reported after the drug has been used for a significant period of time. Weather they are administered at a therapeutic dose or at high levels, NSAIDs can produce various clinical, biochemical and structural changes (Irena *et al*., 2006).

As they are largely prescribed drugs in adults and children so their toxic effects are also frequent in both groups of population. Present study is therefore designed to evaluate the effects of diclofenac sodium (a NSAID) on kidneys of young albino rats, with respect to histological manifestation and underlying pathophysiological mechanism.

MATERIAL AND METHODS

This study was carried out during the period from March to August 2002. For this experimental study 16 young albino rats aged 2 weeks, weighing 20 to 30 gm were used. They were originally obtained from Charles River breeding laboratories, Brooklyn, Massachusetts, USA, and were cross bred at the animal house of Basic Medical Sciences Institute, JPMC Karachi. The animals were kept in the animal house on a balanced diet. They were put under observation for one week, prior to the experimental procedure.

The animals were divided into two groups: a control group-1 (n=8), and the diclofenac sodium group-2 (n=8). The rats in the control group were given 10ml/kg body weight normal saline orally by feeding tube while the animals in group-2 received diclofenac sodium at a dose of 2mg/kg/day (Manocha and Venkatarama 2000) dissolved in distilled water and administered orally by feeding tube for a period of two weak.

On day 15 the animals were sacrificed kidneys were removed, bisected in two halves, one half fixed in 10% formalin and second in alcoholic formalin. The tissues were sectioned and mounted on slides. They were stained by Haematoxylin & Eosin (H&E), silver methamine and Periodic acid schiff' stain (Bancroft and Cook, 1984).

The morphological changes in renal parenchyma were observed under light microscope. Five observations for each parameter were recorded in each animal. Proximal and distal tubular counts were made under 8x ocular and 40x objective with counting reticule in randomly selected five fields in the cortex of the kidney. Tubular diameter was measured with the help of ocular micrometer and the number of cells was also counted under same magnification and with the help of reticule. The data was

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Table 1: Comparison of Proximal Tubular Count, Diameter and Number of Cells per unit area between Group-1 and Group-2

Animal #	No. of observations	Proximal Tubular Count		Proximal Tubular Diameter		Cell Count	
		Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
		Control	Diclofenac Sodium	Control	Diclofenac Sodium	Control	Diclofenac Sodium
1	5	28	19	41.7	47.5	104	76
2	5	27	19	44.7	50.7	103	74
3	5	29	17	40.7	45.6	106	81
4	5	24	16	44.1	49.2	109	92
5	5	28	20	43.2	48.1	117	103
6	5	36	17	40.6	51.8	100	85
7	5	30	18	42.3	49.2	102	75
8	5	27	19	44.6	52	105	93
Mean		28.6	18.1	42.7	49.3	105.8	84.9
S.E.M.		1.2	0.5	0.6	0.8	1.9	3.7

Statistical Analysis

Group	P value	S / NS / HS	P value	S / NS / HS	P value	S / NS / HS
1 Vs 2	≤0.001	HS	≤ 0.001	HS	≤0.001	HS

Table 2: Comparison of Distal Tubular Count, Diameter and Number of Cells per unit area between Group-1 and Group-2

Animal #	No. of Observations	Distal Tubular Count		Distal Tubular Diameter		Cell Count	
		Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
		Control	Diclofenac Sodium	Control	Diclofenac Sodium	Control	Diclofenac Sodium
1	5	27	16	25.41	36	113	67
2	5	26	17	26.7	36.6	100	62
3	5	24	15	25.2	38.4	109	72
4	5	23	15	27	36.3	102	62
5	5	25	15	29.27	33.5	108	64
6	5	22	16	28.11	37.4	110	63
7	5	22	14	25.3	38.2	105	68
8	5	26	17	25	35.1	106	65
Mean		24.4	15.6	26.5	36.4	106.6	65.4
S.E.M.		0.7	0.4	0.6	0.6	1.5	1.2

Statistical Analysis

Group	P value	S / NS / HS	P value	S / NS / HS	P value	S / NS / HS
1 Vs 2	≤0.001	HS	≤0.001	HS	≤0.001	HS

subjected to statistical analysis [(]Bland, 1987) Student 't' test was employed to see the significance of the results.

RESULTS

Group-1

Regarding their general behavior the animals in this group remained active, quick to respond, and their food intake was normal.

In H&E stained sections the histological structure in the cortical and medullary portion appeared absolutely normal without any change in either glomeruli or tubules. No sign of degenerative change was observed in the cytoplasm of renal tubular epithelial cells. The interstitium of the renal cortical and medularly area was sparse and contained small capillaries filled with RBCs (fig. 1). The brush border on the apical surface of proximal tubular epithelial cells stained magenta in colour and almost filled the tubule. The glycogen content of the

cytoplasm of proximal tubular cells was quite normal. The basement membrane of proximal and distal tubules also stained magenta, which was distinct and regular.

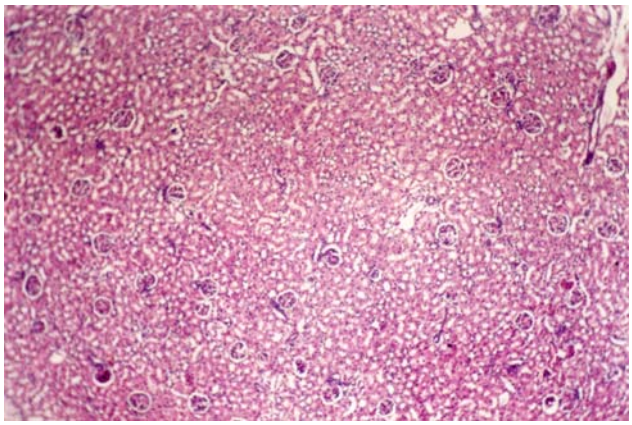

Fig. 1: Photomicrograph of 4µm thick H&E stained paraffin section of rat kidney from group-1 (control), showing normal architecture of renal cortex, 101 times magnified.

Silver methenamine stained sections revealed basement membrane of glomeruli, Bowman's capsule, and proximal and distal tubules which was faint in outline, and un-measurable by light microscopy.

The mean value of number of proximal convoluted tubules per unit area as noted in group-1 were 28.6±1.2 which when compared with group-2 a highly significant increase ($P \leq 0.001$) was noted (table 1).

The mean value of diameter of proximal tubules measured in unit area was 42.7±0.6 um which when compared with group-2 statistically highly significant decrease was ($P \leq 0.001$) noted.

The mean values of total cell count per unit area in proximal tubules observed in group-1 was 105.8±1.9 which when compared with the respective value in group-2 a highly significant increase ($P \leq 0.001$) was observed (table 1).

The mean value of number of distal tubules per unit area was 24.4±0.7, which when compared with group-2 a highly significant increase ($p \leq 0.001$) was observed. The mean value of diameter of distal tubules per unit area was 26.5±0.6, which when compared with group-2 a highly significant decrease ($p \leq 0.001$) was noted.

The mean value of number of cells per unit area in distal tubules in group A was 106.6±1.5 which when compared with that in group B statistically a highly significant increase ($p \leq 0.001$) was noted.

Group-2

The animals in this group were treated with diclofenac sodium. They looked ill and weak from the day-4 of experimental period. They were lethargic; reluctant to food intake and response to stimuli was also sluggish.

In this group the proximal tubules in juxtaglomerular region appeared dilated, circular oval or elliptical in section and filled with cellular debris. The lining epithelial cells of the proximal convoluted tubules were low columnar, many of these cells showed degenerative changes. The nuclei of the intact cells were either central or towards the apical portion of cells. The nuclei of some of the cells appeared condensed indicating pyknosis leading to cell death. Many of the cells in proximal tubules showed vacuolation obscuring all cytoplasmic details.

The distal convoluted tubules also appeared dilated and circular oval or elliptical in outline. Some of them contained cellular debris in their lumen. The lining epithelial cells were cuboidal, some of these cells showed degenerative changes. The nuclei of the intact cells were either central or towards the luminal aspect but some nuclei of the cells appeared condensed.

The interstitium of renal cortical area was sparse with few inflammatory cells but no marked oedema. Many of the dilated and congested blood vessels were frequently observed (fig. 2).

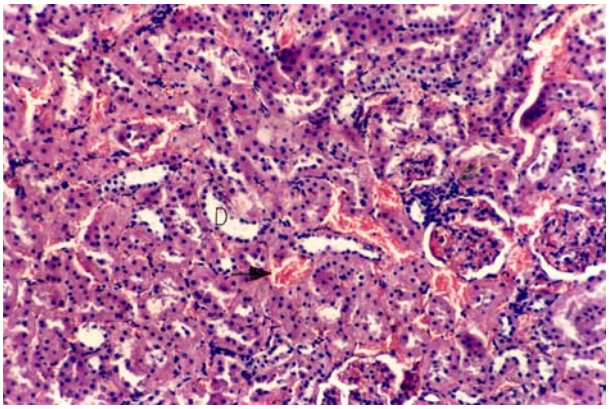

Fig. 2: Photomicrograph of 4µm thick H&E stained paraffin section from group-2 treated with diclofenac sodium, showing dilated blood vessels with marked infiltration of inflammatory cells and damaged tubules, 205 times magnified.

The medulla showed infiltration of mononuclear cells as well as marked congestion of blood vessels. In PAS stained sections the brush border at the luminal surface appeared scanty and indistinct and at some places it was completely absent. The intracellular glycogen content of the proximal as well as distal tubules was moderately

depleted. However, the basement membrane of proximal and distal tubules was regular and intact.

In silver methenamine stained sections the basement membrane was visible as intensely stained black line around proximal and distal tubules which was quite thickened in some tubules but still not measurable by light microscope.

The mean values of number of proximal convoluted tubules per unit area observed in group-2 were 18.1±0.5. The mean value of diameter of proximal tubules was 49.3±0.8. The mean value of number of cells in proximal tubule per unit area under high magnification in group-2 was 84.9±3.7.

Mean values of distal tubular count per unit area as observed under high magnification was15.6±0.4. Mean values of diameter of distal tubules per unit area in group-2 was 36.4±0.6 um. Mean values of total cell count of distal tubules per unit area in group-2 were 65.4±1.2 (table 2).

DISCUSSION

Non-steroidal anti inflammatory drugs (NSAID) are the integral part of the therapy in arthralgia. It not only relieves the pain effectively but is also helpful in decreasing the inflammatory process. Their nomenclature is easy to remember for the common man, so the substantial number of patients without the advice of doctors is indulged in the use of this drug (justified or unjustified) Diclofenac sodium, belonging to the NSAID is the commonly used drug of this group. It acts by inhibiting the enzyme cyclooxygenase hence preventing the synthesis of prostaglandin.

In this study the effects of NSAID in general and diclofenac sodium in particular are being observed on the morphology of renal parenchyma in young albino rats.

The proximal and distal convoluted tubules in the cortex of kidney were damaged and the inflammatory changes in medulla were also observed. Diclofenac sodium inhibits the synthesis of prostaglandin by inhibiting the enzyme cyclooxygenase in body cells. The prostaglandins are synthesized from the precursor arachidonic acid by the action of cyclooxygenase which converts arachidonic acid to prostaglandin (Giff and Itskovitz 1973). Inhibition of prostaglandin synthesis was the initiating factor for the pathological changes in kidney tissue.

After treatment with diclofenac sodium, general behavior of animals changed to ill, and sluggish. Decreased food intake was also noted which may be attributed to unwanted effects of diclofenac sodium, in this context our results are in agreement with the Bjarrason *et al* (1983); Gabriel *et al* (1991) and Graham *et al* (1993) in which it is stated that administration of diclofenac sodium was associated with increased GI. toxicity including mild dyspepsia or cachexia as well as more serious GI reactions.

There was a significant decrease in number of proximal and distal tubules per unit area. This may be attributed to damage to the tubular epithelial cells by ischemia due to vasoconstriction of renal arterioles.

This vasoconstriction is in turn the result of decreased prostaglandin synthesis. These results are in agreement with gray *et al* (1980) and Clive and Stoff (1984) who observed vacuolar degeneration of proximal tubules and focal tubular atrophy in NSAID associated renal failure so the no of proximal tubules per unit area appeared significantly decreased.

The highly significant increase in the diameter of proximal and distal tubules in group-2 as compared to group-1 was noted which may be attributed to degeneration of cells in these tubules resulting in apparent increase in their diameter. These findings are in conformity with Scott *et al* (1966) who observed renal tubular cells in urine after ingestion of NSAID. This degeneration of cells may be explained as renal vascular tone is determined by autonomous intrinsic activity of the renal arterioles and continuous production of prostaglandin which is inhibited by this drug and thus causing unopposed constriction of arterioles resulting in ischemia of tubules and epithelial cell death.

Finally it may be concluded that diclofenac sodium at the dose of 2mg/kg body weight i.e. in therapeutic dose causes damage to the kidney, so it should be given cautiously in patients having renal insufficiency and further research is required to find out an agent having protective role for kidneys against NSAIDs.

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