HISTOPATHOLOGICAL AND ULTRASTRUCTURAL STUDIES ON THE TOXIC EFFECT OF PRIANIL C-R (LITHIUM CARBONATE) IN MOUSE KIDNEY

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ABSTRACT

This study was carried out to investigate the histopathological and ultrastructural alterations in the kidney of Swiss albino mice in response to the administration of prianil C-R (lithium carbonate) at a dose level of 2.32 mg/mouse daily for 30 days (therapeutic dose) and 0.58 mg/mouse daily for 90 days (quarter the therapeutic dose). Our results demonstrated severe changes involved both glomeruli and renal tubules in mice treated with high dose of lithium. The histopathological changes of the glomeruli included hypertrophied glomeruli, shrunked urinary space and increased mesangial matrix. Altered podocytes, ballooning, hypertrophy or focal fusion of the secondary foot process and thickening of the glomerular basement membrane were recorded. The proximal convoluted tubular cells were severely affected. They have pleomorphic irregular shaped nuclei, swollen mitochondria with vesicular cristae, dilated rough endoplasmic reticulum and hypertrophied Golgi apparatus. The brush border was lost in some areas of proximal tubular cells. The changes in the distal convoluted tubular cells are less pronounced and included dilated nuclear membrane, hypertrophied Golgi apparatus, abnormal mitochondria and vaculated cytoplasm. The pathological alteration induced in the renal tissue after low dose of prianil C-R were approaching those induced by the high dose, however, they were less remarkable.

Key-words: Histopathology, ultrastructure, prianil, mouse, toxicity

INTRODUCTION

In 1949 Cade reported the use of lithium in control prevention of manic episodes. There is general agreement that lithium is effective clinically in the treatment of certain kinds of mental illness, especially manic-depressive psychosis (Bowden, 2000). In addition to its demonstrative therapeutic utility, lithium is also valuable as a prophylactic agent in mania-hypomania and depression (Lenox et al., 1998). It has additional uses both in the prophylaxis of unipolar and biopolar depressive disorder and in conjunction with antidepressant for the treatment of resistant depressive illness (Johnson and Mc Farland, 1996).

The successful use of lithium has been hampered by a variety of side effects. In the late 1950’s, an alarm was triggered by reports of kidney damage. Many investigators suggested that severe renal damage may occur quite frequently in lithium treated men (Coskunol et al., 1997) as well as in experimental animals (Moussa et al., 2001). Histological changes including interstitial fibrosis, tubular atrophy and glomerulosclerosis were reported in patients treated with lithium carbonate for long-term (Pospishil, 1998). Christensen et al. (1986) reported lithium induced chronic renal failure in newborn Wistar rats. The characteristic associated morphological changes were large cortical cysts found by dilated tubules and collecting ducts and widespread interstitial fibrosis. Proximal tubular mass was reduced by 50% and the glomerular volume was also significantly reduced.

In comparison with the available data on the toxic effect of lithium on the structure and function of the kidney, it becomes obvious that the effect of this drug on the cellular level has been rarely tackled. Hence, it was felt that, it could be of some importance to carry out histological and ultrastructural studies on the effect of lithium carbonate on the kidney of Swiss albino mice, as an example of mammals, in order to declare more recognition of the adverse changes that occur at the cellular level as a result of the prolonged use of this drug.

MATERIALS AND METHODS

Experimental animals:

The 120 adult male and female Swiss albino mice Mus musculus (20-25g), each were used. Animals were allowed free access of food and water throughout the experimental period (30 and 90 days). Animals were assigned randomly to “one” control and “two” experimental groups and kept in large cages in environmentally controlled room [22-24°C]. The first group (control animals) is subdivided into tow sub-groups of 30 animals each and injected with 1 ml mammalian saline daily for 30 & 90 days respectively. Animals of the second group (30 mice) were injected intraperitoneal with Prianil C-R solution at a dose level of 2.32 mg / mouse, daily for 30 days. This dose represents the human therapeutic dose and referred to as the high dose in the present study. Animals of the third group (30 mice) received an intraperitoneal daily dose injection of 0.58 mg / mouse Prianil C-R for 90 days. This
dose is equivalent to quarter of human therapeutic dose and referred as low dose. The equivalent doses of the given lithium were determined according to Paget and Barnes formula (1964). Lithium carbonate was purchased from El-Nil Pharmaceutical and Chemical Industries Co. Cairo, A.R.E. It is available under the international trade name Prianil C-R.

Preparation of samples for microscopical study:

At the end of the experimental periods (30 and 90 days) mice from every group were killed by decapitation at the occipital condyles. For histological studies small pieces of kidney were excised, immersed in neutral formalin and stained with Haematoxylin and eosin (H and E) (c.f. pantin, 1964)
For electron microscopy small blocks of kidney from different groups were fixed with 2% glutaraldehyde buffered with 0.1M phosphate buffer (pH 7.6) at 4°C. The specimens were post-fixed in 1% OsO₄, dehydrated in increasing ethanol concentrations, treated with propylene oxide and embedded in Epon-Araldite mixture. Sections were cut on an LKB ultramicrotome. Semithin sections were stained with toluidine blue, while thin sections were double stained with uranyl acetate and lead citrate and investigated in a 100 CX electron microscope.

RESULTS

Control animals :

a-Light microscopical observations:

Histological examination of control kidney revealed that, it is distinguishable into an outer cortical region and an inner medullary region. The parenchyma of the cortex is formed of a large number of urinefrous tubules and numerous round Malpighian corpuscles, while the medulla contains mainly collecting tubules (Fig. 1)

Malpighian corpuscle consists of double walled Bowman’s capsule surrounding a capillary tuft, the glomerulus. A preserved urinary space is found between the inner wall (visceral layer) and the outer wall (parietal layer) of Bowman’s capsule (Fig. 2).

The glomeruli show moderatecellularity consisting of glomerular blood capillaries lined with endothelial cells and the mesangium. The endothelial cells are characterized by flattened nuclei with prominent nucleoli. The intramesangial cells were observed filling the region between glomerular blood capillaries in a homogeneous matrix (mesangial matrix) and have nuclei with large size and irregular shape (Fig. 2).

The proximal convoluted tubules of normal kidneys have narrow lumen. And a regular basal lamina lined by a single layer of cuboidal cells which are broader at their bases, than at their free margins. A striated brush border can be seen on the free surface of each proximal convoluted tubular cells while the basal part possesses closely packed abundant acidophilic striations representing the mitochondiral granules (Figs. 2, 3).

The cells of the distal convoluted tubule possess abundant cytoplasm with basal acidophilic striations while their nuclei have nearly equal size and are rounded in shape and central in location. Some nuclei are located at the apical part of the cells near the lumen and prominent nucleoli can be seen in the nucleus (Fig. 3).

b-Electron microscopical observations:

1- The Malpighian corpuscles:

Electron micrographs of the kidney of control mice showed that the parital layer of Bowman’s capsule consists of squamous epithelial cells and underlying basement membrane while the visceral layer of Bowman’s capsule consists of special epithelial cells with long branching cell processes (the podocytes) (Fig. 4). From each podocyte extends several primary foot processes which in turn give rise to numerous small bell shaped secondary foot processes called pedicles (little feet). The secondary foot processes are in direct contact with the glomerular basement membrane and the endings of these processes interdigitate to cover the glomerular basement membrane and are separated by gaps of uniform width called the filtration slits. The pores, between pedicles, open into the urinary space and have filtration slit diaphragms. The cellular body of podocyte contains eccentric, mostly kidney shaped, heterochromatic nucleus and fairly dense cytoplasm.

The urinary space of Bowman’s capsule exists between the two layers of Bowman’s capsule and ramifies throughout the glomerulus (Fig. 4). The glomerulus consists of blood capillaries lined by an endothelial layer which is closely applied to the luminal surface of the glomerular basement membrane i.e. there is a fusion between the basal lamina of podocytes and the endothelium. The cells of this layer possess flattened nuclei and thin fenestrated cytoplasm with no slit diaphragm. Several capillary loops can be recognized by their content of uncompressed
erythrocytes (Fig. 4).

The glomerular basement membrane (Fig. 5) consists of a thick electron-dense central layer, the lamina densa, and thinner electron-lucent peripheral layers, the lamina rara interna and lamina rara externa. The entire glomerular tuft is supported by mesangial cells (intercapillary cells), each of which is surrounded by a membrane with cytoplasmic processes (phyllopodia). The nuclei of mesangial cells are large, heterochromatic and occupying the majority of the cell. The heterochromatin is distributed both centrally and peripherally. Their cytoplasm is dense and was found to be located along the cell membrane (Fig. 4).

2- The proximal convoluted tubules:

The plasma membrane of the proximal convoluted tubular cells possess well developed microvilli consisting of regularly oriented and closely packed finger-like projections that line the tubular lumen at border. In addition, endocytic vesicles are present in the cytoplasm, beneath the microvilli (Fig. 6).

The nuclei of the proximal convoluted tubular cells are nearly spherical in shape and occupy a central position. They are surrounded by a distinct nuclear envelope and is perforated by distinct nuclear pores (Figs. 6,7). The chromatin content exhibits dispersed euchromatin and condensed heterochromatin, located throughout the nucleus. Also, areas of heterochromatin are especially conspicuous along the nuclear peripheral zones facing the inner nuclear membrane (Fig. 6). In addition, the nucleus possesses one and sometimes two prominent nucleoli.

The mitochondria are separate round-ovoid at the apical and mid regions, of the tubular cells. At the basal part of the tubular cells, mitochondria are mostly elongated and are lodged in between basilateral infoldings, arranged in rows in such a way that they are brought into very close relationship to a large area of the basal cell surface (Fig. 6). The mitochondrial cristae appear numerous in number and lamellar in shape (Fig. 7).

Golgi apparatus is made up of three to six flat saccules or elongated cisternae, in close parallel array having juxtanuclear position (Fig. 7). The rough endoplasmic reticulum was noticed in longitudinal profiles having the form of dense heavily stained flattened parallel cisternae, scattered randomly throughout the cytoplasm, and were studded with numerous ribosomes (Fig. 7). There are other groups of ribosomes present free in the cytoplasm single, or in aggregates polyribosomes.

3- The distal convoluted tubules:

They have more wide lumen and their lining cells lack the presence of microvilli at the apical surface, instead they show only few short micrervilli projections (Fig. 8). The cells lining the distal convoluted tubule have central or basal round-ovoid shaped nuclei (Fig. 8). The nucleus is surrounded by the nuclear envelope that consists of two parallel membranes. Some cells of the distal convoluted tubules have nuclei that contain two nucleoli (Fig. 8). Golgi apparatus is located near the nucleus and consists of parallel cisternae. The rough endoplasmic reticulum is represented by scant longitudinal profiles. Ribosomes were also observed free in the cytoplasm (Fig. 9). At the apical and mid regions the mitochondria, appeared mostly rounded and small, they have distinct membranes, tubular cristae and electron dense matrix (Fig. 9). The basilateral domain of the proximal tubular cells is deeply infolded into numerous basilateral infoldings that penetrate the basal substance of the cell subdividing this portion of the tubular cell into compartments (Fig. 6).

Prianiil C-R treated groups

a. Light microscopical observations:

Some hypertrophied glomeruli with increased cellularity were observed in the renal cortex with severe congestion. Increased mesangial matrix and obliterated shrunk urinary space were also detected (Fig. 10). The proximal convoluted tubules showed abnormal morphology. They have irregular basal lamina and vacuolated cytoplasm. The renal tubular lumen appeared more wide and contained cytoplasmic mass and cellular debris. Some of these tubules show unusual contour with different sized open face nuclei and terminalized nucleoli. Disrupted tubular cells with pyknotic nuclei surrounded by a large cytoplasmic area, which show degeneration and loss of their basal striations were also detected (Fig. 10). They were observed containing numerous homogeneous sections probably hyaline droplets that fill the renal tubular lumen and obscure the details of many lining cells (Fig. 11). The nuclei of the distal convoluted tubules were with different size, decreased amount of heterochromatin and terminalized nucleoli. While their cytoplasm show degenerative areas (Fig. 11). The basal striations could not be easily distinguished in their cells and the accumulation of many tiny vacuoles in their cytoplasm (Fig. 11).
Fig. 1. Light micrograph of a transverse section of control kidney, showing the fibrous capsule (arrow), cortex (c) and medulla (Me)(H&E) (X 100).

Fig. 2. Light micrograph of a semithin section of control kidney, showing normal structure of Malpighian corpuscle with Bowman’s capsule parietal epithelium (Pe) and arrow points at basal acidophilic striations and arrow’s head points at glomerular blood capillary. Dt: distal convoluted tubule, Mc: mesangial cell, Mx: mesangial matrix, Pt: proximal convoluted tubule, Us: urinary space (X 1,000).

Fig. 3. Light micrograph of a semithin section of control kidney, showing normal structure of the proximal convoluted tubule (Pt) with regular basal lamina, acidophilic brush border at the luminal surface and basal acidophilic striations (arrow). The nuclei (arrow’s head) show condensed face type and contain deeply basophilic nucleoli. Dt: distal convoluted tubule with regular basal lamina (X1000).
Fig. 4. Transmission electron micrograph of a part of control kidney, showing epithelial cell (Ep) possessing heterochromatic nucleus with nuclear pores (arrow’s head) and primary foot processes(*). Note the secondary foot processes (FP) resting on the glomerular basement membrane (Bm), wall of endothelial cell with fenestra (F) and the obvious association between blood capillary (Bc) and mesangial cell (Mc). Arrow points at erythrocytes. Us: urinary space (X 15,000).

Fig. 5. Enlarged part of a normal three components of glomerular filter. The glomerular endothelium (En) is perforated by fenestra (arrow) with no slit diaphragm. The basement membrane is consisting of lamina rara-external (1), lamina densa (2) and the lamina rara interna (3). The secondary foot processes (Fp) are separated by filtration slit of uniform width with filtration diaphragm (arrow’s head) Bc: blood capillary, Us: urinary space (X60,000).

Fig. 6. Part of the proximal convoluted tubule of control mouse, illustrating cells possessing closely packed microvilli (Mv), intercellular space (arrow), nucleus (N) with distinct nuclear envelope (arrow’s head), euchromatin (Eu), heterochromatin (He) and nucleolus (Nu). Note, the numerous mitochondria (M), basilateral infoldings (B1) and the regular tubular basement membrane (Bm). Ev: endocytic vesicle, (X 10,000).
Fig. 7. Part of the proximal convoluted tubular cell of control mouse, showing part of the nucleus (N) with nuclear pores (arrow’s head), Golgi complex (G), mitochondria (M), rough endoplasmic reticulum (rER) and ribosomes (R). (X 40,000).

Fig. 8. Part of the distal convoluted tubule of control mouse, showing cells with short microvillous projections (arrow), apical nucleus (N) with two peripheral nucleoli (Nu) and nuclear pores (arrow’s head). (M): Mitochondria (X 10,000).

Fig. 9. Part of the distal convoluted tubule of control mouse, showing cells with nuclear pores (Np), rough endoplasmic reticulum (rER), Golgi complex (G) and mitochondria (M) with electron dense matrix. Note, the intercellular space (arrow) and ribosomes (R). (X 40,000).
Fig. 10. Light micrograph of a semithin section of the kidney in a mouse treated with high dose of lithium carbonate, illustrating increased intercellular space, hypertrophied glomerulus (G) with increased mesangial matrix (Mx) and shrunk urinary space (Us). The proximal convoluted tubule (Pt) has unusual contour and contains different sized open face nuclei (arrow). Arrow’s head points at pyknotic nucleus surrounded by a degenerated cytoplasm. (X 1000.).

Fig. 11. Light micrograph of a semithin section of the kidney in a mouse treated with high dose of lithium, demonstrating distal convoluted tubule (Dt) with different sized of nuclei, decreased amount of heterochromatin and terminalized nucleoli (arrow). Note the widespread of tiny vacuoles in the cytoplasm and loss of the basal striations. Arrow’s head points at hyaline droplets filling the lumen of proximal convoluted tubule (Pt), (*) point at degenerative cytoplasm (X 1,000).

Fig. 12. Transmission electron micrograph of Malpighian corpuscle of the kidney in a mouse treated with high dose of lithium, demonstrating hypertrophied secondary foot processes (Fp) resting on a thickened glomerular basement membrane (Bm). The epithelial cell contains vacuoles (V) and depicting nucleus with blebbing of the outer membrane of the nuclear envelope (arrow) and margined heterochromatin (He). Note, the mesangial cell nucleus (N) has slightly irregular nuclear envelope (arrow’s head). M: dense mitochondria, (X15,000).
Fig. 13. Proximal convoluted tubule of a mouse, treated with high dose of lithium, illustrating thickened tubular basement membrane (Bm), presence of peculiar bodies at the contiguous surface between neighboring cells (*); pleomorphic mitochondria (M) and various sized nuclei. Arrows point at hyperchromatic nuclei surrounded by degenerated cytoplasm and arrow’s head point at pyknotic nucleus. Note, the loss of microvilli at certain sites (double arrow) and the presence of myelin figure (Mf) in the tubular lumen. Mv: microvilli, Ev: numerous vacuoles (X 6,000).

Fig. 14. Part of the proximal convoluted tubule of a mouse treated with high dose of lithium, demonstrating part of the nucleus (N) with nuclear pores (Np), orthodox-configurated mitochondria (M) with indistinguishable membranes, divided mitochondria (arrow), mitochondria with vesicular cristae pattern (*) and rupture of the mitochondrial membrane (arrow’s head). Note, Golgi apparatus (G) with dilated vesicles, shedding of ribosomes from the surface of the rough endoplasmic reticulum (rER) and increased number of free ribosomes (X 40,000).

Fig. 15. Part of the distal convoluted tubule of a mouse treated with high dose of lithium, showing emission of flocculent material in the tubular lumen (L), widened interorganellar space, decreased content of nuclear heterochromatin that tends to clump at the margin of the nucleus (N) and pleomorphic mitochondria (M). It: interstitium (X6,000).
Fig. 16. Part of the distal convoluted tubule of a mouse treated with high dose of lithium demonstrating the nucleus (N) with dilated space between nuclear membranes (arrow) and hypertrophied Golgi apparatus (G). M: mitochondria, rER: rough endoplasmic reticulum, R: free ribosomes (X40,000).

Fig. 17. Part of the Malpighian corpuscle of a mouse treated with low dose of lithium, showing epithelial cell nucleus (N) with increased number of nuclear pores and irregular nuclear envelope (Ne). Note, the capillary lumen (Bc) is filled with compressed erythrocytes (arrow). (*) demonstrates the loss of secondary foot processes (X 10,000).

Fig. 18. Part of the proximal convoluted tubule of a mouse treated with low dose of lithium demonstrating widening of the intercellular space (arrow), nucleus (N) with terminalized nucleolus (Nu), altered basilateral infoldings (B1) and divided mitochondria (M). Mv: microvilli, Ev: endocytic vesicle (X 10,000).
Fig. 19. Part of the distal convoluted tubule of a mouse treated with low dose of lithium, illustrating the nucleus (N) with increased euchromatin content and normal mitochondria (M). Arrow points at vacuolated mitochondria. Bm: tubular basement membrane, Ly: lysosome. (X10,000).

b. Electron microscopical observations:

The Malpighian corpuscle:

The nuclei of the epithelial cells (podocytes) appeared to be altered by possessing irregular nuclear envelope, blebbing of the outer membrane of the nuclear envelope and margination of the heterochromatin (Fig. 12). In addition, the cytoplasm of some epithelial cells contained many vesicles and numerous electron dense granules (Fig. 12). Alterations in the secondary foot processes involved ballooning, hypertrophy, focal fusion to form blunted sheet of epithelial cytoplasm (Fig. 12). The glomerular basement membrane was markedly thicken, and the three layers consisting the membrane can no longer be distinguished. The filtration slits, between secondary foot processes, were lost along wide area of the glomerular basement membrane. Loss of fenestrae in the glomerular endothelium was also evident (Fig. 12).

The proximal convoluted tubules:

There was a development of luminal extensions of some cells; such extensions are structureless, devoid of microvilli, and contained few organelles except of sparse mitochondria and ribosomes (Fig. 13). The lumen was characterized by the presence of myelin figures and there was a widening of the lateral intercellular space between proximal convoluted tubular cells that contained peculiar bodies. The nuclei appear pleomorphic and exhibit irregular shape and increased fine chromatin granules, that occupy most of the nucleoplasm, forming darker chromatin areas situated in variously sized aggregations on the inner surface of the nuclear envelope. Some pyknotic nuclei were also observed (Fig. 13).

The mitochondria showed increase in their size and have matrix with a decreased electron opicity. Rupture of the mitochondrial membranes were also detected (Fig. 14). The rough endoplasmic reticulum exhibits dilatation of most profiles and loss of ribosomes from their surface. As a result, the number of free ribosomes in the cytoplasm increases. Hypertrophied Golgi apparatus with dilated vesicles was also detected (Fig. 14).

The distal convoluted tubules:

Flocculent materials were exhibited in the lumen of some distal tubular cells. In addition, the cytoplasm of the epithelial lining was electron-lucent with increased spacing between cellular organelles (Fig. 15). Some nuclei contained slightly decreased amount of heterochromatin and that the fine chromatin granules tend to clump at the margin (Fig 15). Dilated space between nuclear membranes was also recorded (Fig. 16). However, the number of the mitochondria appeared to be altered and Golgi apparatus appeared to be hypertrophied (Fig. 16). The ultrastructural alterations induced in the renal tissue after low dose of Prianil C-R administrations were approaching these induced by the high dose, however, they were less remarkable (Figs. 17-19).
DISCUSSION

Although Prianil C-R (lithium carbonate) is well known as antidepressant drug, used extensively in the treatment of manic-depressive psychosis, it has a unique relationship to renal pathology. Treatment Swiss albino mice with prianil C-R at therapeutic or quarter the therapeutic dose, resulted in many histopathological and ultrastructural changes in different elements of the kidney of treated animals. Severe congestion of glomeruli and hypertrophied glomeruli with shrunk urinary space were observed in mice treated with prianil C-R, either at high or low dose. Badawy et al. (1989) who detected the presence of vascular congestion of some glomerular tufts of mice after Lithium administration. Also Hagi et al. (1982) stated that about 10% of glomeruli showed focal cellular proliferation of the endothelial cells in rat kidney treated with lithium.

In contrary, Moussa et al. (2001) demonstrated the presence of shrunk glomeruli in kidney of experimental animals after lithium administration. According to Glassok (1985) hypercellularity is a common phenomena in different pathological conditions and is suggested to contribute to the development of segmental glomerulosclerosis. This is in agreement with the reports given by Santella et al. (1988) and Markowitz et al. (2000) who stated that there is an association between focal segmental glomerulosclerosis and lithium treatment in human. Other authors mentioned that, hypercellularity is an inflammatory disease of the glomerulus associated with an increase in the number of cells in the glomerular tufts and accompanied by proteinuria (Cotran et al., 1994).

Our electron micrographs showed fusion of foot processes that form blunted sheet of epithelial cytoplasm. Similar results were obtained by Bosquet et al. (1997) who reported a case that developed nephrotic syndrome after lithium therapy and showed fusion of foot processes. In many diseases associated with proteinuria, the foot processes are replaced by a continuous cytoplasmic band along the glomerular basement membrane (Tisher and Madsen, 1991). In this regard, numerous studies have long established the fact that lithium administration is accompanied by appearance of protein in urine (Markowitz et al., 2000).

In the present work, loss of foot processes (in certain sites) from glomerular basement membrane was observed in mice treated with either high or low dose of Prianil C-R. This finding is in agreement with the results obtained by Hagi et al. (1982) in lithium treated rats. An increase in the thickness of glomerular basement membrane was observed in mice administrated with high dose of Prianil C-R. Thickening of the basement membrane was suggested to be due to increased deposition of glycoproteins (McManus, 1948) or due to increased new formation of basement membrane material of normal composition (Klinger, 1968).

In addition, our results demonstrated that, the cellular body of podocyte contains altered nuclei with irregular nuclear envelope, blebbing of the outer nuclear membrane and margination of the heterochromatin. Reaggregation and redistribution of nuclear chromatin correspond in all respects to the classic “karyolysis” in cells injured in a wide variety of ways (Trump et al., 1965). On the other hand, mice treated with low dose of Prianil C-R showed some epithelial cells that exhibited irregular shaped nuclei with increased number of nuclear pores and dilated space between nuclear membranes. Dilatation of the nuclear envelope can be interpreted in the view of the data reported by Faith and Trump (1965) in necrotic in vitro systems. They suggested that with necrosis, aggregation of chromatin fibers and interchromatin granules occurred in early stages. Chromatin tended to collect along the nuclear envelope and around nucleoli, this was followed by fragmentation of chromatin strands and dissolution of interchromatin granules. Concomitant with these changes the space within the nuclear envelope became dilated.

Our results demonstrated that Prianil C-R induced amitotic division in the mesangial cells of mice treated with the high dose while, in mice treated with the low dose, it resulted in an increase of the amount of mesangial matrix and the appearance of mesangial cells with clefted or segmented nuclei. These results may confirm the hypertrophy of glomeruli seen histologically in the current study and are in line with the findings of other research workers in different pathological instances (Osterby et al., 1993; Yamanouchi et al., 1994; Stacchiotti et al., 2002). On the other hand, it was noticed that Prianil C-R treatment resulted in the appearance of Hyaline droplets in proximal convoluted tubular lumen and this is in the line with Singhal and Jain (1997) who reported that, the presence of hyaline droplets within the cells of the proximal convoluted tubules can often appear to displace the nucleus causing nuclear necrosis and represent the protein which has been reabsorbed from the glomerular filtrate.

In mice treated with high dose of lithium, the proximal convoluted tubules showed a development of luminal extensions of some cells that devoid of microvilli. The dilatation of the proximal tubules and loss of the brush border was described as ‘distalization’ in which the proximal tubules resemble the distal ones (Tisher and Brenner, 1989).

Myelin figures were also observed in the lumen of proximal convoluted tubules of treated mice and this may be attributed to the impairment of lysosomal enzyme activity. This finding is in agreement with that of Badawy et al. (1989) who showed an increase in the acid phosphatase (lysosomal enzyme) of renal tubules in lithium treated mice.

Our electron micrographs demonstrated swollen mitochondria, destruction of the mitochondrial membranes.
and retraction of the mitochondrial cristae in mice treated with high dose of Prianil C-R. In the opinion of Ghadially (1975), the swelling of mitochondria which takes place under chemical administration or drug's treatment might be the result of an increased uptake of water or solutes leading to mitochondrial swelling as a subsequent event. Destruction of mitochondrial membranes and retraction of mitochondrial cristae were described also in renal tubules of rats treated with lithium (Evan, 1973). Hypertrophied Golgi apparatus was one of the main ultrastructural alterations observed in the present study. Dilation of the Golgi complex was observed in renal tubular cells of mice after captopril and captopril with furosemide treatment (Ahmed, 2000). We believed that the increase in the size of Golgi apparatus, observed in the present study, may be due to an increased activity of the apparatus to overcome the effect of lithium.

The most striking change involving the rough endoplasmic reticulum (rER) during the course of this investigation was the partial loss of ribosomes from some areas of the cisternal membranes of the rER with dilatation of cisternae. Similarly, Beskid et al. (1989), reported that the rough elements of the endoplasmic reticulum had the form of dilated irregular canaliculi in the proximal convoluted tubular cells of rats treated with lithium carbonate. However, report by Roncero et al. (1992), revealed that this change might be consequence of final hyperactivity prior to cell necrosis.

Other ultrastructural change observed during this study was the shedding of ribosomes from the surface of the rER cisternae, leading to an increase in free ribosomes. Hoffman et al. (1975) stated that, detachment of ribosomes and an apparent increase in free ribosomes and polyribosomes in the cytoplasm of rat liver cells were frequent findings following acute cadmium acetate intoxication. They suggested that this apparent increase in ribosomes could be the result of toxic disorganization of rER or of an increase in the production of polysomes by the nucleus.

In the present investigation, clear peripheralization and clumping of heterochromatin granules, indicating the pyknosis of the cells, were observed in nuclei of some proximal tubular cells of mice treated with high dose of Prianil C-R. This is in conformity with observations reported by Bosquet et al. (1997) who noticed two small foci of tubular atrophy in a case who developed nephrotic syndrome after lithium treatment. The nuclear changes observed in the present study may be secondary to the derangement of mitochondrial morphology (swollen), dilatation of the rough endoplasmic reticulum and hypertrophy of Golgi apparatus.

In the distal convoluted tubules of Prianil C-R treated mice cytoplasmic vacuolation was noticed. This goes well with Walker et al. (1983 & 1986) who reported a distinctive cytoplasmic vacuolation in cells lining the distal convoluted tubules after lithium treatment. According to Asztalos et al. (1990), the focal development of empty vacuoles might be the starting point of the cellular autolytic process. This agree with Zhu et al. (1996) who found cross-sections containing necrotic cells in the distal convoluted tubules of Wistar rats after administration of lithium chloride.

Treatment of mice with low dose of Prianil C-R resulted in the flattening of the epithelium that lines the distal convoluted tubules. This coincides with the results of Olessen et al. (1980) who reported that lithium administration led to flattening of the tubular epithelium of the distal convoluted tubules and collecting duct of treated rats. Similarly, Christensen et al. (1985) demonstrated that, in the developing rat kidney, the distal tubular epithelium was flattened and often showed bulging of the luminal surface over the nuclei after lithium administration.

Moreover, Matthopoulos et al. (1995) observed damage occurred in kidney during long lithium treatment. They reported that, at early stages, cells of kidney originate after exposure to lithium chloride, become flatter on their substrate and upon longer than 4 days treatment, these cells begin to detach from their substrate and eventually cell death becomes in a concentration dependent manner. Widening of the tubular lumen was also detected in Prianil C-R treated mice, either with high or low dose. This finding is consistent with the data reported by Tisher and Brenner, (1989) who reported that, dilatation of the distal convoluted tubules is a prominent feature of ischaemic tubular necrosis.

Collectively, the present investigation revealed that the most severe changes, involved both glomeruli and renal convoluted tubules, were observed in mice treated with high dose of Prianil C-R, whereas mice treated with low dose showed relatively milder lesions in glomeruli, but the renal convoluted tubules had undergone damage. Moreover, the proximal convoluted tubules appeared to be more affected by lithium administration than the distal ones. This may be due to the fact that the proximal convoluted tubules are highly specialized regions of the nephron and it is the first site coming in contact with the toxic agent after its filtration by the glomeruli (Herptinstall, 1985).

REFERENCES


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