ORIGINAL ARTICLE

Morphological Study of Lead Induced Nephrotoxicity with Role of Zinc in Albino Rats

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ABSTRACT

Introduction: Damage to the kidneys is one of the primary toxic actions of the metals and nephrotoxicity by heavy metals has been the focus of much research. Lead is a heavy metal widely distributed in the environment. Its toxicity is a matter of concern as very low levels in the environment are found to effect under-nourished population. Entering the body through multiple routes it gets distributed in almost every organ including kidneys altering its structure and functions.

Objective: Heavy metals such as Lead are known to interact with the essential trace elements at the level of absorption and also during metabolism. The present study was designed to observe morphological changes in renal tissue with special reference to Proximal tubules following concomitant administration of essential micronutrient zinc with lead.

Design: Experimental study.

Materials & Methods: 45 young adult albino rats selected for the study were distributed into 3 main groups of 15 rats each. Group A served as control, Group B rats received Inj. Lead acetate 8 mg/kg intraperitoneally daily and Group C in addition to lead received Inj. Zinc chloride 0.21 mg/kg intraperitoneally daily. Each group was further subdivided into three sub-groups according to the period of treatment given i.e. 2,4 & 6 weeks, at the end of which animals were sacrificed. The kidneys after processing and staining (PAS-Haematoxylin) were subjected to detailed morphological examination of proximal tubules.

Results: The morphologic study in lead treated subgroups revealed changes indicating progressive distortion of renal cortical tissue with increasing time periods so that at six weeks a number of necrotic tubules with pyknotic nuclei were seen. Histological picture was close to that of Control and showed minimum distortion in rats co treated with zinc.

Conclusion: Based on the study, it can be stated that lead induced nephrotoxicity particularly damages the structure of proximal tubules and the damage is more pronounced with increasing time period. Concomitant treatment with essential micronutrient zinc reduces or delays the toxic effects of lead.

Key words: Heavy Metal, Proximal tubules, Nephrotoxicity, per oxidative damage, anti oxidant

INTRODUCTION

Over the last few decades, it has become increasingly obvious that kidney is adversely affected by an array of chemicals. Man is exposed to these nephrotoxic agents as medicines, industrial and environmental chemicals, and a variety of naturally occurring substances.[1]

Environmental chemicals such as lead are capable of inducing nephrotoxicity.[1] Many studies show a strong association between lead exposure and renal effects. [2-4] Proximal renal tubular cells are particularly vulnerable to the toxic action of chemicals; owing to their high energy demand such as re-absorptive and secretory functions.[1]Dose-related proximal tubular dysfunction has been observed in rats exposed to

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lead by researchers.[5,6] Lead is highly toxic and persistent in the environment and thus, a major concern for public health.[7]

Exposure to Lead can occur from multitude of sources. Lead used as an anti-knock during the manufacturing of gasoline is the main source of Tetraethyl lead, the end product found in the emission of vehicles getting into air.[8] These lead isotopes are very stable and do not decay for millions of years [9] contaminating air, water, soil and food.[10] Although lead has been removed from gasoline in western countries, leaded gasoline continues to be used in developing countries.[11] Exposure to lead may also occur from poorly controlled industrial emissions at metal refineries & battery recycling plants and demolition of old houses. Products that contain lead include pipes, solders, electric cables, paint, ceramics and ayurvedic medicines.[9]

Exposure to lead enhances per oxidation[12] of membrane phospholipids, accompanied by a concomitant decrease in the activity of antioxidant enzymes, such as superoxide dismutase[12] and glutathione reductase.[4,13] Superoxide dismutase is an enzyme responsible for detoxification of highly reactive and potentially toxic free radicals to less toxic hydrogen peroxide. The results of a study[4] indicated an inhibition of SOD in kidneys of lead treated animals. Lead accumulates in the mitochondria and causes both structural and functional alterations by inhibiting respiratory function and energy (adenosine triphosphate) production.[11]

Zinc is an essential element and a micronutrient with antioxidant effects, it plays a biochemical role in stabilizing membrane structure and thus reducing per oxidative damage to sulfhydryl group. [14] Moreover S.O.D which forms a major protective system against free radical injury is a zinc dependent enzyme.[15] In one study, co treatment with zinc greatly reduced mercury induced renal toxicity.[16] Zinc supplementation can effectively compete for and reduce the availability of binding sites for lead uptake.[17]

MATERIALS& METHODS

The study was conducted in the Department of Anatomy, Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre (JPMC) Karachi. A total of 45 active, young adult Albino rats with a fully developed urinary system were obtained from the Animal House of J.P.M.C.

The animals at the onset of experimental study, were divided into three groups comprising 15 rats; each group was further sub-divided into three sub-groups according to the period of treatment they received, i.e. 2 weeks, 4 weeks and 6 weeks; each subgroup comprised of 5 animals.

Ø Group-A rats with subgroups A1, A2, A3 served as control ,received injection normal saline 1 ml intra-peritoneally daily for 2,4 and 6 weeks respectively.

Ø Group-B rats with subgroups B1, B2, B3 received Injection Lead Acetate (Merck, Germany) at a dose of 8 mg/kg body weight intra-peritoneally daily for 2,4 and 6 weeks respectively

Ø Group-C rats with subgroups C1, C2, C3 received Injection Zinc Chloride (Merck, Germany) at a dose of 0.21 mg/kg body weight intra-peritoneally two hours before administration of Lead Acetate at a dose of 8 mg/kg body weight intra-peritoneally, daily for 2,4 and 6 weeks respectively.

Throughout the period of experimental study the animals were kept under observation to note any change in their general condition, behavior and their activities.

The animals were sacrificed under ether anesthesia at the end of experimental period (fig.1).

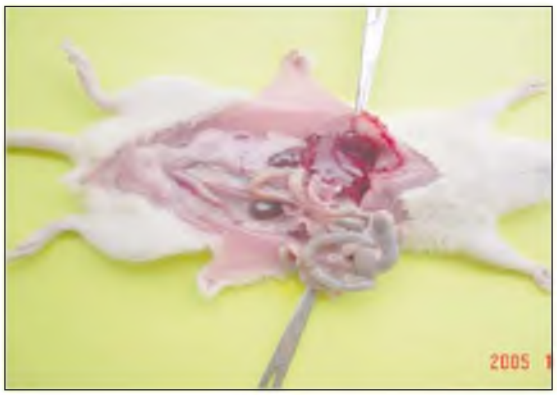

Figure – 1 : A photograph of opened and exposed abdominal cavity of group-A rat showing kidneys before excision

The abdominal cavity was opened by giving a midline incision and kidneys were dissected out, cut into two longitudinal halves and were fixed in Alcoholic Formalin for 24 hours. Later they were processed in ascending strengths of alcohol, cleared in xylene, infiltered and embedded in paraffin .After this process sectioning was performed in order to obtain 5μ thick longitudinal sections with the help of a rotatory microtome, which were then mounted on albumenized glass slides. The slides were stained with Periodic Schiff haematoxylin technique in order to visualize not only the cytoplasm and nuclei of the cells but also the structures containing a high proportion of macromolecules i.e the brush border and basement membrane. The morphological study of proximal tubules was done in detail under light microscope using 8x ocular and 100 x oil immersion objectives.

OBSERERVATIONS & RESULTS

The morphological examination of Periodic Schiff-Haematoxylin stained sections of kidneys belonging to Control groups-A1, A2 & A3 revealed a normal intact cortical and medullary architecture. The proximal tubules were closely packed and appeared circular, oval or elliptical in sections & were mostly confined to the cortex particularly in the vicinity of glomeruli .The cells appeared low, columnar with fine and granular cytoplasm. They were regularly arranged on an intact and well defined basement membrane. The spherical nuclei located centrally or towards the basal portion of the cell presented fine and evenly distributed chromatin .The luminal surface of these cells presented a distinct and regular brush border. The lumina of the tubules were devoid of any cellular or nuclear debris. The interstitium in the cortical area showed no signs of acute or chronic inflammatory cell infiltration. (fig.2).

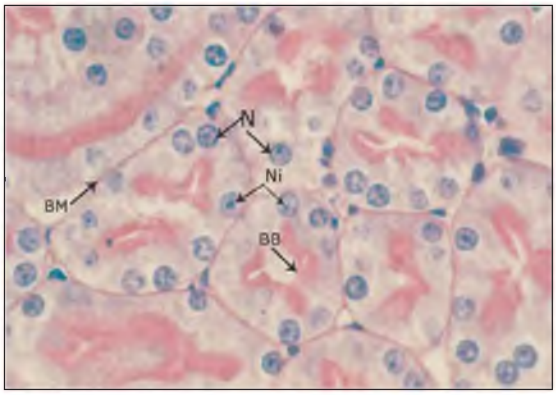

Figure – 2 : PAS-Haematoxylin stained, 5 μm thick, longitudinal section of kidney from group-A (control) rat showing proximal tubules with intact brush border (BB), basement membrane (BM), Nuclei (N).

The histological examination of lead treated rats revealed an irregular cortical architecture which was found highly distorted at 6 weeks in subgroup B3.The proximal tubules mostly confined to the cortex appeared dilated in all the lead treated subgroups(fig.'s 3&4) and this dilatation was accompanied by severe sloughing and degeneration in B3 (fig.5).

In subgroup B1(2 weeks lead treated) the lining cells of many tubules appeared enlarged while in B2(4 weeks lead treated) the enlarged cells were mostly vacuolated obscuring cytoplasmic details (fig.4) .In

subgroup B3 in addition to large vacuolated cells many cells appeared almost flattened (fig. 5).

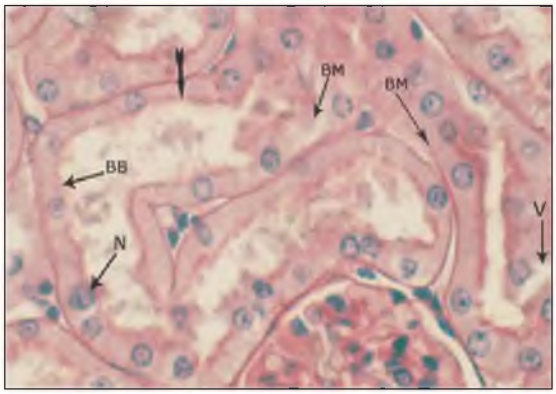

Figure – 3 : PAS-Haematoxylin stained, 5 µm thick, longitudinal section of kidney from group-B1(2 weeks lead treated) rat showing dilated proximal tubules with scanty brush border (BB), distorted basement membrane (BM), enlarged and displaced nuclei (N), and cytoplasmic vacuole (V).

The nuclei in many proximal tubular cells appeared enlarged and displaced from usual basal or central portion of cell. These enlarged nuclei (fig. 4) were more numerous in subgroup B2 which often presented a condensed and clumped chromatin. In subgroup B3 most of nuclei appeared pyknotic with condensed and darkly stained chromatin (fig.5). In some of the cells no nuclei were seen.

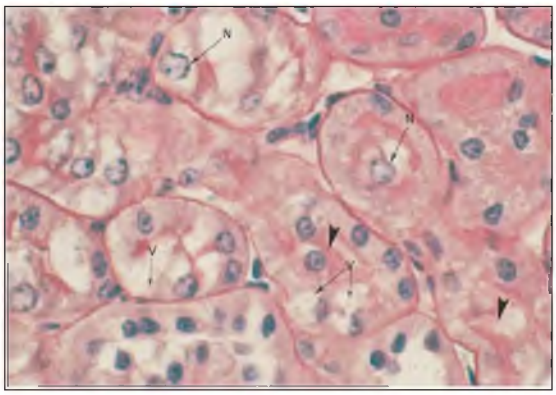

Figure – 4 : PAS-Haematoxylin stained, 5 µm thick, longitudinal section of kidney from group-B2 (4 weeks lead treated) rat showing enlarged nuclei (N), vacuolated cytoplasm (V), and epithelial debris (↑) in lumen.

The brush border on apical surfaces was found scanty at 2 weeks of treatment (fig.3) with few areas of complete loss at 4 weeks in B2. In subgroup B3 the distorted apical surface of cells presented almost a complete loss of brush border (fig. 5)

The underlying basement membrane was found disorganized in initial periods of treatment; few areas of distortion were seen in subgroup B2 while in subgroup B3 basement membrane was completely distorted in a number of tubules (fig.3-5).

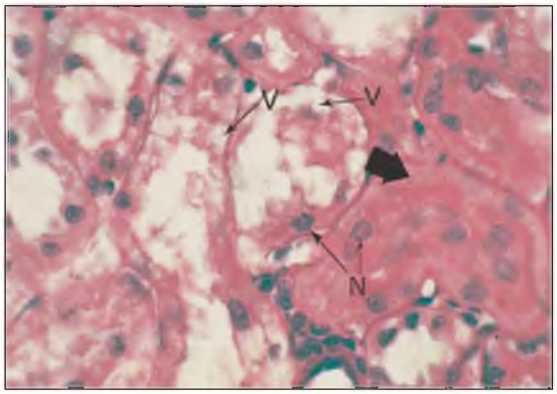

Figure – 5 : PAS-Haematoxylin stained, 5 µm thick, longitudinal section of kidney from group-B3 (6 weeks lead treated) rat showing necrotic tubules (↑) with pyknotic nuclei (N), severe vacuolar degeneration (V),distorted brush border and basement membrane,mononuclear infilteration .

The Lumina of tubules showed cast off materials in all the lead treated subgroups especially at 4 and 6 weeks in B2 & 3 (fig. 4) the epithelial and nuclear debris were found in numerous tubules. The interstitium surrounding the distorted tubules showed large areas of mononuclear cell infiltration (fig. 5). In 6 weeks lead treated subgroup B3 (fig.5) in addition to the tubules described above, groups of proximal tubules showing obvious necrotic changes were seen in the cortical region. They showed shrinkage in size and appeared as a homogeneous, glassy, eosinophilic material lying within the basement membrane. The cell boundaries were not recognizable and lumina were obscured. There were few pyknotic and irregularly arranged nuclei. Few tubules of same

features were also found scattered in renal cortices of subgroup B3.

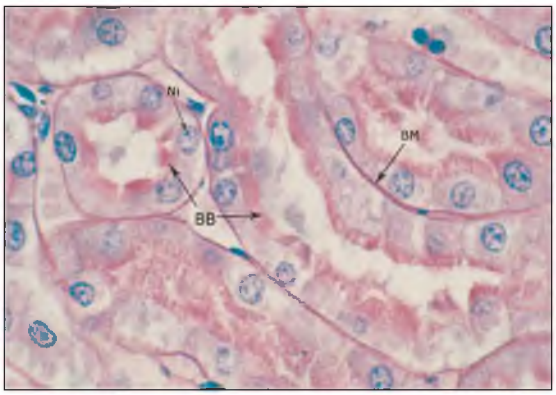

Figure – 6: PAS-Haematoxylin stained, 5 µm thick longitudinal section of kidney from group-C3 (6 weekslead + zinc treated) rat showing proximal tubules with regular basement membrane (BM) brush border (BB), nucleus (Ni) and occasional cytoplasmic vacuoles. Photomicrograph x1000

The study of the proximal tubules in subgroups C1& C2 (2&4 weeks lead+zinc treated respectively) showed a picture better than the corresponding lead treated subgroups. Few tubules showed epithelial casts in later weeks of treatment with lead + zinc. The study of sections belonging to subgroup C3 also showed a better picture than corresponding B3 as there were occasional cells with vacuolated cytoplasm and nuclei with darkly stained, clumped chromatin. The basement membrane and brush border were comparatively regular (fig. 6). Only a few tubules with scanty brush border were also seen.

DISCUSSION

Several chemicals both therapeutic and non-therapeutic have toxic effects on one or more anatomical elements of kidney. Proximal renal tubular cells are particularly vulnerable to the toxic action of chemicals.[1]

Environmental chemical such as lead is capable of inducing nephrotoxicity.[1] The kidneys are particularly exposed to the untoward toxic effects of lead as they form its major route of excretion.[3]

With increasing concerns about environmental pollution, the interaction of micro-nutrients with toxic metals is of great interest.[15] Zinc has been proved to have a protective role against the nephrotoxic action of many agents. In 2002, a study [16] concluded that co-treatment with zinc protected against mercury induced renal toxicity in mice.

It was thought worthwhile to carry out a study using experimental induction of nephrotoxicity in albino rats by administration of lead. Moreover attempts have been made to study the protective effects of concomitant administration of zinc on lead induced nephrotoxicity on different groups of animals at different time period. In that respect a detailed morphological examination of proximal tubular cells was done under light microscope in Periodic Schiff stain.

In the present study lead was used in a dose of 8 mg/kg body weight, while zinc was used in a dose of 0.21 mg/kg body weight. Both the lead acetate and zinc chloride were administered to the animals by means of intraperitoneal injections to allow accurately calculated doses of solutions to be administered to the animals and to enable uniform absorption.

The morphological examination of renal cortical tissue in lead treated group-B showed dilated proximal tubules as compared to the corresponding control groups. This could be attributed to inflammatory changes following lead exposure.[6]

The lining epithelial cells in the majority of tubules showed enlargement due to vacuolar degeneration of cytoplasm [2] which obscured cytoplasmic details and shifted the nuclei to atypical positions. Lead accumulation in mitochondria causes them to swell resulting in decreased activity of Na-pump & increased influx of Ca^{++}, H_2O and Na^{+} and an efflux of K^{+} causing cellular swelling & loss of microvilli. This results in scanty & indistinct brush border on the luminal surfaces of cells in all the lead treated subgroups. After 6 weeks of lead treatment there

was almost complete loss of brush border so that dilated tubules with flattened epithelia were observed. The basement membrane underlying the proximal tubular cells was found disrupted and discontinuous from the beginning of experimental period which can be correlated to the previous work [15] with disorganized basement membrane in the seminiferous tubules of rats treated with lead acetate. This can be attributed to decreased ATP which results in disruption of protein synthetic apparatus due to swelling of endoplasmic reticulum & detachment of ribosomes.[18]

There were enlarged nuclei with progressive clumping of chromatin in variable periods of treatment in group-B. The finding is in agreement with previous studies [5-6] and may be attributed to the presence of lead induced nuclear inclusion bodies and pseudo-inclusions or nuclear invagination of cytoplasmic contents.[11] The decrease in cellular ATP causes an increased rate of anaerobic glycolysis, which results in accumulation of lactic acid, reducing the cellular pH, which in turn causes clumping of nuclear chromatin.

In our study lead treated subgroups receiving lead for 6 weeks showed irregular & pyknotic nuclei, in contrast with the study [5] in which enlarged nuclei were observed even after two months treatment with lead acetate. However, pyknotic nuclei were seen in proximal tubular cells after exposure with other heavy metals such as cisplatin[19]at earlier stages of treatment. Presence of irregularly shaped pyknotic nuclei in our observations might be related to the degenerative changes found in renal tubular cells with continued lead treatment.

In such groups we also observed tubules showing shrinkage in size, with necrotic changes. Such changes were also described by other researchers[11] according to whom areas of dilated tubules alternate with necrotic & atrophic tubules.

The presence of sloughed material and epithelial casts and nuclear debris in the lumina of tubules in lead treated group-B indicates loss of cytoplasmic contents and nuclei from the distorted apical surface of the tubules which also explains the absence of nuclei in many cells.

The morphological examination of the renal cortical tissue in lead plus zinc treated group-C revealed a picture that was comparable to control group-A. The histology of the tubular cells with their apical and basal surfaces, their nuclei were all comparable to their corresponding controls, except occasional vacuoles observed in the cytoplasm of a few cells of subgroup C3. Moreover the tubules with obvious necrotic changes were not seen in group-C, as were found in late periods of treatment of group-B.

On the whole these results strongly suggest that the anti-oxidant role of zinc helps to ameliorate the effects of lead induced nephrotoxicity.

Several explanations have been clearly proposed to account for this effect of zinc in animals. Zinc supplementation could significantly compete for and effectively reduce the availability of binding sites for lead uptake.[17] Zinc has been clearly shown to have an anti-oxidant role and it acts by protecting the sulfhydryl groups against oxidation and prevent the production of hydrogen peroxide and super oxide radicals by transition metals.[20] Pharmacological doses of zinc by altering various biochemical pathways can induce proteins and enzymes, affect the metabolism of other metals and stabilize cellular membranes[20]

CONCLUSION

In the present study it was observed that administration of lead severely damages the proximal tubular cells indicating its nephrotoxic effects on renal cortical tissue. The concomitant administration of anti-oxidant zinc protects the proximal tubules by reducing or delaying the toxic effects of lead.

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