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EFFECT OF DICLOFENAC SODIUM ON THE BASEMENT MEMBRANE OF KIDNEY TUBULES IN ALBINO RATS

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ABSTRACT

OBJECTIVE: *NSAIDs are widely used analgesics worldwide. Diclofenac Sodium is one of the members of this group. They are the most widely prescribed drugs. As large population is exposed to this drug, its toxic effects are also commonly encountered. GIT toxicities are well known even by the patients, but the physicians as well as patients are unaware of the renal effects of Diclofenac sodium. The objective of this study is to find out the renal effects of Diclofenac sodium after taking the therapeutic dose.*
STUDY DESIGN: *It is an experimental study, which lasted for two weeks.*
MATERIAL AND METHODS: *In this experimental study 16 albino rats were used. They were divided into two groups, A and B. Group A acted as control received normal saline 10ml/kg orally daily, while group B received Diclofenac sodium at therapeutic dose i.e. 2mg/kg body weight orally daily through feeding tube. Animals were sacrificed after the last dose. The kidneys were isolated, fixed and processed for light microscopy. The slides were stained with H&E and silver methamine and were examined for the basement membrane of proximal and distal tubules.*
RESULTS: *In Diclofenac sodium treated group the silver methamine stained sections revealed much thickened basement membrane as compared to non-treated group; the p value is less than 0.000.*
CONCLUSION: *Diclofenac sodium causes the thickening of basement membrane of kidney tubules, thus impairing the renal function.*

KEY WORDS: Diclofenac sodium, Kidney, Basement membrane.

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INTRODUCTION

Non-Steroidal Anti-Inflammatory drugs (NSAIDs) are commonly used analgesics. Nowadays different new NSAIDs have been introduced in last three decades but the use of individual drug varies between countries[1]. They are the most widely prescribed medications worldwide[2]. Although their toxic effects are not increased but as their use has been increased, number of people exposed to these medications is larger.
Diclofenac sodium is also one of the members of NSAIDs. It is a non-selective COX inhibitor[3] having the properties of being analgesic, anti-inflammatory, antirheumatic and antipyretic agent. It has an inhibitory effect on prostaglandin synthesis[4].
It is more potent than indomethacin, naproxen and several other agents[5]. It has been found effective in musculoskeletal disorders such as rheumatoid arthritis, osteoarthritis, ankylosing spondylosis and other painful conditions like dysmenorrhoea. Its preoperative use controls the postoperative pain[6]. In the case of patent ductus arteriosus it has been successfully used to close it[7]. The toxicities of NSAID in general and Diclofenac sodium in particular are well known. Gastrointestinal tract is the first target of its toxicities while kidney is the 2nd most important organ for untoward effects. The unwanted renal effects include renal failure[8], chronic renal injury, nephrotic syndrome[9], interstitial nephritis and abnormalities of water metabolism and sodium and potassium homeostasis. Damage to kidney tubules at ultrastructural level has also been shown experimentally[10].

MATERIAL AND METHODS

This study was carried out in Basic Medical Sciences Institute (BMSI) of Jinnah Postgraduate Medical Centre Karachi. 16 albino rats, weighing 190-200 gm were used in this study. They were originally obtained from Massachusetts, USA and cross bred in the animal

house of BMSI.

They were divided in two groups, comprising 8 animals in each group. Rats in group- A received normal physiological saline 10 ml/kg body weight orally daily through feeding tube. The rats in the group-B received Diclofenac sodium at therapeutic dose i.e. 2mg/kg/day[11] orally through feeding tube for 14 days.

Twenty four hours after the last dose the rats were sacrificed by the overdose of ether anesthesia in a glass jar. The animals were dissected through midline abdominal incision. The kidneys were identified and examined in situ for an obvious change in size, shape, color, contour and consistency. They were bisected into two halves one half fixed in 10% formalin and other in alcoholic formalin. Tissue samples were processed through graded alcohol and xylene and embedded in paraffin block. Tissue sections of 4µm were made at multiple levels.

Tissue sections were stained with haematoxylin and eosin (H&E), and silver methamine[12] stains. Mounted slides were then examined under low power (10x) and high power (40x) objectives.

H&E stained sections were examined for the general architecture of the cortex and medulla while silver methmine stained sections were examined for any change in the basement membrane. The changes in the thickness of basement membrane were graded as 0 no change, +, ++, +++ for mild, moderate and intense thickening of basement membrane respectively. The results were subjected to statistical analysis. Mann Whitney test was applied to see the significance of results and P value was calculated. The level of significance was kept below 0.05.

TABLE-1

Thickness of Basement membrane in control and experimental animals

Animal No.	Group A (Control)	Group B (Diclofenac Sodium)
1	0	+
2	0	++
3	0	+
4	0	+++
5	0	+++
6	0	++
7	0	+
8	0	++

Mann Whitney Test

Statistical Analysis

	GROUP	N	Mean Rank	Sum of Ranks
DATA	Control	8	4.50	36.00
	Total	8	12.50	100.00
	Experimental	16		

Exact Sig. 0.000	P. value Highly significant

RESULTS

The animals in both groups remained alive during experimental period. The kidneys in both A and B groups appeared oval in shape dark in color and soft in consistency with smooth shining surface, having delicate fibrous capsule that stripped off with ease. The cut surfaces showed normal thickness of the cortex.

Microscopic Observation

The kidneys were observed with the help of light microscope. The morphological observations were based on the study of tissue sections 4µm thick, stained with H&E, and silver methamine stains.

Group A

The kidney tissue in this group revealed no significant deviation from the normal histological structure.

Under low power: In H&E stained sections the cortical and medullary portion appeared absolutely normal without any change either in golmeruli or tubules. Brush border appeared as lining on the apical surface of proximal tubular cells almost filling the lumen of the tubule. Silver methamine stained sections revealed basement membrane of golmeruli, Bowman's capsule, proximal and distal tubules. It was indistinct.

Under high power: In H&E stained sections the glomeruli, Bowman's capsule and tubules appeared quite normal. In silver methamin stained sections the basement membrane was stained as faint outline which was un-measureable by light microscopy (Fig-1). In all the animals the thickness of basement membrane was graded as 0.

Group B

The histological appearance of cortex and the medullary architecture was intact under (H&E) stain.

Under low power: In H&E stained sections both the cortex and medulla appeared normal. There were no remarkable changes in glomeruli, Bowman's capsule, proximal and distal tubules. The silver methamin stained sections revealed basement membrane of proximal and distal tubules.

Under high power: The H&E stained sections revealed no any remarkable change in glomeruli, Bowman's capsule, proximal and distal tubules. In silver methamine stained sections the basement membrane of proximal and distal tubules was visible as intensely stained thick black line around proximal and distal tubules but still unmeasureable by light microscopy (Fig-2).

To apply the Mann Whitney test the thickness of membrane was assigned as 0 for normal thickness, and 1, 2 and 3 for +, ++, and +++ respectively. In three animals it was 1, in three animals it was 2 and in two animals the thickness of membrane was 3 (Table-1). The P-value came to be 0.00 which is highly significant.

DISCUSSION

The common ability of NSAIDs is to inhibit cyclooxygenase, a major enzyme in the biosynthesis of all prostaglandins. Prostaglandins are ubiquitous in their distribution throughout the body, and they function for the most part as "local

hormones". Their biological activity is exerted primarily at the site of their synthesis, since they have a short half life in the circulation. Further more they are probably not stored but rather synthesized immediately before release – a process initiated by the liberation of arachidonic acid from the phospholipids pool of cell membranes[13].

The kidneys are extremely active in the regulation and metabolism of prostaglandin. These compounds participate in several processes in renal physiology including auto regulation of blood flow, glomerular filtration and urinary excretion of electrolytes and water[14].

Prostaglandins are potent vasodilators, they are vital to the maintenance of resting blood flow to the kidney and act as determinant of intra renal distribution of blood flow[15]. NSAIDs are potentially nephrotoxic and their effects include salt and water retention, acute tubular necrosis, acute interstitial nephritis with heavy proteinuria and chronic renal failure.

In this study we found comparable thickening of basement membrane in group B. This thickening of basement membrane in group B treated with Diclofenac sodium may be attributed to decrease synthesis of prostaglandin in renal tubular cells. Prostaglandin E_2 increases the metalloproteinase 2 activities, metalloproteinase is an enzyme that degrades the basement membrane and inhibits its excessive deposition. When prostaglandins are inhibited by NSAID there is decrease activity of this enzyme and therefore excessive deposition of basement membrane. Our results are in conformity with Singhal[16] who also observed decrease metalloproteinase activity with the treatment of NSAID.

Our results are also comparable with dixit mp who reported a case of acute tubule-interstitial nephritis (ATIN) in which he found deposits in tubular basement membrane in a child after receiving the therapeutic dose of NSAID[17].

CONCLUSION

In conclusion Diclofenac sodium induces excessive basement membrane deposition in kidney tubules. Nephrotoxicity of Diclofenac is well known but effects on tubular basement membrane are not studied extensively. Currently contribution of metalloproteinase in the presence of Diclofenac Sodium in human is simply not known. Where's some other factors may be worth studying as cause of human toxicity. Further study is required to determine the exact mechanism and individual variation in response to drug.

Fig – 1

4 µm thick Silver Methamine stained section of rat kidney from group-1, showing very thin and regular basement membrane in proximal and distal convoluted tubules.

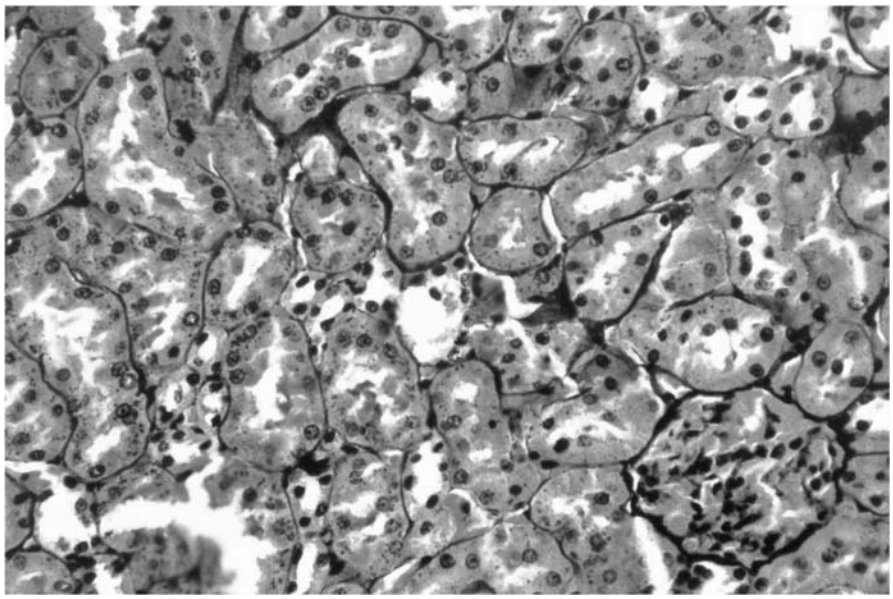
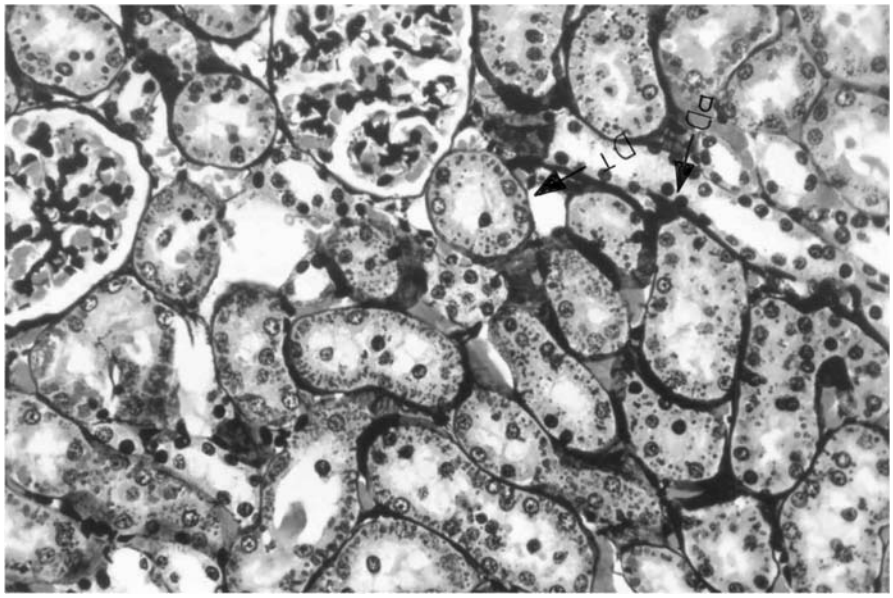


Fig – 2

4 µm thick Silver Methamine stained section of rat kidney from group –B treated with Diclofenac sodium showing thick basement membrane in proximal and distal convoluted tubules.



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