Original Article

Adverse effects of Diclofenac sodium on renal parenchyma of adult albino rats

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Abstract

Objective: To see the toxic effects of NSAID on kidney tissue of albino rats.

Methods: For this experimental study, 16 albino rats were taken. They were divided into two groups; A and B. The animals in group-A were given Normal Saline. Group-B received Diclofenac Sodium 2 mg/kg/day by feeding tube for 14 days. On day-15 all animals were sacrificed. Kidneys were removed, fixed, embedded in paraffin, section cut at 4 µm thick and stained with H&E, PAS, and silver methenamine. Renal histology was done under light microscope to see the renal tubular diameter, count and cellular detail.

Results: The result of present study revealed that diclofenac sodium in single daily dose of 2 mg/kg for a period of two weeks effectively produced destruction of proximal and distal convoluted tubules in adult albino rats showing the dilatation of tubule and flattening of tubular epithelium, disruption of brush border in proximal tubule and thickening of basement membrane around proximal and distal tubular epithelium.

Conclusion: Diclofenac sodium induced nephrotoxicity causes the destruction of proximal and distal convoluted tubules showing the dilatation of tubule and flattening of its epithelium in albino rats (JPMA 57:349:2007).

Introduction

Diclofenac sodium, an analgesic, anti-inflammatory and antipyretic compound, nowadays, is one of the commonest used non-steroidal anti-inflammatory drugs (NSAID).[1] It is used in a variety of painful conditions like osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, renal colic, in dentistry and preoperatively[2] to reduce postoperative pain. Under such conditions if the use is prolonged, these drugs sometimes produce moderate to marked degree of nephrotoxicity. Kidney is an important target site for untoward effect of diclofenac sodium in humans as well as in animals.[3]

The usage of this drug by large number of patients indicates its efficacy; however it also indicates that a large population is at risk.[4]

Present study is therefore designed to study the gross and microscopic changes in the kidney following administration of therapeutic dose of diclofenac sodium in albino rats.

Material and Methods

The animals used in this experimental study were albino rats of 12 weeks age, weighing between 180~200 grams. They were originally obtained from Charles River Laboratories, Brooklyn, Massachusetts, USA, crossbred and kept at the Animal House of Basic Medical Sciences Institute, JPMC, Karachi. The study period extended from June 2002 to November 2002.

Sixteen animals were used in this study; they were divided into two groups; A and B. The animals in each group were kept in a separate cage and labeled. Each animal was weighed prior to the treatment. The animals in group-A served as control and received Normal Saline, 10 ml/kg body weight orally daily for two weeks. The animals in group-B received diclofenac sodium at a dose of 2 mg/kg/day[5], dissolved in distilled water and administered orally by a feeding tube once daily for two weeks. On day-15 the animals were sacrificed by deep ether anesthesia. They were dissected; their kidneys were removed and weighed with the help of Sartorious balance. Each kidney was bisected into two halves; one half fixed in 10% the

formalin and other half in alcoholic formalin.

The tissue was then processed for paraffin embedding, sectioned on a rotary microtome, 4 μm thick longitudinal sections were cut, and were mounted on gelatinized slides. The tissue sections fixed in 10% formalin were stained with H&E to access the general architecture of renal parenchyma and with silver methenamine for basement membrane. The sections of tissue fixed in alcoholic formalin were stained with PAS.[6] The morphological changes in renal parenchyma were observed. Micrometry was done and the data was subjected to statistical analysis.

The parameters were proximal and distal tubular count, their diameter and number of cells per unit area. Tubular count was made under 8 ocular and 40 objective with counting reticule in randomly selected five fields in the cortex of kidney, while tubular diameter was recorded with the help of ocular micrometer. Nuclear count, cytoplasmic and nuclear details were made under low power, high power and oil immersion objectives.

Results

Regarding the general behaviour, the animals in group- A remained active, quick to respond, and their food intake was normal. In group- B the animals looked ill and weak from day- 4 of experimental period. They were lethargic, response to stimuli sluggish, and were reluctant to take food.

The H&E stained sections in group-A showed the histological structure in the cortical and medullary portion to be absolutely normal without any change in either glomeruli or tubules. No sign of any degenerative change was observed in the cytoplasm of renal tubular epithelial cells. The interstitium of the renal cortical and medullary area was sparse and contained small capillaries filled with RBCs. 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.

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 number of proximal convoluted tubules per unit area in group-A was 23.750±0.559, which when compared with group-B, a highly significant increase ($P<0.001$) was noticed.

The H&E stained sections in group-B revealed the proximal tubules in juxtaglomerular region to be 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 dilated and congested blood vessels were frequently observed.

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

Table 1. Comparison of Proximal Tubular Count, Diameter, and number of Cells between Control and Diclofenac Sodium treated animals.

Animal No.	No. of Observ-ations	Tubular Count		Tubular Diameter		No. of Cells	
		A	B	A	B	A	B
		Control	D.S.	Control	D.S.	Control	D.S.
1	5	23	17	49.5	51.3	109	81
2	5	24	17	56.7	58.8	111	85
3	5	26	15	48.9	50.7	114	93
4	5	22	15	51.6	54.0	118	85
5	5	26	19	50.2	52.3	122	80
6	5	24	13	52.3	56.5	117	92
7	5	22	18	51.4	55.8	107	80
8	5	23	20	49.7	53.7	121	97
Mean		23.750	16.750	51.287	54.137	114.875	86.625
SD		1.581	2.314	2.479	2.765	5.540	6.566
P-value		<0.001		<0.05		<0.001	

Key: D.S. = Diclofenac Sodium.

Table 2. Comparison of Distal Tubular Count, Diameter, and number of Cells between Control and Diclofenac Sodium treated animals.

Animal No.	No. of Observ-ations	Tubular Count		Tubular Diameter		No. of Cells	
		A	B	A	B	A	B
		Control	D.S.	Control	D.S.	Control	D.S.
1	5	23	14	38.80	55.50	119	70
2	5	25	16	39.90	53.80	113	70
3	5	23	14	39.30	53.10	111	79
4	5	22	15	37.90	57.00	115	71
5	5	24	14	36.25	54.20	112	75
6	5	20	14	39.75	56.10	115	70
7	5	23	14	38.15	57.00	120	72
8	5	22	16	39.10	52.00	117	75
Mean		22.750	14.625	38.643	54.837	115.25	73.142
SD		1.488	0.916	1.194	1.849	3.240	3.338
P-value		<0.001		<0.001		<0.001	

Key: D.S. = Diclofenac Sodium.

some tubules but still not measurable by light microscope.

Table 1 and 2 show significant difference between tubular count, diameter and number cells between control and diclofenac sodium treated animals.

Discussion

The main effect of diclofenac is like all other NSAIDS, to prevent the synthesis of prostaglandin by inhibiting the enzyme cyclooxygenase in the cells of the body. The kidney is extremely active in the synthesis and metabolism of prostaglandin. These compounds participate in several processes in renal physiology including auto-regulation of renal blood flow and glomerular filtration, modulation of renin release, tubular ion transport and water metabolism.[7] It is not surprising that diminished prostaglandin synthesis may be an initiating event in the patho-physiologic process of diclofenac sodium induced renal dysfunction.[8]

After treatment with diclofenac sodium in group-B, the general behaviour of the animals changed to ill, sluggish, and food intake decreased, which may be attributed to loss of appetite due to side effects of diclofenac sodium on GIT. Our findings are in conformity with Beun et al[9] who also observed anorexia in patients receiving diclofenac sodium for arthritis.

A significant decrease in number of tubules per unit area of the kidneys was noticed in group-B animals, which may be attributed to damage to the tubular epithelial cells by ischaemia produced by inhibition of prostaglandin in renal arterioles causing their constriction. These results are in agreement with Gray et al[10] and Clive and Stoff[4] who observed vacuolar degeneration of proximal tubule and focal tubular atrophy in NSAID (indomethacin) with renal failure.

A highly significant increase in the diameter of proximal tubules in group-B animals as compared to group-A was noted, which may be attributed to degeneration of cells in proximal tubules resulting in apparent increase in their diameter. Our findings are in conformity with Scott et al[11] who observed renal tubular cells in urine after ingestion of salicylates and concluded that NSAID (diclofenac sodium) causes transient shedding of renal cells. The damage to renal tubules may be attributed to decrease in blood supply to kidney tissues as the renal vascular tone is determined by autonomous intrinsic activity of the renal arterioles and continuous production of renal prostaglandin which was inhibited by this drug and caused unopposed constriction of arterioles resulting in ischaemia of tubules and epithelial cell death.

The interstitial nephritis was noted in the cortex and medulla of the kidneys in group-B animals. This may be attributed to decrease in cyclooxygenase by NSAID, lead to shunting of arachidonic acid precursor into lipooxygenase pathway, favouring the production of inflammation inducing metabolites of eicosapentaenoic acid which functions as lymphokinase, leading to recruitment of T-lymphocytes and perpetuation of the inflammatory process.

Finally, it may be concluded that diclofenac sodium produces changes in kidney, which may be attributed to ischaemia induced by inhibition of prostaglandin synthesis resulting in tubular necrosis.

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