

Morphological and Biochemical Changes in Kidneys of Gentamicin Treated Rats

WAQAS IQBAL BUTT¹, MAIRA BHATTI², SABAHAAT GUL³

ABSTRACT

Aim: To observe morphological and biochemical changes in the kidneys of gentamicin treated rats.

Methods: It is an Experimental study conducted in the experimental research laboratory of University of Health sciences, Lahore. 16 albino rats 6-8 weeks old weighing 175-200 grams were divided into two groups having 8 animals each. Group A served as control and was given 1ml distilled water intraperitoneally for 8 days. While group B served as the experimental group and was given 100mg/kg body weight of gentamicin dissolved in 1ml distilled water intraperitoneally for 8 days. At the end of experimental period; animals were anesthetized. Blood samples were drawn by cardiac puncture for biochemical analysis, and kidneys were removed for histological examination.

Results: The histological picture of kidneys showed loss of brush border, pyknosis, karyolysis, and presence of protein casts in lumen of proximal convoluted tubules in treated group.

Conclusion: Gentamicin is nephrotoxic with discernable damage to kidney tubules, producing statistically significant rise in serum urea and creatinine.

Key words: Gentamicin. Proximal convoluted tubule. Urea. Creatinine.

INTRODUCTION

Aminoglycosides are a class of antimicrobial agents which are protein synthesis inhibitors. Streptomycin, neomycin, kanamycin, amikacin, gentamicin, tobramycin and netilmicin belong to this class¹.

Gentamicin is an aminoglycoside antibiotic derived from a bacterium, *Micromonospora purpurea*². It is commonly used to treat infections caused by aerobic gram negative bacteria. It acts by irreversibly inhibiting protein synthesis. It passes through the outer membrane of bacteria by passive diffusion through porin channels. It is then transported to the cytoplasm passing through the cell membrane by an oxygen dependent process¹. Once inside the cell, gentamicin binds to the 30S subunit of bacterial ribosomes and interferes with protein synthesis by making the ribosomes misread the genetic code, preventing the process of polysome disaggregation and assembling³.

Gentamicin is poorly absorbed from the gastrointestinal tract; if administered orally it is entirely excreted in feces. The preferred route of administration is intramuscular, in which case peak concentration is reached in 30 – 90 minutes; it can also be given intravenously. Gentamicin shows bactericidal effect directly related to its blood concentration levels. When administered as a single large dose it showed strong bactericidal effect as compared to when given in small divided doses¹.

Due to its polar nature Gentamicin is not uniformly distributed in most tissues of the body and is reported to be present in significant quantity in body secretions. However it tends to accumulate in the renal cortex and in the endolymph and perilymph of inner ear, where high concentrations of gentamicin may be found. Half-life of gentamicin in serum of normal individuals is 2 – 3 hours. It might increase to 24 – 48 hours if the renal function is significantly impaired as it is mainly excreted by the kidneys¹.

Gentamicin penetrates the cell membranes poorly; however the proximal tubular cells of kidneys tend to accumulate the drug many fold as compared to the plasma. Gentamicin binds to anionic phospholipids of the cell membrane and enters the cell. Once inside the cell it binds to organelles and accumulates in the lysosomes. Thus the accumulation of gentamicin within the renal cortex is reported to be related intimately to its nephrotoxicity⁵. Gentamicin acts as an iron chelator. The drug iron complex leads to the generation of reactive oxygen species causing cell damage⁶. It damages membranes of cells and organelles causing increase in their permeability. Damage to lysosomal membranes results in release of acid hydrolases in the cytoplasm leading to cell damage⁷. Accordingly acute renal failure ensues following necrosis of proximal tubular cells⁸. Serum urea and creatinine levels rise due to the failure of kidney to function properly⁹.

METHODS

Sixteen albino rats, 6-8 weeks of age were procured from University of Health Sciences Lahore, They were weighed and their blood samples were collected, from tail vein, for estimation of serum urea and creatinine levels before starting the experiment. They were housed under controlled room temperature ($23 \pm 2^\circ\text{C}$) and humidity ($50 \pm 5\%$) with cycles of light and dark of 12 hours each. Standard rat diet was given to the animals with water ad libitum. Animals were left to acclimatize for four days before starting the experiment.

Two groups of animals were made with 8 animals each. Group A was the control group and was given 1 ml distilled water intraperitoneally for 8 days. Group B was given gentamicin 100 mg/kg/day dissolved in 1 ml distilled water intraperitoneally for 8 days¹⁰.

On the 9th day each rat was anaesthetized using chloroform and a blood sample from each rat was collected by cardiac puncture for serum urea and creatinine estimation. Each animal was then sacrificed. The kidney of each animal was removed for gross and histological examination. The details of macroscopic features including color, lobularity and weight of kidneys were recorded. Kidneys were cut longitudinally into two pieces and fixed in

¹Asst Professor Anatomy, Sharif Medical & Dental College, Lahore

²Asst Prof Pharmacology, Sharif Medical & Dental College, Lahore

³Assistant Professor Anatomy, QA M C, Bahawalpur

Correspondence to Dr. Waqas Iqbal Butt, Email:

drwaqasiqbal@gmail.com @gmail.com Cell: 0312-4203543

10% formalin solution for 48 hours.. The processing of kidney pieces was done in an automated processor for 18 hours. The tissue was passed through ascending grades of alcohol for dehydration. They were then cleared in xylene and infiltrated with paraffin wax. After processing the tissues were embedded in paraffin blocks. Five micron thick sections were made with the help of rotary microtome and stained with hematoxylin and eosin stains. Microscopic parameters included brush border, cytoplasmic vacuolation, presence of intraluminal protein casts, desquamation of epithelial cells, pyknosis and karyolysis and were recorded for proximal convoluted tubules.

At least 10 randomly selected non-overlapping fields per section were used. The results were graded as the percentage of the damage, as previously described by Erdem et al⁵. The tubules were regarded as normal (-) which showed no damage to the tubules, mild (+) when less than quarter of the total number of tubules showed damage (<25%). The damage was considered moderate (++) when damage was observed in 26-50 % tubules and severe (+++) more than 50% of tubules showed damage.

Data was analyzed using SPSS software, version 21. Mean \pm SD was given for quantitative data and frequency and percentages were given for qualitative data. For analysis of qualitative data Fischer's Exact test was performed and for quantitative data One way ANOVA and Post Hoc Tukey's test were performed. P value of ≤ 0.05 was considered statistically significant.

RESULTS

On gross examination the kidneys of both groups appeared reddish brown with smooth surface. Mean weight of animals of group A at the start and end of experiment was 179.63 \pm 61.6 gm and 203 \pm 5.39 gm respectively and of group B was 184.13 \pm 9.35 gm and 158.75 \pm 4.20gm respectively. The mean body weight decreased significantly (p value <0.001) in group B as compared to group A at the end of experimental period.

When mean weight of both right and left kidneys combined was compared among groups A and B, the difference was statistically insignificant. P value = 0.654

In groups A and B, the values of mean diameter of renal corpuscles were 63.66 \pm 2.37, 82.52 \pm 2.68 respectively. Increase in size of renal corpuscle in group B when compared to group A was significant (p value <0.001).

The proximal convoluted tubules of kidneys in animals of group A showed normal epithelium with a prominent brush border (Fig. 1). While the proximal convoluted tubules in group B exhibited, patchy loss of brush border, pyknotic and karyolytic nuclei, intraluminal protein casts and desquamated epithelial cells in their lumina (Fig. 2). Fisher's exact test showed statistically significant association between groups regarding percentages of above mentioned histological parameters (p < 0.001).

In group A the mean values of serum urea at the start and end of experimental period were 30.62 \pm 5.12 mg/dl and 29.25 \pm 3.99 mg/dl respectively. While in group B the mean values of serum urea at the start and end of experimental period were 29.37 \pm 5.12 mg/dl and 122.62 \pm 8.78 mg/dl respectively. There was a statistically significant rise in the mean values of serum urea (p value <0.001) in group B when compared with those of group A.

In group A the mean values of serum creatinine at the start and end of experimental period were 0.50 \pm 0.07 mg/dl and 0.50 \pm 0.10 mg/dl respectively. While in group B the mean values of serum creatinine at the start and end of experimental period were 0.48 \pm 0.08 mg/dl and 2.67 \pm 0.48 mg/dl respectively. Significant rise in the mean values of serum creatinine (p value <0.001) were observed in group B as compared to those in group A.

The rise in mean values of serum urea and creatinine was observed in group B, as compared to group A, which indicate a decline in kidney function in animals of group B.

Fig 1: Photomicrograph of kidney from group A. H&E stain X400.

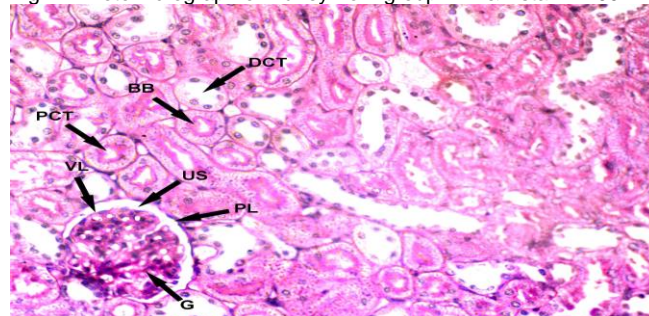
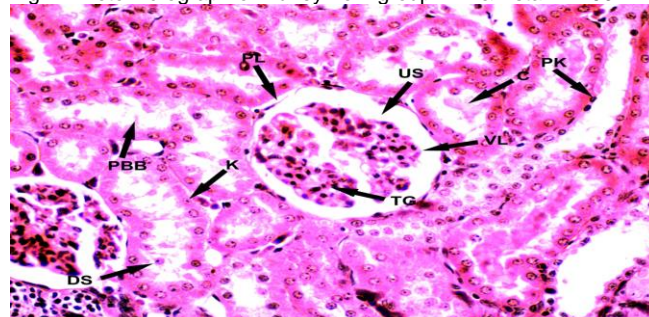


Fig 2: Photomicrograph of kidney from group B (gentamicin treated) stained with H&E at 400x magnification. The image shows significant damage to the proximal convoluted tubule (PCT) with loss of brush border (BB), pyknotic nuclei (PK), and intraluminal protein casts (C). Other labeled structures include the urinary space (US), parietal layer (PL), and glomerulus (G).



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Conflict of Interest: There is no conflict of interest.

DISCUSSION

In the current study, on comparing the mean body weights of animals in groups A and B it was observed that at the termination of experiment group B animals showed decline in body weight when compared with animals of control group A. A similar decrease in body weight after gentamicin administration was observed by Kumar et al¹¹, Tavafi et al¹² and Nivetha and Prasanna¹³.

When the mean weights of both right and left kidneys together were compared between groups A and B statistically insignificant difference was observed showing that gentamicin treatment did not produce significant effect in the weight of kidneys. This finding is in accordance with the findings of Singroha et al¹⁴. Our findings however differed from the findings of Nivetha and Prasanna¹³ who observed a decrease in weights of kidneys after treatment with gentamicin, and Noorani et al¹⁵ who observed increase in kidney weight after gentamicin treatment.

Kidneys of animals in groups A and B showed smooth surface and reddish brown color. These findings differed

from those observed by Alarifi et al¹⁶ who observed whitish spots on the surface of the kidneys.

The mean size of renal corpuscle was significantly increased in gentamicin treated group B when compared with those from group A. These findings were also observed by Noorani et al¹⁵. Who also reported swelling of renal corpuscles after administration of 80 mg/kg/day gentamicin intraperitoneally for 7 days.

Current study showed significant loss of brush border and presence of intraluminal protein casts in proximal convoluted tubules in group B when compared to group A. Guzmán et al¹⁸ and Azab et al¹⁷ also observed loss of brush border and presence of protein casts in proximal convoluted tubules upon treatment with gentamicin. Protein casts in lumina of proximal tubules were also observed by Frazier et al¹⁹, Kang et al²⁰ and Lakshmi and Sudhakar²¹.

Gentamicin treated group B showed significant desquamation of epithelial cells when compared with group A. these findings are similar to the findings of Padmini and Kumar²² and Lakshmi and Sudhakar²¹, who also observed tubular epithelial cell desquamation.

The present study showed significant Pyknosis and Karyolysis in group B indicating tubular necrosis. Comparable findings were also observed by Kang et al²⁰, Lakshmi and Sudhakar²¹, and Qadir et al²³ on treatment of animals with Gentamicin.

A significant rise in levels of serum urea and creatinine was observed in animals of group B when compared with animals of control group. Similar findings were also reported by Nivetha and Prasanna¹³, Kumar et al¹¹ and Erdem et al⁵.

CONCLUSION

The Current study showed gentamicin induced nephrotoxicity in rats as manifested by histological and functional changes. There was significant damage to the proximal convoluted tubules as evidenced by the loss of brush border, cytoplasmic vacuolation, pyknosis, karyolysis and presence of protein casts in the lumina of proximal tubules. The renal corpuscles also showed glomerular swelling and increased glomerular capillary wall thickening. The values of urea and creatinine were elevated indicating damage to kidney function.

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