MEDICAL CHANNEL

Original Article

A MORPHOMETERIC STUDY OF THE ROLE OF L-ARGININE IN NEPHROTOXICITY (INDUCED BY LITHIUM CARBONATE) IN ALBINO - RATS

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ABSTRACT

OBJECTIVE: To observe the effects of the L-Arginine in the nephrotoxicity (induced by Lithium carbonate) in albino rats.
DESIGN: A prospective experimental study.
PLACE: Department of Anatomy, Basic Medical Sciences Institute, Jinnah Post Graduate Medical Centre Karachi
DURATION: July 2007 to November 2007
METHODOLOGY: Forty five adult albino rats of either sex were taken and divided into three groups as A, B and C, which were further divided into three subgroups according to period of the treatment they received i.e. two, four and six weeks respectively. Group A animals served as control, Group B received lithium carbonate (Neurolith, Adamjee Pharma) at dose of 20 mg/kg/day with feed and Group C animals received lithium carbonate 20 mg/kg/day with feed plus L-Arginine (Arginine, General nutrition corporation, Pittsburg USA) 300 mg/kg/day with feed. After end of their respective period of treatment animals were sacrificed and their kidneys were removed, fixed in alcoholic formalin, and after processing were embedded in paraffin and 5µ thick sections were cut and stained with PAS-Haematoxylin.
RESULTS: PAS-Haematoxylin stained sections revealed significant and highly significant (i.e. $P < 0.05$, $P < 0.001$) decrease in proximal tubular count and proximal tubular nuclear count in sub group B2 and B3 animals respectively. A significant and moderately significant ($P < 0.05$, $P < 0.01$) decrease was also noted in nuclear diameter of proximal tubular cells in subgroup B2 and B3 respectively. Results of Group 'C' were found insignificant when compared with control group.
CONCLUSION: After morphometeric study it appeared that L-Arginine minimizes the toxic effects of Lithium carbonate on proximal tubules of kidney.

KEY WORDS: Lithium carbonate, Nephrotoxicity, L-Arginine.

INTRODUCTION

Arginine is an important aminoacid. Infants are unable to effectively synthesize it therefore it is nutritionally essential for infants. Adults are however able to synthesize it in urea cycle. The L- form is one of 20 most common natural aminoacids [1]. In 1932 L-Arginine was found to be required for generation of urea, which is necessary for the removal of toxic ammonia from the body [2]. Its precursor is nitric oxide. Demonstration of nitric oxide in 1987 as an enzyme in vessel endothelial cells opened up what can now be considered an era of biological research with in endothelial cells [3]. Preliminary evidences suggest that arginine may be useful in the treatment of medical conditions that are improved by vasodilatation such as angina, arthrosclerosis, coronary artery disease, erectile dysfunctions, heart failure, and intermittent claudication/peripheral vascular disease.
Arginine may stimulate protein synthesis and has been used for wound healing, body building, and enhancement of sperm production (spermatogenesis) and prevention of wasting in people with critical illness [2]. Supplemental dietary arginine enhances wound

healing in normal mice [4]. L-Arginine can ameliorate kidney dysfunction induced by cyclosporin via a mechanism which involves production of nitric oxide. In addition, L-Arginine may therefore be a beneficial for cyclosporin nephrotoxicity and can be used to improve the therapeutic index of cyclosporin [5].

In 1970 the U.S.A. food and drug administration F.D.A. approved lithium as a treatment for mania. Four years later the F.D.A. also approved the use of lithium as preventive or prophylactic treatment for manic depressive illness [6]. Lithium is capable of causing a major disturbance in water balance, manifest as polyuria and secondary polydipsia [7]. In long term use of lithium in therapeutic concentration have been thought to cause histological and functional changes in kidneys. The significances of such changes are not clear, but are of sufficient concern to discourage long term use of lithium unless it is definitely indicated [8].

MATERIAL AND METHODS

The present study was conducted in the Department of the Anatomy Basic Medical sciences institute and Jinnah postgraduate medical centre Karachi. 45 adult albino rats of either sex between 90-120 days were selected for present study. Before the commencement of study animals were observed for a week for any abnormality. The animals were divided into three groups A, B, and C. Each group was further subdivided into three subgroups according to period of treatment they received, i.e. 2, 4 and 6 weeks respectfully. Each subgroup comprised of 5 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 received lithium carbonate 20mg/kg/day plus L-Arginine (Arginine General Nutrition Corporation, Pittsburg USA) 300mg /kg per day with feed.

The animals were anaesthetize with ether in a glass chamber and were sacrificed and then fixed on dissecting board after complitation of their respective periods of treatment. Abdomens were opened by midline incision. Kidneys were excised and fixed in alcoholic 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 micron thick longitudinal sections were cut on rotatory microtome. Sections were stained with PAS-Haematoxylin technique.

The proximal renal tubular count was done under 8 × ocular and 40 × objective with counting reticule in randomly selected 10 fields from cortex of each kidney of each animal. Nuclear count of proximal tubule was done under 8 × ocular and 100 × oil immersion objective with help of a counting reticule in randomly selected 10 fields in cortical area of kidney of each animal. Micrometry was done with ocular micrometer and counting reticule. The statistical analysis was done by student's't' test and P-value less than 0.05 was considered as significant.

RESULTS

Mean number of proximal tubules in cortical region of kidney in group "A", are 17.800 ± 0.199, 18.200 ± 0.374 and 18.000 ± 0.181 microns , in group "B" are 14.200 ± 0.373, 12.400 ± 0.244 and 9.000 ± 0.316 microns and group "C" are 17.400±0.244, 17.800±0.375 and 18.400±0.244 microns respectively. When group 'A' and 'B' compared the results are significant ($P < 0.05$) to highly significant ($P < 0.001$). Insignificant ($P > 0.05$) results are obtained when group "C" is compared to control.

Mean number of nuclei of proximal tubular cells of kidneys in group "A" are, 18.066 ± 181, 18.266 ± 0.593 and 18.400 ± 0.130 microns, in group "B" are 15.133 ±0.165, 12.312 ± 0.204 and 10.800 ± 0.222 microns group "C" results are 17,933±0.248, 17.600±0.272 and 18.133±0.199 microns respectively. When group 'A' and 'B' compared the results are significant ($P < 0.05$), to highly significant ($P < 0.001$). Insignificant ($P > 0.05$) results are obtained when group "C" is compared to control.

Mean nuclear diameter of proximal tubular cells of kidney in group "A" are 6.730 ± 0.118, 6.933 ± 0.153 and 7.066 ± 0.153 microns in group "B" are 7.266 ± 0.181, 5.866 ± 0.125 and 5.333 ± 0.126 microns in group "C" are 6.666±0.159, 6.800±0.200 and 7.666±0.126 microns respectively. When group 'A' and 'B' compared the results are insignificant increase ($P > 0.05$), significant ($P < 0.05$) and moderately significant ($P < 0.01$) respectively. Insignificant ($P > 0.05$) results were obtained when group C was compared to control.

DISCUSSION

There are many causes of nephrotoxicity such as salts of heavy metals (e.g. mercury and gold), carbon tetra chloride, ethylene glycol, poisonous mushrooms, radio contrast media, insecticides, herbicides, and drugs particularly aminoglycosides [9,10]. We can define nephrotoxicity as dysfunction that arises as a direct or indirect result of medicines and environmental chemicals [11]. Proximal tubular cells are particularly vulnerable to the toxic actions of chemicals owing to their high energy demands such as secretory functions [10]. A therapeutic agent currently in wide spread use for Bipolar affective disorder is lithium but a frequent side effect of it is renal toxicity [12]. Renal toxic effects include nephrogenic diabetes inspedius, chronic interstial nephritis and minimal change glomerulopathy [13].

L-Arginine dilates resistant blood vessels of perfused rat kidney by a mechanism in which there may be the involvement of release of endothelium derived relaxing factor (nitric oxide). L-arginine administration lowers mean arterial pressure. It increases GFR and renal plasma flow [14]. Depending on nutritional status and develmental stage normal arginine concentration in human and animals range from 9-250µ mol/L [15]. Keeping this in mind the present study was designed to observe the effects of lithium carbonate on proximal tubules of albino rats and use of L-Arginine to prevent its toxicity. It is speculated that the increased lipid peroxidation as a result of endogenous antioxidant capacity may be the initial event in producing renal damage [16]. Thus damage to antioxidant enzymes (Superoxide dismutase "SOD", Catalase "CAT" and Glutathion Peroxidase "GSH-Px" is initial event in cell damage. It leads to damage to mitochondria and disturbances of ATP production causing decrease in activity of Na-pump which results in an increased influx of Ca , H2O, Na and efflux of K which results in cellular swelling, loss of microvilli,and swelling of endoplasmic reticulum(17).

Recent data point strongly to the antioxidant role of nitric oxide component of L - Arginine provides protection against lipid peroxidation induced by Lithium carbonate (18). Analysis of data depict, significant ($P<0.05$), moderately significant ($P< 0.01$) and highly significant ($P<0.001$) values between lithium carbonate treated group with the control. When L-Arginine plus Lithium carbonate treated group is compared with control group it becomes apparent that there is no difference of P-value between two groups.

FIGURE 1

PAS - haematoxylin stained 5 micron thick, section of kidney from group A rat showing proximal tubules with intact brush border (BB) basement membrane (BM), nuclei with prominent nucleoli (Ni).
Photomicrograph × 1000.

GRAPH – 1

Mean Number of Proximal Tubules in Cortical Region of Kidneys of Albino Rats in Different Groups at Variable Time Interval

FIGURE 2

PAS - haematoxylin stained 5 micron thick, section of kidney from group B rat showing epithelial casts and variable shape of nuclei
Photomicrograph × 1000.

GRAPH – 2

Mean Number of Nuclei of Proximal Tubular Cells of Kidneys of Albino Rats in Different Groups at Variable Time Interval

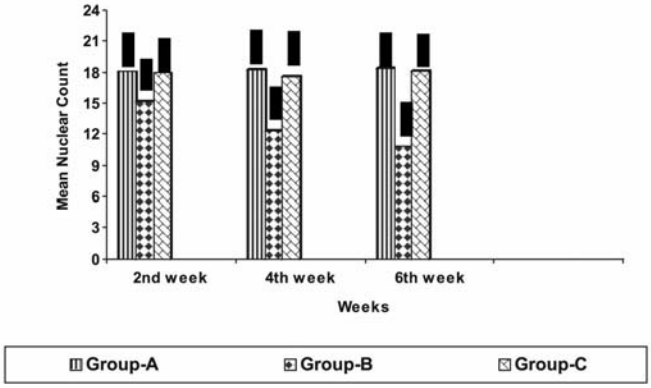
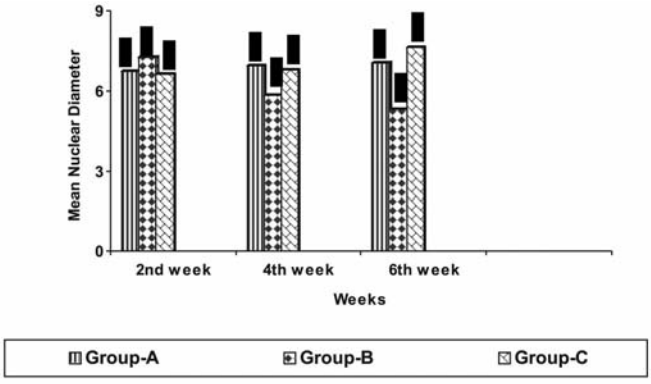


FIGURE 3

PAS - haematoxylin stained 5 micron thick, section of kidney from Group C rat showing proximal tubules with well defined brush border (BB) and intact basement membrane (BM)
Photomicrograph × 400.

GRAPH – 3

Mean Nuclear Diameter of Proximal Tubular Cells of Kidneys of Albino Rats in Different Groups at Variable Time Interval



CONCLUSION

L-Arginine minimizes the toxic effects of Lithium carbonate on proximal tubules of kidney.

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