

MICROSCOPIC CHARACTERIZATION OF GENTAMYCIN-INDUCED NEPHROTOXIC EFFECTS IN ALBINO RATS

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DOI: <https://doi.org/10.5281/zenodo.17378387>

Abstract

Drug-induced nephrotoxicity is an important cause of renal failure. Aminoglycosides throughout the endocytic pathway are taken up into the epithelial cells of the renal proximal tubules and stay there for a long time, which leads to nephrotoxicity. Wistar albino male rats weighing 125–150gms, are utilized for the present study. Blood samples were collected with cardiac puncture for biochemical investigations like blood urea, uric acid, creatinine, serum Na, K, Ca, determination. By using one way ANOVA the results are significant at .001. Tubular epithelial necrosis and dilatation is observed affecting less than half of cortical tubules when rats treated with 60mg/kg b.w. Hyaline cast formation is observed in PCT with atrophic glomeruli affecting half of the cortical region when rats treated with 80mg/kg b.w. gentamicin must be given in the lowest effective therapeutic doses in patients with normal kidney function.

Keywords: gentamycin, glomeruli, hyaline cast, proximal convoluted tubules, urea

Introduction

Drug-induced nephrotoxicity is an important cause of renal failure. Aminoglycosides throughout the endocytic pathway are taken up into the epithelial cells of the renal proximal tubules and stay there for a long time, which leads to nephrotoxicity. Acidic phospholipids, broadly distributed in the plasma membranes in various tissues, were considered to be the binding site of aminoglycosides in brush-border membrane of proximal tubular cells (Nagai and Takano, 2004[1]; Nagai, 2006[2]). Hydroxyl radicals play a role in the pathogenesis of gentamicin nephrotoxicity, gentamicin can induce suppression of Na(+)-K(+)-ATPase activity and DNA synthesis in rats proximal tubules leading to renal injury; this injury may be relevant to reactive oxygen metabolites generated by gentamicin. Renal cortical mitochondria is the source of reactive oxygen metabolites, which induces renal injury (Nephrol Dial Transplant. 1994;9 Suppl 4:135-40[3]).

Very few studies of histopathology were reported in literature regarding histopathology of gentamicin induced renal failure in Albino rats. So, the present study is taken up to record the nephrotoxicity by biochemical investigation and histopathological damage in the structure of rat kidney.

Materials and Methods

Wistar-albino male rats weighing 125–150gms, are utilized for the present study. Experiments were performed with the permission of the institutional ethics committee. In the present study, male albino rats were used and are grouped as follows:

- ⊙ I. controls with normal saline (group I-6 rats) for 10 days
- ⊙ II. gentamicin 60mg/kg b.w (group II-6 rats) for 10 days
- ⊙ III. gentamicin 80mg/kg b.w. (group III-6 rats) for 10 days

All rats were kept under observation for 1 week prior to the experiments to permit the animals to adjust to the environment. All animals were fed standard rat chow and were provided tap water to drink ad libitum. They were housed in a facility with 12–12 h light–dark cycle that is maintained at 25°C. All animals were weighed before the injections. The animals were anaesthetized with ether inhalation. Blood samples were collected with cardiac puncture for biochemical investigations like blood urea, uric acid, creatinine, serum Na, K, Ca, determination. Bilateral periumbilical vertical incisions were made. Right and left kidneys were removed quickly and weighed and preserved in 10% formalin.

2.1. Blood sampling:- At the end of each experimental period (10 days) and after overnight fasting, animals were given high dose of chloroform . Blood samples were collected by cardiac puncture and animals are decapitated. Blood samples are centrifuged for 10 minutes at 3000 rpm within an hour and the sera was obtained. All specimens of sera were stored at -20°C until use.

2.2. Determination of serum urea, creatinine and uric acid:- The serum parameters were analyzed spectrophotometrically by using double beam UV Visible spectro-photometer (UV-Visible spectrophotometer, Elico, model SL 150). Estimation of blood urea, and creatinine were carried out using respective diagnostic kits purchased from Transasia Bio-medicals Ltd (HP) in collaboration with Erba diagnostics Mannheim GmbH (Germany) according to the methods of Talke and Schubert et al (1965[4]), Tiffany et al (1972[5]) respectively. Estimation of serum sodium and potassium was done by using Excel diagnostic kit method.

2.3. Determination of serum electrolytes:- Sodium (Na) and Potassium (K) analysis were accomplished by Excel diagnostic kit method according to the method of (Terri A.E et al 1958[6]). Serum calcium was determined colorimetrically using commercial kits (Erba, Germany) according to the method of Moorehead W R et al 1974[7].

2.4. Histopathological examination: Kidneys from first three groups were fixed in 10% neutral buffered formalin and processed to paraffin wax. 5 microns Sections are stained with Haematoxyllin and Eosin, Massons trichrome, and Periodic Acid Schiff and are examined under light microscope at 100 and 400 magnification.

I. Results

The values obtained are very much significant as shown in Table 1.

Table (1): Nephrotoxic effects of gentamicin on some biochemical parameters in rats when compared to controls.

	Control G I=6 rats	gentamicin 60mg/kgb.w GII=6 rats	gentamicin 80mg/kgb.w GIII=6 rats
Body weight	125-150mg	115mg	115mg
PARAMETERS	MEAN+SD	MEAN+SD	MEAN+SD
Urea (mg/dl)	16.8+.6	60.28+2.3	74.18+2.03
Creatinine (mg/dl)	.48+.02	1.82+.09	2.15+.30
Uric acid (mg/dl)	.36+.06	.81+.10	1.05+.16
Na (meq/L)	125.63+2.14	121.24+.97	118.67+2.14
K (meq/L)	4.11+.09	4.83+.31	5.51+.17
Total calcium (mg/dl)	9.31+.56	8.76+.29	7.55+.50

Mean, Standard deviation and one way ANOVA was done to know the significance. Values are expressed as means $\pm$ S.D. By using one way ANOVA the results are significant at .001

1. Histopathological Observations

3.1.1. Group-I treated with normal saline

Male albino rats with intake of normal saline showed normal architecture of renal glomeruli with intact bowmans capsule(fig.1) Brush bordered cuboidal epithelium lining the proximal convoluted tubules(fig.2) Simple cuboidal epithelium lining the distal convoluted tubules .macula densa is very prominent(fig.3 H&E, fig.4 Massons Trichrome).



fig.1



fig.2



fig.3



fig.4

fig.1 Control rat-glomeruli in the cortex of kidney, H&E, 40X10
fig.2 Control rat—showing showing longitudinal sections of proximal convoluted tubules lined by ciliated cuboidal epithelium,H&E, 40X10
fig.3 Control rat—showing macula densa,H&E, 40X10
fig.4 Control rat—showing showing macula densa , Massons trichrome,40x10

1.2Group-II – rats treated with 60mg/kg b.w

Epithelial cell degeneration and granular deposits in tubular lumens with evidence of tubular epithelial cell desquamation and lymphocytic infiltration around PCT (fig.5) Tubular epithelial necrosis and dilatation is observed effecting less than half of cortical tubules under 100 magnification (fig.6).

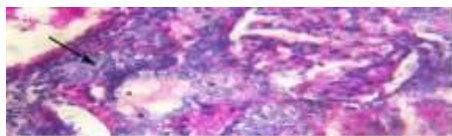


Fig.5 rats treated with 60mg/kg b.w - lymphocytic infiltration around PCT . Periodic Acid Schiff, 40x10



Fig.6 rats treated with 60mg/kg b.w -showing tubular epithelial necrosis and dilatation . Periodic Acid Schiff, 40x10

3.1.3. Group-III -rats treated with 80mg/kg b.w

Close examination of these sections at high magnification, revealed the appearance of cells with alterations typical of apoptosis (cell shrinkage and cytoplasm eosinophilia and presence of a small and shrunken nucleus with chromatin condensation (fig.7). Break down of glomerular capillaries and vacuolar appearance in tubular lumen is observed(fig.8).The use of the periodic acid-Schiff reaction confirmed that these apoptotic cells were almost exclusively found in proximal tubules causing obstruction of PCT „S(fig.9). Tubular basement membrane is interrupted. Glomerular congestion, disruption of glomerular capillaries,vacuolar degeneration of tubular epithelial cells is observed with hyaline cast formation is observed in PCT(fig.10). Atrophic glomeruli are present effecting half of the cortical region. Lymphocytic infiltration has increased (fig.11).



Fig.7--cell shrinkage and shrunken nucleus,PAS,40x10



Fig.8-breakdown of glomerular capillaries, PAS, 40x10



Fig.9-showing apoptotic cells in proximal tubules causing obstruction of PCT, PAS,40x10

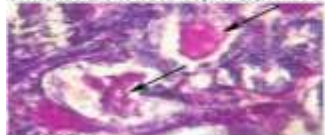


Fig.10



Fig.11

Fig.10-showing disruption of glomerular capillaries,vacuolar degeneration of tubular epithelial cells with hyaline cast formation . PAS,40x10
Fig.11-showing atrophic glomeruli .PAS,40x10

II. Discussion

The incidence of renal dysfunction following amino-glycoside administration was detected by many workers (Garetz and Schacht 1996 [8]; Baliga et al., 1997[9] and Abdel Naim et al., 1999[10]). Gentamicin is aminoglycoside broad spectrum antibiotic used against pathogenic gram negative and positive bacteria (Taha, 1993[11]). Its administration into rats induced impairment of renal function through liberation of oxygen free radical (Heibashy & Abdel Moneim, 1999 [12] and Heibashy et al., 2009[13]). Acute renal failure is characterized by disorders in some biochemical parameters in gentamicin treated rats as shown in the first experiment presented in Table -1. Rats treated with 60mg/kg b.w and 80mg/kg b.w. Gentamicin produced increase in the concentration of serum urea, creatinine and uric acid.

These results confirmed that gentamicin produced nephrotoxicity as previously reported by Ali et al., 2003[14], Goto, 2004 [15] and Heibashy et al., 2009[13]. These changes reflected the severity of renal insufficiency which occurred in association with the sudden fall in glomerular filtration rate because of the majority of administered GM enters specifically the proximal tubular epithelial cells, binds to anionic

phospholipids in the target cells inducing abnormalities in the function and metabolism of multiple intracellular membranes and organelles then developed injury in the proximal tubular epithelial cells of kidney that caused acute renal failure (Swan, 1997[16]). More than half of proximal tubules showing desquamation of necrosis but involved tubules easily found, complete or almost complete tubular necrosis. Serum electrolytes were disturbed in GM treated rats as compared with control animals.

Lower value of serum sodium indicated inability of kidney to conserve sodium and chloride. Haemodilution too may be involved in the fall of sodium value via excess of water intake and or increased production of endogenous water. Increase of Potassium may be due to reduced excretion of K aggravated by leakage of intracellular potassium into blood stream as a result of gentamicin induced lesions in renal tubular epithelium. The present results are in harmony with the data obtained by Heibashy & Abdel Moneim (1999)[12] and Heibashy et al. (2009)[13]. Apoptosis plays a major role in kidney embryogenesis, resulting in large-scale cell death during development (Coles H S R et al 1993[17]). By contrast, in the adult and under normal circumstances, evidence of apoptosis is seldom found in the kidney, where the rate of cell turnover is very low. However, there are a number of documented cases related to kidney insult in both pathology and toxicology where the renal tissue, in particular the tubular epithelium, exhibits a substantial increase of apoptotic cells (Conaldi P G et al 1998[18], Davis M A et al 1998[19]).

Thus, apoptosis is clearly involved in ischemic renal atrophy (Gobe G C et al 1987[20]) Gentamicin treated rats show tubular epithelial damage with intense granular degeneration involving > 50% of renal cortex. Some of the tubular epithelium contains tubular casts as observed by K.Vijay Kumar et al 2000[21])

Renal tissue specimens obtained 30 minutes after the 8th day of administration were examined histologically by Yoshiyama et al 1992 [22] with similar findings as observed by other authors.

Rats given GM in midlight (1:00pm) showed: tubular necrosis, degeneration, dilation together with regeneration of proximal tubular cells, cell infiltration in interstitium, and hyaline cast formation in the tubular lumen.(Yoshiyama et al 1992[22]) .Gentamicin renal cell damage as induced by tubular necrosis ie, marked congestion of the glomeruli with glomerular atrophy, degeneration of tubular epithelial cells with casts in the tubular lumen and infiltration of inflammatory cells in the interstitium was confirmed on histopathological examination by Shirwaikar A et al 2003[23].

In the present study also shrunken glomeruli and glomerular atrophy is observed.

Conclusion

Daily intraperitoneal injection of rats with 80 mg gentamicin /kg b.w for 10 days causes serious harmful effects and is evident on renal function tests. Thus, it could be suggested that gentamicin must be given in the lowest effective therapeutic doses in patients with normal kidney function. Also, gentamicin therapy should be preceded by antioxidant administration and renal function tests must be done to detect any early functional alterations.

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