Original Article

Anatomy

Alterations in Mitochondria of Kidney Tubules by Different Doses of Diclofenac Sodium in Rabbits

1. Talat Yasmeen 2. Irfan Ashraf Siddiqui 3. Zaheer Amjad 4. Amir Ali Shoro 5. Talat Mirza

1. Prof. of Anatomy, JSMU, Karachi 2. Asstt. Prof. of Anatomy, JSMU, Karachi 3. Senior Demonstrator Anatomy, DUHS, Karachi 4. Prof. of Anatomy, LNMC, Karachi 5. Prof. of Pathology, DUHS, Karachi

ABSTRACT

Introduction: Diclofenac sodium, a Non-Steroidal anti-inflammatory agent (NSAIDs), is being prescribed since many decades for the treatment of rheumatic diseases as well as for the relief of pain and fever. It is the inhibitor of enzyme cyclooxygenase. Unfortunately its use is often accompanied by gastro-intestinal renal and hepatic side effects. Renal dysfunction is characterized from acute renal failure to chronic injury. We herein report the damaging effect of diclofenac sodium on the ultrastructure of PCT of rabbit kidney by increasing the doses above the recommended.

Study Design: Experimental Study

Place and Duration of Study: This study was conducted at the Institute of Basic Medical Sciences (IBMS), Dow University of Health Sciences from March 2009 to March 2010.

Material and methods: In this study 88 male albino rats were selected, they were divided into 4 groups group A received normal saline 2 ml/kg, group B diclofenac sodium 2mg/kg body weight group C 4 mg/kg and group D 6 mg/kg for two weeks. At the end of experiment animals were sacrificed, dissected, kidneys were identified, fixed in 4% gluterldehyde than 1% osmium tetraoxide and passed through graded alcohols, infiltrated and embedded in resins. Semithin 3~4 µm sections were stained with toluidine, ultrathin (1µm) with uranyle acetate. Tissue sections were observed under transmission electron microscope. Tissue changes were graded as 0, +, ++ and +++, no change, mild moderate and severe changes respectively. The results were then analyzed statistically.

Results: There were non-significant changes in the cell organelles of PCT in group A and B, while significant changes were observed in group C and highly significant in group D.

Conclusion: Diclofenac sodium has damaging effect on the mitochondria of PCT cells far before the light microscopic changes. So its use should be restricted only in very painful conditions. Secondly in case of prolong treatment follow up with regular renal function test should be carried out.

Key Words: Diclofenac sodium, kidney, ultrastructure.

INTRODUCTION

NSAIDs (Non-Steroidal Anti-Inflammatory Drugs) are very commonly prescribed drugs. During the past thirty years there has been a substantial increase in the number of NSAIDs, but their availability varies markedly between countries[1.] These drugs when grouped by generic categories they are the most widely prescribed of all medications[2]. As large population is exposed to these compounds their adverse effects are also rapidly expanding.

Diclofenac sodium, a commonly prescribed analgesic also belongs to the group non-steroidal anti-inflammatory drugs (NSAID). It is a non-selective COX inhibitor[3] and probably one of the most common non-steroidal compounds with analgesic, anti-inflammatory, anti-rheumatic and antipyretic properties with inhibitory effect on prostaglandin synthesis[4].

Its potency is substantially greater than that of indomethacin, naproxen and several other agents[5]. Chief clinical application of Diclofenac sodium is an anti-inflammatory agent in the treatment of musculoskeletal disorders such as rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, and in dysmenorrhoea. It is also found effective for the control of postoperative pain when used preoperatively[6], pre-operative rectal diclofenac is found to delay the onset of post-operative pain and is adequate as an analgesic for early post-operative period[7].It has also been used in neonates to close the ductus arteriosus when it has remained patent[8].

The gastrointestinal toxicities are well known, while kidney is also an important site for untoward effects. In fact these agents have been known to produce a wide array of untoward renal effects that may be divided into several distinct nephrologic syndromes, including acute renal failure[9], chronic renal injury, nephrotic syndrome[10], interstitial nephritis, abnormalities of water metabolism, and abnormalities of sodium and potassium homeostasis. Renal papillary necrosis and nephritic syndrome have been reported in many patients taking Diclofenac sodium[9]. Experimental studies have also shown damage to kidney tubules at ultrastructural level[11]. Hepatotoxicity though uncommon but when occurs it is lethal.

MATERIALS AND METHODS

For this experimental study 88 male rabbits were taken from the Institute of Basic Medical Sciences (IBMS), Dow University of Health Sciences. They were about three months of age and 900-1000 gm. in weight. They were divided into four groups A, B, C, and D each comprising of 22 animals.

The animals were weighed on single pan triple beam balance on day-1 and day-14 of the experimental period. Group A served as control receiving normal saline 2ml intraperitoneally/daily, group B received diclofenac sodium (injection Voren) in the dose of 2 mg/kg body weight daily, group C received diclofenac sodium in the dose of 4mg/kg body weight daily (double the therapeutic dose), and the group D received diclofenac sodium 6 mg/kg/daily (three times the therapeutic dose), given intraperitoneally. These injections were given for 14 days. On day 15 the animals were sacrificed by a blow on head.

The kidneys were dissected out and divided into small pieces with the help of a sharp knife, and were fixed in 4% gluterldehyde for electron microscopy[12].

Tissue Processing for Electron Microscopy

All the tissue processing was done under a fume hood. Pieces of kidney were then chopped to 1 mm^3 pieces again transferred to the same fixative for further 24 hours for proper infiltration of the fixative.

a) The tissue pieces were washed with Sorensen phosphate buffer for ten minutes then post-fixed in osmium tetra oxide at 4 ºC for one hour. They were dehydrated in alcohols and infiltrated with half resin and half acetone at 37 ºC for one hour and embedded in full resin.

b) Semi-thin sections 3~4 µm were cut and stained with toluidine blue for general orientation of the tissue. After areas of interest were identified thin sections 1 µm (100 nm) stained with uranyle acetate and lead citrate were examined under transmission electron microscope (TEM Leica, Germany).

c) Tissue changes were graded as 0 no change, +, ++, and +++ as mild, moderate and sever changes respectively. The results were then analyzed statistically. P value less than 0.05 was taken as statistically significant. SPSS version 17 was used or the analysis of data.

RESULTS

The gross examination of the kidney in the group A, B, C, and D showed normal appearance. The kidney in group A appeared oval in shape, dark red in colour, soft in consistency with smooth shiny surface having delicate fibrous capsule that stripped off with ease. The kidneys of animals in groups B, C, and D were appeared normal, somewhat oval in shape with smooth looking contour, extra soft in consistency and capsule stripped with ease. The cut surface showed normal thickness of the cortex.

Electron Microscopic Observation: The kidney sections from animals of all four groups which were processed for electron microscopy and were mounted on grids; were examined under E/M. The following observations were recorded.

Group A: In group A animals the ultrastructure of kidney tubules revealed normal morphology. The plasmalemma at the microvillar brush border showed normal hazy appearance. In the cytoplasm the cell organelles appeared normal.

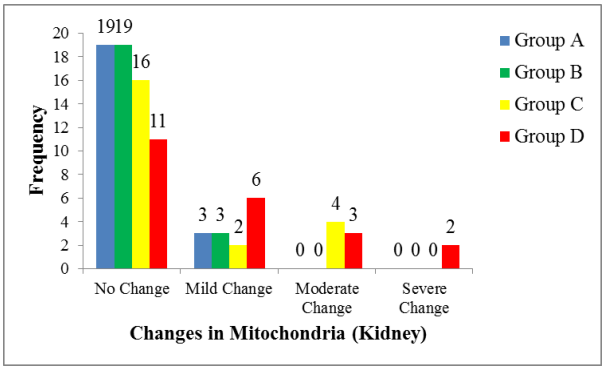
The mitochondria were elongated and rod shape, they were scattered all over the cell but gathered more near the base of the cell. Here they were arranged parallel to the long axis of cell in the basal infolding of plasmalemma. Both the inner and outer mitochondrial membranes were distinctly visible with densities in matrix; while most of the cristae were arranged transversely (Fig-1). The mean value of changes in this group was 0.14 ± 0.34 (Table-1).

Table A.1: Changes in Mitochondria of PCTs

Animal No.	Group A	Group B	Group C	Group D
1	0	0	0	1
2	0	0	0	0
3	1	0	1	2
4	0	0	0	0
5	0	0	2	3
6	0	0	0	0
7	0	1	0	2
8	0	0	0	0
9	0	0	0	1
10	0	0	1	0
11	0	0	0	0
12	1	0	0	1
13	0	0	0	0
14	0	0	2	3
15	0	1	0	1
16	0	0	0	1
17	0	0	2	0
18	1	0	0	0
19	0	0	0	0
20	0	1	0	1
21	0	0	0	2
22	0	0	2	0
Mean	0.14	0.14	0.45	0.82
Std Dev	0.34	0.34	0.78	0.98

Table A.2: Post Hoc Tests – Changes in Mitochondria of PCTs – Multiple Comparisons

	Group B	Group C	Group D
Group A	1.000	0.695	0.033
Group B		0.695	0.033
Group C			0.615



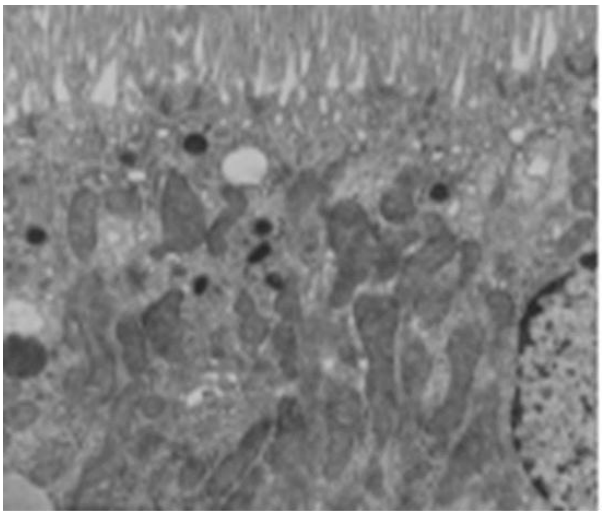


Figure 1: Electron micrograph of the rabbit kidney treated with normal saline (group A) showing a PCT cell with normal brush border, nucleus and other cell organelles x 6000

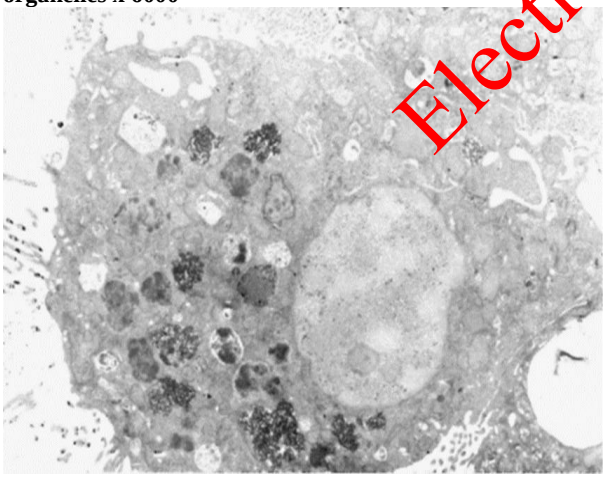


Figure 2: Electron micrograph of the rabbit kidney treated with Diclofenac sodium 6 mg/kg (group D) showing damaged mitochondria and brush border in a PCT cell x 6000

Group B: In group B animals the proximal tubular cells were almost normal under E/M. in cellular details the plasma membrane was intact and clearly visible, the cytoplasmic organelles were also studied and they showed mild to moderate change. Most of the mitochondria were elongated they were very well visualized along the basal part of the cells. They were arranged parallel within the basal infolding of plasmalemma of proximal tubular cells. Some of the mitochondria were swollen and in these mitochondria the outer membrane was not properly visualized. Mean value of the changes in group B was 0.14 ± 0.34.

Group C: The cells in proximal convoluted tubules showed many degenerative changes. Although the plasma membrane was normal bilaminar; the changes were clearly observable in other cell organelles. Mitochondria were swollen in many of the proximal tubular cells. Inner membrane was indistinct, and the cristae were short. In some of the mitochondria cristae structure was lost and it appeared as if mitochondria are filled with flocculent densities. . Mean value of the changes in group C was 0.45 ± 0.78.

Group D: The tubular lining cells showed significant ultrastructural changes. However the proximal convoluted tubules were more affected. Plasmalemma at the basal surface of the cells was almost normal while the apical surface i.e. the brush border revealed some loss.

There was marked swelling of the mitochondria in many of the proximal convoluted tubular cells. In some of the mitochondria the outer membrane was ruptured, there was loss of the cristae in inner membrane while increased densities inside the matrix were noted (Fig-2). Mean value of the changes in group D was 0.82 ± 0.98 (Table-1).

Statistically non-significant changes were observed in the mitochondria of cells in PCT in group A animals, while in groups B and C these changes were significant. Statistically highly significant changes were observed in group D ($p < 0.05$).

DISCUSSION

There are numerous reports describing various morphological and biochemical changes that occur in the kidney following administration of diclofenac sodium[12,13,14,15]. In this report an attempt has been made to study injury to cell organelle specially mitochondria of PCT under the influence of diclofenac sodium. We have tried to correlate the extent of injury with the dose of the drug. The extent of injury with the dose of the drug was also calculated statistically. An attempt has also been made to understand the mechanism of nephrotoxicity.

Rabbits treated with 2 mg/kg diclofenac sodium showed no any remarkable change in the mitochondria of cells in PCT. While those treated with higher doses i.e. 4mg/kg and 6 mg/kg body weight showed mild to severe changes. Nephrotoxicity was statistically significant in rabbits getting the diclofenac sodium 6 mg/kg.

The swelling and rupture of mitochondrial membrane is due to a phenomenon called mitochondrial permeability transition (MPT). In this phenomenon the permeability of mitochondrial membrane is increased to solutes of molecular weight less than 1500 Da. This MPT is formed at the contact sites between the inner and outer mitochondrial membrane. It results in depolarization and there is equilibrium of solutes between cytoplasm and mitochondrial matrix. This ultimately leads to the rupture of mitochondrial membranes.

Many researchers have identified the ability of diclofenac sodium to induce apoptosis and it was found that mitochondria play an important role in this mechanism[16,17]. At therapeutic dose it causes swelling of mitochondria but as the dose is increased there is rupture of mitochondrial membrane. The changes in this study are comparable with that found by Kretz Romel and Boelsterli[18]. They suggested that changes in mitochondria are dose dependent.

The E/M observations are essentially in agreement with those of Hickey EJ[19] also who proved that Diclofenac targets the mitochondria of renal proximal tubules. But he proposes the mechanism in which there is oxidative stress and massive DNA fragmentation.

The present results may have both toxicological and bioenergetic implications. First they show the potential of diclofenac sodium for toxicity in renal PCT mitochondria and secondly this drug is potential inducer of MMPT prevention. In this regard studies are necessary to evaluate the sensitivity to MMPT following diclofenac sodium exposure in vivo[20].

CONCLUSION

Diclofenac sodium has damaging effect on the mitochondria of PCT cells far before the light microscopic changes. So its use should be restricted only in very painful conditions. Secondly in case of prolong treatment follow up with regular renal function test should be carried out.

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Address for Corresponding Author:
Prof. Dr. Talat Mirza
Professor of Pathology
Dow International Medical College (DUHS),
Karachi.
E-Mail: talat.mirza@duhs.edu.pk