ROLE OF DIETARY CALCIUM SUPPLEMENTS OR CALCIUM CHANNEL BLOCKERS IN PROTECTION AGAINST GENTAMICIN NEPHROTOXICITY IN RATS

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INTRODUCTION

Nephrotoxicity associated with aminoglycoside antibiotics remains a common clinical problem (Smith et al., 1983). Many interactions between calcium and aminoglycoside antibiotics are known. Pminogcycoside binding to bacterial membranes (Bryan & Vanden Elzen, 1977), to plasma proteins (Myers et al., 1978) and to isolated phospholipid membranes is inversely related to the ionized calcium concentration in the medium. Calcium may also reverse or prevent aminoglycoside - induced neuromuscular blockade (kornguth et al., 1980). Literature about the possible role of increasing dietary calcium in prophylaxis and / or

treatment of aminoglycosides induced nephrotoxicity show great deal of controversies. Several investigators have reported that in rats, oral calcium is associated with a marked amelioration in gentamicin - induced renal failure (Weinberg & Humes, 1980; Bennett et al., 1982; Humes et al., 1984 and Quarum et al., 1984). In contrast, Tamer et al., (1989) denied any beneficial role of high calcoium diet in gentamicin nephrotoxicity.

The effect of calcium channel blockers on nephrotoxic effect of aminoglycosides is a matter of much controversy. Diltiazem was assumed to enhance gentamicin nephrotoxicity

(Gomez et al., 1989 and Gibey & Henry, 1990). On the other hand, Lee et al., (1987) and Sokol et al., (1989) postulated that nitrendipine and verapamil could significantly ameliorate gentamicin - induced nephrotoxicity in rats, other reports stated that these two calcium channel blockers have no effects (Kacew, 1989 a; Tamir et al., 1989 and Niemczyk et al., 1990). Because of these controversies, the present study was designed to investigate the effect of dietary calcium loading and a calcium channel blockers, nifedipine, on gentamicin induced nephrotoxicity in rats using the same experimental model.

MATERIAL AND METHODS

Male albino rats, weighing 100-150gm each, were caged under similar conditions. They were provided with diet of identical constituents except for calcium carbonate contents. Rats were divided into the following three main groups, each of 12 rats :

Group I (Control group) :

Rats received normal diet containing calcium carbonate 0.5% by weight

to provide sufficient calcium for the growing rats (Corbin, 1976 & Rogers, 1979). for 4 weeks.

Group II :

Rats fed high calcium diet, 4% calcium carbonate by weight, for 4 weeks (Quarum et al., 1984).

Group III :

Rats were supplied with normal calcium diet. They were treated with nifedipine (Adalat capsule, Bayer) in the dose of 2 mg/kgm/day orally for 4 weeks (Puls, 1979).

By the end of the 2nd week, 6 rats of each group were injected intraperitoneally with gentamicin (Garamycin ampoules Memphis, Egypt) 20 mg/kgm body weight twice daily for 2 weeks, the time reported for development of maximal gentamicin nephrotoxicity (Quarum et al., 1984). The remaining rats in each group were injected intraperitoneally with equal volume of normal saline.

At the end of the 4th week, the animals of each group were placed singly

into metabolic cages for urine collection during the last 24 hours before sacrifice. The volume of collected urine was measured and blood samples were obtained from retroorbital sinus and sera were separated. Urine and serum samples were taken for further biochemical analysis. Then, animals were sacrificed and the kidneys were gently dissected out under aseptic conditions.

Biochemical Studies :

- 1) Urinary concentrations of glucose (Trinder, 1969), albumin (Doums & Bleggs, 1972) and alkaline phosphatase activity (King & King, 1954) were estimated.
- 2) Serum and urinary levels of creatinine were estimated by the modified method of (Popper, H, 1937).
- 3) Urinary concentrations of sodium & potassium were measured by flame photometer (Hald, 1947 & Admisen, 1967).
- 4) Urinary & serum calcium concentrations were analysed (Ray & Chauhan, 1967) only for the animals that were not injected

with gentamicin.

- 5) Creatinine clearance was calculated according to the following equation.

$$\text{Creatinine clearance} = U \times V / P$$

Where :

U : Concentration of creatinine in one ml of urine.

V : Volume of urine/min.

P : Concentration of creatinine in one ml of serum.

Histopathological & Histochemical Study :

The other kidney of each rat was immediately divided into two identical parts. The first part was fixed in 10% formalin to be processed as paraffin section and stained with Hematoxylin and Eosin. The second part was cut as frozen sections for histochemical study of renal enzyme activity. Frozen sections were stained by Gomori's methods (1952) for alkaline phosphatase, Azo dye method of Baraka (1960) for acid phosphatase and Pearse's method (1980) for succinic dehydrogenase.

RESULTS

Control Rats :

The data obtained from rats that were not treated with gentamicin in the three groups are presented in tables (1&2). Rats in the three groups had similar creatinine clearance, serum & urine creatinine levels, urine output, urinary excretion of sodium and potassium. The only significant difference is a higher urinary calcium excretion in high dietary calcium group.

Histopathological examination of these rats showed more or less normal renal tissue with no significant pathological changes in these three groups (Fig. 1).

Histochemical examination demonstrated that :

- The alkaline phosphatase enzymatic activity appears strong in the proximal tubules and absent in collecting tubules. The enzymatic activity is restricted at the brush borders of the lining epithelium of the tubules (Fig. 4).
- Acid phosphatase enzymatic activity appears moderate in the

proximal tubules and absent or weak in the collecting tubules.

The enzymatic activity occurs more at the basal part of the lining epithelium (Fig. 7).

- Succinic dehydrogenase enzymatic activity appears strong in proximal convoluted tubules but moderate in distal tubule and mild in collecting tubules (Fig. 10).

Gentamicin Treated Rats :

The data of gentamicin treated rats are illustrated in tables (3&4). The results show that i. p. gentamicin injection alone for 2 weeks in rats fed normal calcium diet produced classical manifestations of aminoglycosides nephrotoxicity including significant elevation of serum creatinine with reduction of creatinine clearance, increased urinary alkaline phosphatase activity, increased urinary excretion of sodium & potassium and marked glucosuria and albuminuria, while volume of urine output is maintained more or less equal to control value.

Histopathological examination of this group revealed diffuse and

marked tubular lesion mostly in proximal tubules where cloudy swelling, hydropic degeneration and necrosis of epithelial cells were marked. Some tubules showed desquamation of their lining epithelium, and their lumina were plugged with cast of eosinophilic material (Fig. 2). The histochemical examination showed that alkaline phosphatase enzymatic activity was lost from the lining epithelium of many of the tubules. Only scattered tubules showed enzymatic activity that appeared weaker than normal (Fig. 5). Acid phosphatase enzymatic activity in the proximal tubules appeared strong, i. e. higher than normal control (Fig. 8). Succinic dehydrogenase enzymatic activity appeared weaker in some tubules than normal (Fig. 11).

Dietary calcium supplementation in group (II) and nifedipine treatment in group (III) are associated with marked preservation of creatinine clearance, reduction of serum creatinine value, lower urinary alkaline phosphatase activity and decreased urinary excretion of sodium, potassium, glucose and albumine while the volume of urine

remained unchanged.

The histopathological examination, in these two groups, demonstrated marked improvement in the histological picture of gentamicin induced tubular damage with only mild scattered degenerative changes of proximal tubule epithelium. There was no evidences of necrosis (Fig. 3). In addition, the histochemical examination revealed marked restoration of the renal tissue enzymatic activity which appeared more or less similar to the normal controls that had not received gentamicin. Only there were few scattered tubules of altered enzymatic activity (Fig. 6, 9, 12).

DISCUSSION

The laboratory, histopathological and histochemical findings demonstrated in the present study are consistent with the typical pattern of aminoglycosides induced nephrotoxicity described by many investigators (Emerson & Pryse, 1964; Luft et al., 1975; Cohen et al., 1975; Beck et al., 1977; Mondorf et al., 1978; Cronin et al., 1980; Quarum et al., 1984;

kitasato et al., 1989).

The initial renal manifestation of gentamicin nephrotoxicity is enzymuria. The marked increase in urinary loss of the brush border membrane alkaline phosphatase in the present study may be due, in part, to the effect of gentamicin on membrane phosphatidylinositol required for the binding of alkaline phosphatase (Low & Zilversmit, 1980) as well as to the focal loss of brush border membranes of proximal renal tubule epithelial cells rich in this enzyme (Just & Habermann, 1977; Walshe & Venuto, 1979; Lipsky et al., 1980; Sastrasingh et al., 1982a and Kacew, 1989 a, b). These factors could also explain the decrease in renal cortical alkaline phosphatase activity demonstrated by histochemical examination in the present study. The increase of the lysosomal enzyme acid phosphatase activity in proximal tubular epithelial cells on histochemical examination could be explained by the electron microscopic studies of Mondorf et al., (1978) and Regec et al., (1989) who demonstrated an increase in the number and size

of lysosomes with changes in their structure accompanied with lysosomal enzymuria and increased cortical content of some lysosomal enzymes. Histochemical examination also revealed a reduction of succinic dehydrogenase activity this finding is a supportive evidence for gentamicin induced mitochondrial dysfunction as demonstrated by Simmon et al., (1980).

Aminoglycosides nephrotoxicity was reported to be characterised in its early stages by a number of tubule transport abnormalities such as proteinuria and glucosuria (Emerson & Pryse-Davies, 1964; Ginsburg et al., 1976; Appel & Neu, 1977 and Cronin et al., 1980) this is consistent with our result. Gentamicin inhibits sodium - dependent glucose uptake in isolated renal brush border membrane that is antagonised by the divalent cations (Sastrasingh et al., 1982 b).

The increase in urine sodium and potassium excretion demonstrated during gentamicin toxicity in the present study might be attributed to the aminoglycosides - induced cellular

calcium dyshomeostasis (Humes & Weinberg, 1986 and Robert & Thomas, 1988). Since, Taylor & Windhager (1979) reported that any increase in cytosolic calcium reduces net sodium transport because the cytosolic calcium plays a regulatory role for sodium transport due to the existence of $\text{Na}^+ - \text{Ca}^{++}$ exchange mechanism across the basolateral cell membrane of proximal and collecting tubules as originally proposed by Blaustein (1974). Accordingly, active transport of potassium is inhibited because large amounts of potassium are reabsorbed as a result of potassium co-transport with sodium in both the proximal tubules and the thick ascending limbs of the loops of Henle (Guyton, 1986). Moreover, acidic phospholipids, major plasma membrane aminoglycoside binding sites (Sastrasinh et al., 1982 a), are required for optimal activity of $\text{Na}^+ - \text{K}^+$ ATPase sodium pumping protein in the basolateral membrane of the distal tubule. Aminoglycosides inhibit this enzyme in vitro and in vivo (Lipsky & Lietman, 1980; Williams et al., 1981; Sastrasinh et al., 1982 a and Guyton, 1986).

These factors may explain the increase in urine sodium and potassium losses demonstrated in the present study.

In accord with the present findings, gentamicin nephrotoxicity is classically associated with a nonoliguric type of acute renal failure, so that excretory failure may develop in the presence of normal urine output. This may be attributed, at least in part, to a water diuresis resulting from urinary concentration defect and insensitivity to antidiuretic hormone due to inability of injured kidney to maintain its hypertonic medullary interstitium (Cohen et al., 1975; Luft et al., 1975 and Cronin et al., 1980). Inhibition of ADH activity at the level of adenylate cyclase may be an additional factor (Gordon et al., 1981).

In agreement with our histopathological findings, Baylis et al., (1977); Apple & Neu, (1977); Schor et al., (1981); Humes & weinberg., (1983) and Matsuda et al., (1988) demonstrated that injury of renal proximal tubule epithelium is a key event in

the pathogenesis of gentamicin nephrotoxicity. These findings may be explained by rapid concentration of gentamicin in renal cortex as a result of avid proximal tubular brush border membrane binding, pinocytotic uptake and lysosomal storage (Morin et al., 1980 and Quarum et al., 1984).

The demonstrated elevation of serum creatinine with concomitant reduction of creatinine clearance indicate a decline in glomerular filtration rate. It is thought to be primarily due to decreased capillary ultrafiltration coefficient, although a mild fall in glomerular plasma flow may also be contributory (Schor et al., 1981). This functional derangements in glomerular hemodynamics are partially secondary to intrarenal angiotensin II generation that increases vascular resistance and thereby declines the glomerular ultrafiltration and the glomerular plasma flow (Myers et al., 1975; Blantz et al., 1976; Schor et al., 1981 and Humes & Weinberg, 1986). However, in advanced stages of gentamicin nephrotoxicity when tubular necrosis become marked, the reduction of glomerular filtration is augmented not only due to

glomerular alterations but also due to tubular obstruction and concomitant back leak of filtrate (Neugarten et al., 1983).

The susceptibility of the kidney to aminoglycoside toxicity is derived from its ability to transport and accumulate these antibiotics in toxic concentration (Luft & Kleit, 1974 and Fabre et al., 1976). Although the morphological and whole-organ physiological events of gentamicin-induced acute renal failure have been extensively studied, little is known of the biochemical alterations provoked by gentamicin which lead to renal cell injury and ultimately to renal cell death (Appel and Neu, 1977 and Morin et al., 1980).

The cellular toxicity of aminoglycosides is directly related to their chemical structure. They have cationic properties, therefore, they have high affinity for acidic phospholipids (Humes & Weinberg, 1986). Several studies suggested that the acidic phospholipids are the major binding sites for aminoglycoside in plasma membranes of proximal tubule cells (Lipsky et al., 1980 and Humes et al., 1984).

Membrane phospholipids, in addition to their contribution to membrane structure, play important roles in the regulation of membrane permeability, in the function of membrane bound enzymes and in hormone-membrane receptor interactions. The affinity of aminoglycosides for membrane phospholipids, thus, allows them to potentially influence all these functions.

Gentamicin bounded to cell membrane is then incorporated into apical vesicles inside cytoplasm then the drug is largely sequestered into the lysosomes. Aminoglycosides - acidic phospholipid interactions is not restricted to the outer cell membrane but also it affects other subcellular membranes including lysosomal and mitochondrial membranes (Morin et al., 1980 and Bennett et al., 1988) with subsequent alterations of their permeability associated with significant instability of these membranes, structural and functional changes of these intracellular organelles which may be responsible for the extensive dysfunction of cells of proximal tubules where gentamicin accumulates (Wellwood et

al., 1976 and Morin et al., 1980). These lysosomal and mitochondrial changes occur early before frank cellular necrosis and therefore may be an early biochemical event in the pathogenesis of renal cell injury (Simmon et al., 1980). In addition, binding of gentamicin to ribosomes with subsequent inhibition of protein synthesis is also demonstrated (Bennett et al., 1988). Which cellular or subcellular component is most critical in development of aminoglycosides - tubular cell injury is not known. Undoubtly, no single membrane - toxin interaction is sufficient, rather each is necessary and interrelated (Simmon et al., 1980 and Hymes & Weinberg, 1986).

It is postulated that the toxic injury to the plasma membrane can lead to cellular calcium overload that plays a pathogenic role in both acute and chronic renal diseases (Schanne et al., 1979 and Farber, 1982). Cellular calcium intoxication can occur as a consequence of gentamicin - induced increase membrane permeability to calcium by unknown mechanism. It is reported that if the mitochondrial pool

becomes overloaded with calcium, oxidative phosphorylation is uncoupled, calcium dependent proteases and phospholipases are activated and autolysis of intracellular components takes place causing cell death. The inhibition of cortical mitochondrial oxidative phosphorylation induced by gentamicin is thought to be as a result of gentamicin - induced block of electron transport along cytochrome respiratory chain. Moreover, the early loss of mitochondrial calcium to cytosol activates calcium - calmodulin dependent phospholipase resulting in the degeneration of membrane phospholipids (Simmon et al., 1980 and Robert & Thomas, 1988). However, it is noteworthy to mention that Browning (1990) speculates that Ca^{++} influx into the cytosol and mitochondria is not a requirement for the cell injury in some toxic insults or it is not a necessary common step in lethal processes of all toxic compounds but rather an epiphenomenon which could be a marker of the toxicity.

Our results show that dietary calcium loading ameliorates gentamicin

nephrotoxicity as measured by renal functional biochemical indices and by histopathological and histochemical evaluation of renal cell injury. In agreement with our study several investigators have reported recently in rats that oral calcium loading is associated with a marked attenuation in the severity of gentamicin nephrotoxicity (Simmon et al., 1980; Goldszer et al., 1981; Humes et al., 1984 and Corrado et al., 1989). In contrast, Tamir et al., (1989) denied any beneficial effect of high calcium diet in reducing gentamicin nephrotoxicity. However, in Tamir's study, they stated that additional nutritional factors may be responsible for this controversy since dietary deficiencies of sodium, potassium & magnesium have been shown to potentiate gentamicin nephrotoxicity (Bennett et al., 1976 and Kohlhepp et al., 1982).

The mechanism by which increased dietary calcium protects against gentamicin nephrotoxicity remains speculative. Based on the experience in animal models, calcium present in adequate concentrations acts as a

calcium channel blocker controlling its own flux rate across the cell membrane (Eckert and Eward, 1982 and Hurwitz et al., 1982). This could decrease gentamicin toxicity which was proposed to be associated with cellular calcium overload (Holohan et al., 1988). Moreover, calcium is found to be an effective competitive inhibitor of gentamicin binding to biological membranes (Humes et al., 1984). Therefore, increasing dietary calcium will increase delivery of calcium to renal tubules which in turn suppress ionic binding of gentamicin to tubular brush border membranes. Since this binding is preliminary step for uptake of gentamicin by renal tubular epithelium (Kohlhepp et al., 1982), gentamicin uptake by tubular epithelium will be restricted and its concentration in renal tissue will be decreased in rats fed high calcium diet and gentamicin toxicity will be attenuated (Humes et al., 1984 and Quarum et al., 1984).

A less direct inhibition of renal tubular cell gentamicin uptake in rats on high calcium diet could result from the parathyroid suppression that occurs

during calcium loading, since parathyroid hormone has been shown to increase gentamicin phospholipid receptors in renal tubules and to enhance gentamicin nephrotoxicity (Nakahama et al., 1990). Parathyroid suppression thus may restrict the number of receptor sites and thereby limit drug uptake. Moreover, parathyroid suppression might exert an additional protective effect during gentamicin administration, since parathyroid hormone increases cytosolic free calcium by augmenting mitochondrial calcium efflux (Borle, 1973) and by a direct calcium ionophoric action on cell membrane (Sabau et al., 1982). Accordingly in cell with increased calcium permeability because of gentamicin toxic injury.

Parathyroid suppression may be beneficial in preventing cellular calcium overload which was assumed to have a primary importance in pathogenesis of gentamicin acute renal failure (Burke et al., 1982 and Holohan et al., 1988). On the other hand, Humes et al., (1984) found that calcium supplements are not accompanied with any change in cortical gentamicin

concentration, therefore, they assumed that the protective action of calcium is not essentially extracellular to prevent gentamicin membrane binding and uptake and it is possible that the protective effects of calcium are mediated by inhibiting gentamicin action within the renal cells rather than on their surface or that some other metabolic alterations occurring during calcium loading may play a major role in the protection (Simmon et al., 1980).

The possibility that high calcium diet exert a nonspecific salutary effect on proximal tubular cell integrity has not been excluded. This salutary effect may be not limited to the setting of gentamicin nephrotoxicity and further studies of the effect of dietary calcium on other models of acute tubular injury is necessary (Quarum et al., 1984).

The present study revealed a significant protective effect of nifedipine against gentamicin nephrotoxicity in rats. The effect of calcium channel blockers on aminoglycosides nephro-

toxicity is a matter of much controversy (Donoker et al., 1976; Rogers, 1979; Lee et al., 1987; Tamir et al., 1989; Gomez et al., 1989; Sokol et al., 1989 and Gibey and Henry, 1990). These controversies could be hardly explained, however the type of calcium channel blocker used may be a factor since these drugs are known to differ in their affinity to different receptor sites in the calcium channels (Murphy et al., 1983 and Ruth et al., 1985) and in their affinity to the same type of calcium channels in different tissues (Glossman, 1985). Moreover, the distribution of binding sites is somewhat different in various tissues (Glossman et al., 1985). Also the difference in molecular structure of these drugs could alter some critical chemical configuration essential for competitive inhibition with gentamicin. Difference in the dose administered or species of the used animals are other possibilities which have to be examined.

It is not clearly documented whether the mechanism (s) of the protective effect of calcium channel blockers are

exerted on vascular, epithelial or both types of renal tissue (Papadimitriou et al., 1983 and Goldfarb et al., 1985).

Studies of Burke et al., (1984); Goldberg & Schrier (1984) demonstrated the potential vascular protective effects of calcium channel blockers. They reported that these drugs, especially nifedipine & verapamil, may attenuate the increase in vascular resistance and correct the decrease in glomerular ultrafiltration coefficient and renal blood flow in angiotensin treated rats by inhibiting the angiotensin-induced increase in smooth muscle or mesangial cell calcium concentration responsible for the vasoconstrictor effect of angiotensin. Since, it is likely that gentamicin nephrotoxicity often occurs in a setting in which renal angiotensin generation is stimulated, calcium channel blockers may reserve gentamicin-induced derangements in glomerular haemodynamics that are proposed to be responsible for the reduction in glomerular filtration rate at early stages of gentamicin nephrotoxicity (Schor et al., 1981). In support to this concept

Schor et al., (1981) reported that chronic concurrent administration of the angiotensin I converting enzyme inhibitor, captopril, could prevent gentamicin induced glomerular dysfunction.

An additional factor that may contribute to improvement in glomerular ultrafiltration by calcium channel blockers is perhaps through stimulation of intrarenal vasodilator prostaglandins synthesis (Zacharieva et al., 1989). Mehta (1985) suggested that the calcium channel blockers-induced increase in PGs may be a result of concomitant inhibition of thromboxan A₂ leading to diversion of cyclic endoperoxides to the prostacyclin pathway with subsequent increase of the antiagregant and vasodilator prostacyclin.

The other effects of calcium channel blockers in protecting renal functions may reside at the level of injured renal tubule at non vascular epithelial tissue (Holohan et al., 1988). It is demonstrated that the protective effects of calcium channel blockers are

associated with normalization of renal histology (Zusman & keiser, 1977; Henrich et al., 1978; and Robert & Thomas, 1988). Also, it is found that nifedipine or verapamil could largely improve mitochondrial respiratory dysfunction induced by gentamicin in vitro (Stacey & kappus, 1982).

It is possible that calcium channel blockers could protect against gentamicin renal cell injury by blocking gentamicin induced increase of calcium influx (Holohan et al., 1988), since it is assumed that cellular and/or mitochondrial calcium overload may be largely responsible for the deleterious effects of gentamicin on renal tubule epithelium (Simmon et al., 1980 and Robert & Thomas, 1988).

Another proposed cellular protective mechanism of calcium channel blockers is based upon chemical nature of gentamicin and calcium channel blockers, both are weak bases and have cationic properties at physiological pH (Corrado et al., 1989). Accordingly, calcium channel blockers could compete with gentamicin for

biological membrane acid phospholipids known as specific receptors for gentamicin binding (Schacht, 1979; Lipsky et al., 1980; Sastrasinh et al., 1982 a and Humes et al., 1984). Since binding of gentamicin to phospholipid is essential for its cellular uptake and is responsible for the gentamicin induced disturbances of structure, function and permeability of cell membranes, calcium channel blockers could therefore limit membrane binding of gentamicin and prevent its membrane toxic effects (Simmon et al., 1980). Moreover, it could be assumed that competition between calcium channel blockers and gentamicin on binding to biological membranes, is not restricted to cell surface but it might to suspected to interfere with binding of gentamicin to subcellular membranes thus, could protect cell organelles from the injurious effects of this drug.

Finally, it was stated that lipid peroxidation may play a significant role in acute nephrotoxicity of gentamicin. Since calcium channel blockers, were proved to have an antioxidant property both in vitro (Shrida & Robak, 1982).

and in vivo (Yegen et al., 1990), nifedipine may act through this antioxidant property to prevent lipid peroxidation and thereby can protect against gentamicin nephrotoxicity.

In summary, nutritional and/or pharmacologic interventions that specifically modify calcium metabolism appear to be specific means for reversing the abnormalities of calcium metabolism associated with gentamicin nephrotoxicity. The possible clinical relevance of these observations is unclear. Patients prone to gentamicin toxicity may have deficiencies of dietary calcium, hypocalcaemia or hypocalciuria (Quarum et al., 1984). Conceivably, correction of these calcium abnormalities could decrease the likelihood or severity of the toxic injury. It is tempting to further speculate on a possible role of calcium or calcium channel blockers as a therapeutic modality in this common nephrotoxic insult.

SUMMARY

A great deal of controversies is present about the effects of dietary

calcium supplements or calcium channel blockers on gentamicin induced nephrotoxicity. The present study was designed to evaluate the protective effect of either of them against gentamicin nephrotoxicity in rats using the same experimental model.

Daily I. P. injection of gentamicin 40 mg/kgm/day for 2 weeks in albino rats produced the characteristic pattern of aminoglycosides nephrotoxicity in rats as judged by the significant increase in urine alkaline phosphatase excretion accompanied with a significant decrease in renal tissue alkaline phosphatase and succinic dehydrogenase activities meanwhile cortical renal tissue acid phosphatase activity was increased. Also there was significant glucosuria, albuminuria and increased urine sodium and potassium excretion. Histopathological examination revealed that gentamicin produced extensive tubular degenerative and necrotic changes which were almost restricted to the proximal convoluted tubules. In addition, gentamicin produced a significant elevation of serum creatinine with reduction of

creatinine clearance meanwhile volume of urine output was maintained unchanged.

Feeding rats a diet with high calcium content (4% calcium carbonate by weight) for 2 weeks before gentamicin and for another 2 weeks concurrently with gentamicin produced a significant protection against gentamicin induced nephrotoxicity as evidenced by the significant correction of all the laboratory measures estimated and almost complete normalization of the histopathological and histochemical alterations produced by gentamicin. Oral administration of nifedipine 2 mg/kgm/day for 2 weeks before gentamicin and for another 2 weeks concurrently with gentamicin injection

produced similar protection to that produced by dietary calcium supplements. The possible clinical relevance of these observations is unclear. Patients prone to gentamicin nephrotoxicity may have deficiencies of dietary calcium, hypocalcaemia or hypocalciuria. Conceivably, correction of these calcium abnormalities could decrease the likelihood or severity of the toxic injury. It is tempting to further speculate on a possible role of calcium or calcium channel blockers as a therapeutic modality in this common nephrotoxic insult. However, any conclusion must await further informations concerning the specificity of the protective effect of calcium or calcium channel blockers.

Table 1 : Effect of high calcium diet or nifedipine on urinary excretion of calcium & creatinine and serum concentration of calcium & creatinine in normal rats.

Group \ Parameter	Control (Normal Ca ⁺⁺ diet*)	Nifedipine (2mg/kgm for 4 weeks)	High Calcium Diet *
- Calcium Excretion (mg/24 hr.)	0.215	0.203	0.319
P	±0.04	±0.029	±0.02
P ₁		N. S	<0.001
			<0.001
- Serum Calcium (mg %)	10.950	11.000	10.730
P	±1.071	±2.183	±1.208
P ₁		N. S	N. S
			N. S
- Creatinine Excretion (mg/24 hr.)	2.045	2.049	2.047
P	±0.35	±0.612	±0.45
P ₁		N. S	N. S
			N. S
- Serum Creatinine (mg %)	0.575	0.510	0.493
P	±0.064	±0.099	±0.025
P ₁		N. S	N. S
			N. S

All value represent the mean $\pm$ standard deviation.

Number of rats in each group = 6

* Normal calcium diet contain 0.5% calcium carbonate by weight.

** High calcium diet contain 4% calcium carbonate by weight.

P : As compared with control group.

P₁ : As compared with nifedipine group.

N. S. : Not significant.

Table 2 : Comparison between effect of high calcium diet or nifedipine on urine volume and urinary excretion on Na^+ , K^+ and alkaline phosphatase in normal rats.

Group Parameter	Control	Nifedipine	High Calcium Diet
- Urine Volume (ml/24 hr.)	7.186	7.412	7.025
P	± 1.121	± 1.211	± 1.184
P ₁		N. S	N. S
- Na^+ Excretion (mEq/24hr)	0.558	0.546	0.554
P	± 0.046	± 0.069	± 0.096
P ₁		N. S	N. S
- K^+ Excretion (mEq/24 hr.)	0.182	0.170	0.180
P	± 0.02	± 0.034	± 0.031
P ₁		N. S	N. S
- Alkaline Phosphatase Excretion (IU/24hr)	0.170	0.173	0.171
P	± 0.015	± 0.021	± 0.033
P ₁		N. S	N. S

P : As compared with control group.

P₁ : As compared with nifedipine group.

N. S. : Not significant.

Table 3 : Effect of high calcium diet or nifedipine on urine volume, urinary creatinine excretion, serum level of creatinine and creatinine clearance in gentamicin-affected rats.

Group Parameter	Control	Gentamicin 40 mg/day I. P. for 2 W.	Gentamicin + Nifedipine	Gentamicin + high Ca++ Diet
- Urine Volume (ml/24 hr.)	7.186 ±1.121	7.072 ±1.101	7.057 ±2.001	7.157 ±2.021
P		N. S	N. S	N. S
P ₁			N. S	N. S
P ₂				N. S
- Creatinine Excretion (mg/24hr)	2.045 ±0.35	0.873 ±0.085	1.179 ±0.130	1.263 ±0.135
P		<0.001	<0.001	<0.001
P ₁			<0.001	<0.001
P ₂				N. S
- Serum Creatinine (mg %)	0.575 ±0.064	3.393 ±0.170	0.695 ±0.062	0.710 ±0.079
P		<0.001	<0.01	<0.01
P ₁			<0.001	<0.001
P ₂				N. S
- Creatinine clearance (ml/min.)	0.247 ±0.047	0.018 ±0.002	0.118 ±0.033	0.124 ±0.024
P		<0.001	<0.001	<0.001
P ₁			<0.001	<0.001
P ₂				N. S

All values represent the mean ± Standard deviation.

Number of rats in each group = 6

N. S. : Not significant.

P : As compared with control group.

P₁ : As compared with gentamicin group.

P₂ : As compared with gentamicin + nifedipine group.

Table 4 : Effect of high calcium diet or nifedipine on urinary excretion of Na⁺, K⁺, Glucose, Albumin and Alkaline phosphatase in gentamicin-affected rats.

Group Parameter	Control	Gentamicin .	Gentamicin + Nifedipine	Gentamicin + high Ca++ Diet
- Na ⁺ Excretion (mEq/24hr)	0.558 ±0.026	0.813 ±0.09 <0.001	0.567 ±0.05 N. S <0.001	0.584 ±0.04 N. S <0.001
P				
P ₁				
P ₂				N. S
- K ⁺ Excretion (mEq/24hr)	0.182 ±0.01	0.370 ±0.03 <0.001	0.190 ±0.03 N. S <0.001	0.184 ±0.01 N. S <0.001
P				
P ₁				
P ₂				N. S
- Glucose Excretion (mg/24hr)	0.000	4.560 ±0.685 <0.001	1.569 ±0.24 <0.001 <0.001	1.564 ±0.315 <0.001 <0.001 N. S
P				
P ₁				
P ₂				
- Albumin Excretion (gm/24 hr)	0.005 ±0.001	0.128 ±0.030 <0.001	0.036 ±0.007 <0.001 <0.001	0.031 ±0.009 <0.001 <0.001 N. S.
P				
P ₁				
P ₂				
- Alkaline phosphatase excretion (IU/24hr)	0.170 ±0.01	0.345 ±0.038 <0.001	0.194 ±0.012 <0.01 <0.001	0.187 ±0.015 <0.05 <0.001 N. S
P				
P ₁				
P ₂				

P : As compared with control group.

N. S. : Not significant.

P₁ : As compared with gentamicin group.

P₂ : As compared with gentamicin + nifedipine group.

Fig. 1: Renal cortex of a rat on normal calcium diet (normal control). Shows no pathological abnormalities (Hx. & E. X 100).

Fig. 3 : Renal cortex a rat on highcalcium diet treated with gentamicin for 14 days. Minimal degenerative changes in proximal tubule epithelium with no necrosis or casts (Hx. & E. X 160).

Fig. 2 : Renal cortex of a rat on normal diet treated with gentamicin for 14 days. Marked degeneration and necrosis of proximal tubule epithelium, some have eosinophilic casts in their lumina, (Hx. & E. X 160).

Fig. 4: Renal cortex of a rat on normal calcium diet. Shows normal alkaline phosphatase activity in proximal convoluted tubules (X 160).

Fig. 5 : Renal cortex of a rat on normal calcium diet treated with gentamicin for 14 days. Alkaline phosphatase enzymatic activity is lost in most of the proximal convoluted tubules (X 160).

Fig. 7 : Renal cortex a rat on normal calcium diet. Shows normal tubular acid phosphatase activity (X 160).

Fig. 6 : Renal cortex of a rat of high calcium diet treated with gentamicin for 14 days. Normal alkaline phosphatase activity in most tubules (X 160).

Fig. 8 : Renal cortex of a rat on normal calcium diet, treated with gentamicin for 14 days. Strong acid phosphatase activity in proximal convoluted tubules (X 400).

Fig. 9 : Renal cortex of a rat on high calcium diet treated with gentamicin for 14 days. Acid phosphatase activity within normal (X 100).

Fig. 11 : Renal cortex of a rat on normal calcium diet treated with gentamicin for 14 days. Marked decrease in succinic dehydrogenase activity (X 160).

Fig. 10 : Renal cortex of a rat on normal calcium diet. Shows normal tubular succinic dehydrogenase activity (x 160).

Fig. 12 : Renal cortex of a rat on high calcium diet treated with gentamicin for 14 days. Succinic dehydrogenase activity is more or less similar to normal controls (X 400).

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دور زيادة الكالسيوم في الغذاء أو مغلفات قنوات الكالسيوم في الوقاية من تأثير الجنتاميسين السام على الكلى في الفئران.

نظراً للتناقضات الكثيرة بشأن تأثير كل من الكالسيوم أو مغلفات قنوات الكالسيوم على مدى تأثير الجنتاميسين السام على الكلى، أجرى هذا البحث لدراسة وتقييم التأثير الواقى لكل منهما ضد سمية الجنتاميسين على الكلى في الفئران البيضاء باستخدام نفس النظام التجريبي.

أظهرت نتائج هذا البحث أن حقن الجنتاميسين ١٠ مجم/كجم يومياً في التجويف البريتوني للفئران البيضاء لمدة اسبوعين أحدث التأثيرات المميزة لتسمم الكلى الناتج عن استخدام الأمينو جليكوزيدات وتشمل مظاهر هذا التسمم زيادة نشاط أنزيم الفوسفاتاز القاعدي في البول مع نقص هذا الانزيم في أنسجة الكلى ووجد أيضاً نقص في نشاط أنزيم السكسينك ديهيدروجيناز وزيادة في نشاط أنزيم الفوسفاتاز الحمضى في نسيج الكلى. وكذلك وجدت زيادة ذات دلالة احصائية هامة في كمية اخراج السكر، الزلال، الصوديوم والبوتاسيوم في البول مع زيادة نسبة الكرياتينين في مصل الدم مصحوباً بنقص في معدل ترويق الكرياتينين بينما لم يتغير حجم إخراج البول وذلك بالمقارنة بالمجموعة الضابطة. بالإضافة إلى ذلك أظهرت نتائج الفحص المجهرى اضمحلال وموت في نسبة كبيرة من خلايا أنابيب الكلى المتتوية الأولى (القريبة).

وقد وجد أن زيادة كمية الكالسيوم في الغذاء (٤٪ كالسيوم كربونات من وزن الطعام) لمدة اسبوعين قبل حقن الجنتاميسين ولمدة اسبوعين آخرين أثناء حقن الجنتاميسين له تأثير مفيد في الوقاية من التأثير السام للجنتاميسين على الكلى حيث أن جميع التغيرات التي حدثت نتيجة إعطاء الجنتاميسين قد تحسنت بدرجة كبيرة ذات دلالة احصائية هامة كما ظهرت أنسجة الكلى بحالة شبه طبيعیه عند فحصها مجهرياً. هذا وقد وجد أن إعطاء أحد مغلفات قنوات الكالسيوم (النفيديين) ٢ مجم/كجم يومياً عن طريق الفم لمدة اسبوعين قبل إعطاء الجنتاميسين ولمدة اسبوعين آخرين أثناء حقن الجنتاميسين كان له تأثير واقى ضد الجنتاميسين يماثل ذلك الذى نتج عن زيادة الكالسيوم في الغذاء.

قد يبدو مناسباً أن نتصور امكانية استخدام الكالسيوم أو مغلفات قنوات الكالسيوم في الوقاية من تأثير الجنتاميسين السام على الكلى. ولكن تجدر الاشارة إلى أن هذا الأمر يحتاج لمزيد من المعلومات عن مدى فاعليتها في الانسان.

My dear Mr. [Name],

I have just received your letter of the 10th inst.

and am glad to hear that you are well.

I am writing you a few lines to let you know

that I have received your letter of the 10th inst.

and am glad to hear that you are well.

I am writing you a few lines to let you know

that I have received your letter of the 10th inst.

and am glad to hear that you are well.

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