Original Article

The Role of L- Arginine in Lithium Induced Nephrotoxicity in Albino Rats a Morphological Study

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ABSTRACT

Objective: To observe the protective role of L-Arginine on kidney from toxic effects of Lithium carbonate in Albino rats.

Design: A prospective experimental study.

Place and duration of Study: The study was conducted at Department of Anatomy, Basic Medical Sciences Institute, Jinnah Postgraduate centre Karachi from July 2007 to November 2007.

Materials and Methods: Forty five adult albino rats of either sex were taken and were taken and divided into three groups as A, B and C which were further divided in to three subgroups according to the period of treatment they received i.e. two, four and six weeks respectively. Group 'A' animals served as control and Group 'B' animals received Lithium carbonate (Neurolith, Adamjee Pharma), 20 mg/kg/day with feed. Group 'C' animals received Lithium carbonate 20 mg/kg/day plus L-Arginine (Arginine, General Nutritional Corporation, Pittsburg USA) 300 mg/kg/day with feed. After completion of respective period of treatment, kidneys were removed and fixed in alcoholic formalin and 10% formalin, after processing were embedded in paraffin. 5µ thick longitudinal sections were cut and stained with PAS-Haematoxylin and Gomori's calcium phosphate method for study of cell morphology.

Results: PAS-Haematoxylin stained sections of group 'A' revealed normal renal cortical histology. Gomori's calcium phosphate method stained tissue revealed normal activity of alkaline phosphates. Group 'B' revealed altered renal histology with damage to the proximal tubules on PAS-Haematoxylin stained sections. Gomori's calcium phosphate method stained sections revealed decreased activity of Alkaline phosphatase in proximal tubules. Group 'C' revealed normal cortical architecture except very mild alteration to brush border in subgroup 'C3'. Gomori's calcium phosphate method stained sections revealed normal activity of Alkaline phosphatase.

Conclusion: The present study suggests that even in therapeutic dose lithium carbonate causes damage to the proximal tubules in albino rats and L-Arginine minimizes the toxic effects of lithium carbonate.

Key Words: Lithium carbonate, PAS-Haematoxylin, Gomori's calcium phosphate proximal tubules, albino rats.

INTRODUCTION

Arginine (symbol Arg or R) is an amino acid. The L form is one of 20 most common natural amino acid. Infants are unable to effectively synthesize L-Arginine making it nutritionally essential for infants[1]. Arginine was isolated from a Lupin seedling extract in 1886 by the Swiss Ernest Schulze[1].

The importance of Arginine is attributed to its role as a precursor for nitric oxide that is synthesized in mammalian cells from L-Arginine by nitric oxide synthase[2]. Nitric oxide produces blood vessel relaxation[3]. Arginine gives rise to nitric oxide by the reaction Arginine to citruline + nitric oxide, which is catalyzed by nitric oxide synthase[4]. Preliminary evidence suggest that Arginine may be useful in the treatment of medical condition that are improved by vasodilatation, such as angina arthrosclerosis, coronary artery disease, erectile dysfunction, heart failure intermittent claudication/peripheral vascular disease and vascular headaches[3].

The L-Arginine/nitric oxide pathway seems to have slightly protective effect on kidney after ischaemic renal perfusion in rats[5]. Oxidative damage of vascular endothelium represents an important initiation step in development of arthrosclerosis which can be prevented by L-Arginine/nitric oxide pathway[6].

Nephrotoxic effects of analgesics, antibiotics such as aminoglycosides, anticancer agents such as cisplantin and other chemicals used in industries are known since long time[7]. Lithium therapy has long been associated with nephrogenic diabetes insipedius, chronic interstitial nephritis and minimal change nephropathy[8]. Lithium is now the drug of choice for treating Bipolar affective disorder. It is successful in improving both the manic depressive symptoms in 70-80% of patients. Lithium may also used to treat alcoholism, schizoaffective disorders, and cluster headaches. Thus

lithium is an indispensable pharmaceutical component of modern psychiatric therapy[9]. Proximal renal tubular cells are particularly vulnerable to the toxic actions of chemicals owing to the high energy demands such as reabsortive and secretory functions[7]. With this back ground this study was designed to observe the morphologically role of L-Arginine on lithium induced nephrotoxicity in albino rats.

MATERIALS AND METHODS

The present study was conducted in the Department of the Anatomy, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre Karachi. 45 adult Albino rats of either sex between 90 -120 days were selected for present study. Animals were observed for a week for any abnormality before the commencement of this experimental study. The animals were divided in to three groups A, B and C. Each group was further subdivided in to three subgroups according to the period of treatment they received, i.e. 2. 4 and 6 weeks respectively. Each group comprised of five animals.

Group A animals served as control and Group B animals received Lithium carbonate (Neurolith, Adamjee Pharma) at the dose of 20 mg/kg/day with feed and Group 'C' animals received Lithium carbonate 20 mg/kg/day plus L-Arginine (Arginine, General Nutrition Corporation, Pittsburg USA) 300 mg/kg/day with feed.

After completion of their respective period of treatment the animals were anaesthetized with ether in a glass chamber and were fixed on dissecting board and animas were sacrificed and abdomen were opened by midline incision, and kidneys were excised and fixed in alcoholic formalin and 10% formalin for 24 hours. After fixation tissues were processed in higher grades of alcohol from 70-100%, cleared in xyline infiltrated and embedded in paraffin. 5 µ thick longitudinal sections were cut on rotatory microtome. Sections were stained with PAS-Haematoxylin technique and Gomori's calcium phosphate method.

RESULTS

Control Group;

Kidneys appeared oval or bean shaped dark red to dark brown in color, soft in consistency with a smooth and shiny surface, covered by a delicate fibrous capsule which striped off easily in all the animals of group 'A'. Examination of PAS-Haematoxylin stained sections under light microscope showed proximal tubules closely packed and were circular, oval or elliptical in shape, mostly confined to cortex particularly in vicinity of glomeruli. The lining epithelium of proximal tubules was arranged regularly on intact and well defined basement membrane. The cells appeared low columnar having nuclei located in the centre or basal portion of the cells. Brush border was found distinct on luminal surface of tubules. There was no nuclear and epithelial debris in the lumen. Gomori's calcium phosphate stained sections showed the site of enzymatic activity of alkaline phosphatase in the proximal tubules in the form of brownish black deposits, which were seen regularly arranged with in tubules.

Treated Group:

Group B;

On gross examination of kidney of subgroup B1 appeared reddish brown in color, oval in shape. Their capsules stripped of easily from kidneys. The kidneys of subgroup B2 animals also appeared reddish brown in color, oval in shape but appeared swollen as compared to subgroup A2 and their capsules stripped off with difficulty. The kidneys of subgroup B3 albino rats appeared shrunkened in size and light brown in color and their capsules were removed with difficulty.

The microscopic examination of PAS-Haematoxylin stained sections of kidneys subgroup B1 revealed renal architecture with distorted arrangement. The proximal tubules showed epithelial casts in the lumen. The microscopic examination of subgroup B2 and B3 revealed irregular cortical architecture. The proximal tubules were found dilated and filled with cellular debris, sloughed off material and casts. The cells of proximal tubules were found containing nuclei displaced from centre and appeared irregular in size and shape. Epithelial casts and nuclear debris was prominent in the lumen of proximal tubules. Brush border appear damaged and basement membrane was distorted. There was marked leukocytic infiltration in the interstitium suggestive of inflammatory process. Gomori's calcium phosphate stained sections showed decrease in brownish black deposits in B1, B2 and B3 respectively, suggesting damage to the brush border leading to alterations in pattern of activity of the alkaline phosphates on proximal renal tubular cell .

Group C;

On gross appearance the kidneys of subgroup C1, C2 and C3 which were treated with lithium carbonate and L-Arginine appeared oval or bean shaped dark red in color, soft in consistency with smooth and shinny surfaces covered with delicate capsule which stripped of easily.

Microscopically subgroups C1 and C2 revealed renal architecture with no change in renal tubules and malpighian corpuscles. The lining epithelium of proximal tubules were arranged regularly on intact and well defined basement membrane. Nuclei were present with prominent nucleoli. Brush border was distinct and basement membrane intact. There was no evidence of inflammatory infiltration in the interstitium. The subgroup C3 revealed renal architecture same as compared to the control subgroup A3 apart from brush border was found indistinct and scanty in some tubules.

the Gomori's calcium phosphate stained sections showed distribution of activity of alkaline phosphatase regularly suggestive of intactness of brush border of proximal tubules.

DISCUSSION

L-Arginine is alpha amino acid, in mammals it is classified as semi essential amino acid depending in the stage and health status of the individual[1]. Supplementation is some times required[3]. Infants are some times able to synthesize Arginine making it nutritionally essential amino acid for infants[1]. Arginine is involved in numerous pathways of human metabolism. It serves as precursor for the biosynthesis of proteins and also ornithine, polyamines and nitric oxide. Arginine increases GFR and renal plasma flow[17]. Nephrotoxicity is an inherent adverse effect of certain anticancer drugs for example streptozocine, cisplantin etc[12]. Long term uses of lithium in therapeutic concentrations have been thought to cause histological and functional changes in kidney. The significance of such changes is not clear but is of sufficient concern to discourage long term use of lithium unless it is definitely indicated[13]. In this study the morphological examination of renal cortical tissue in lithium treated subgroup B showed abnormal dilatation of proximal renal tubules. This is in conformation with studies of Christensen S at al., 1982. Vacuolar degeneration was noted in the Group B treated rats this is an adoptive response [14]. Over all reduction in volume of cell was observed when compared to the corresponding controls[15,16]. The histological damage found in lithium treated group was attributed to the biochemical alterations. It is speculated that increased lipid peroxidation as a result of reduced endogenous oxidant capacity may be the initial event in producing renal damage[10]. Damage to antioxidant enzymes led cells unprotected from the effects of lithium as super oxide dismutase (SOD), Catalase (CAT) and Glutathion Peroxidase (GSH-Px) were decreased leading to damage to mitochondria and disturbance of ATP production[10]. This lead to the damage to tubular cells and the nitric oxide component of L-Arginine appear to have protected group C rats from nephrotoxic effects of lithium carbonate.

CONCLUSION

It is concluded from this experimental study that nephrotoxicity produced by lithium carbonate could be minimized by supplementation of L-Arginine.

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