# AGGRAVATION OF GENTAMICIN INDUCED NEPHROTOXICITY BY CIPROFLOXACIN

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## ABSTRACT

***Objective:*** To evaluate histopathological changes of gentamicin induced nephrotoxicity in rabbits receiving the combination of ciprofloxacin and gentamicin compared with rabbits receiving gentamicin alone.

***Study Design:*** Laboratory based Randomized controlled trial.

***Place and Duration of Study:*** Study was conducted in the department of Histopathology with the collaboration of the department of Pharmacology, Army Medical College Rawalpindi and National University of Sciences and Technology (NUST) Islamabad.

***Material and Methods:*** Eighteen rabbits were used in this study. They were divided randomly into three groups. Each group contained six rabbits. Group 1 (control) was injected subcutaneously with 0.9% NaCl. The second group of animals was injected with gentamicin. The third group of animals was injected with the same dose of gentamicin and additionally ciprofloxacin. The findings were entered and analyzed with the help of histopathological (HP) score calculated by summing up the histological parameters through Microsoft excel and Graph pad Instat 3.

***Results:*** Necrosis, congestion and inflammatory infiltrate were graded as absent, mild, moderate and severe. The score assigned was from 0 to 4 accordingly. Highest tubular necrosis grade (grade 3) and maximum inflammatory changes (moderate grade) were observed in 50% of rabbits of group 3, receiving combination of gentamicin and ciprofloxacin. *p*-values were significant <0.01.

***Conclusion:*** Combined administration of ciprofloxacin along with gentamicin in rabbits enhances adverse histopathological effects of gentamicin induced nephrotoxicity.

**Keywords:** Antibiotics, Apoptosis, Toxicity, Tubular necrosis.

## INTRODUCTION

Aminoglycoside antibiotics, including gentamicin, are widely used in the treatment of gram-negative infections. However, their efficacy is counterbalanced by significant toxicity especially nephrotoxicity, which causes kidney damage by a direct dose dependent mechanism[1,2]. Despite rigorous patient monitoring, nephrotoxicity appears in 10–25% of therapeutic courses[3].

Accumulation of aminoglycosides within the renal cortex is known to be related intimately to the pathogenesis of nephrotoxicity. Although the mechanism of gentamicin-induced cell injury and cell death is still unclear, interactions with the cell membrane, mitochondria, lysosomes and microsomes are likely to be involved[4]. Histopathological findings are characterized mainly by tubular necrosis particularly proximal tubules, basal membrane disruption, apoptosis, intracellular edema, glomerular narrowing of Bowman's capsule[5,6]. Gentamicin also induces a reduction in renal blood flow (RBF)[7]. Despite the fact that gentamicin does not generate significant morphological changes in the glomeruli, in high-dose treatment, a slight increase in size, alteration of their round shape and density, and a diffuse swelling of the filtration barrier associated with neutrophil infiltration have been detected[8].

Ciprofloxacin is acommonly used fluoroquinolone either alone or in combination with other drugs including aminoglycosides like gentamicin. Combination of gentamicin and anti-pseudomonal fluoroquinolone (like ciprofloxacin) is in clinical use for treatment of *Pseudomonas aeruginosa* infections. This combination is not the first choice but used clinically only in resistant cases[9]. However, effects of ciprofloxacin on kidney when used in combination with gentamicin have not been

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very well established or documented in either experimental animals or human study.

Thus there is need to explore the histomorphological effects of this drug alone and in combination withother drugs. To address this issue, the present study was designed to evaluate the histopathological(HP) nephrotoxic effects induced by gentamicin alone or when administered in combination with a fluoroquinolone like Ciprofloxacin.

## MATERIALAND METHODS

This laboratory based randomized control trial was carried out in the department of histopathology and animal house of Army Medical College Rawalpindi, National University of Sciences and Technology Islamabad. A total of 18 rabbits was included in this study. They were divided randomly into three groups. Each group contained six rabbits. Rabbits had free access to water and food during the fourteen days time period in the metabolic cages. Group 1 (control) was injected subcutaneously with 0.9% NaCl, for a period of 14 days[10]. The second group of animals was injected with gentamicin at a dose of 20 mg/kg/12 hour intramuscularly for 14 days[11]. The third group of animals was injected with the same dose of gentamicin and additionally ciprofloxacin (80mg/kg/day) for 14 days intraperitoneally[12]. Ciprofloxacin only group is not considered in this study as it has a protective effect in gentamicin nephrotoxicity[10]. At the end of 14$^{th}$ day, all the animals were sacrificed. Kidneys were taken out, sectioned, processed and stained for histopathological analysis. The histopathological changes were subdivided intotubular, interstitialand glomerular changes. The parameters assessed in renal tubules were tubular basement membrane separation due to necrosis, hyaline cast formation, interstitial edema, apoptosis and narrowing of tubular lumen due to sloughed off cells. Interstitial changes consist of interstitial inflammatory infiltrate around the necrotic tubules and congestion. Glomerular changeswere glomerular hypercellularity, glomerular basement membrane thickening. Necrosis, congestion and inflammatory infiltrate were graded as absent, mild, moderate and severe. Score assigned was from 0 to 4 accordingly.

### Statistical Analysis

All the parameters were evaluated by the criteria defined by Houghton[13].All data were collected through specifically designed proforma. The findings were entered and analyzed with the help of histopathological score calculated by summing up the parameters through Microsoft excel and Graph pad Instat[3]. Men with standard deviation and median were also calculated. One way Analysis of Variance (ANOVA) followed by Dunnet's multiple comparison test was applied to compare different groups. The experimental findings were considered statistically significant if *p* value was <0.05.

## RESULTS

Group 1 was controlled and given normal saline. There were no histopathological nephrotoxic changes seen on light microscopy. Histopathological score (HP score) was 0 for this group. In group 2 rabbits were given only gentamicin. The total HP score for this group was 64. In group 3 where gentamicin and ciprofloxacin was administered in combination. The total HP score was 74. Minimum, mean, median, maximum HP score and standard deviation (SD) are given in table 1. P values were significant i.e.<0.01 (table 2). Highest tubular necrosis grade (grade 3) and maximum inflammatory changes (moderate grade) were

**Table-1: Groups based on histopathological (HP) score.**

| Group title | Group 1 C(n=6) | Group 2 G(n=6) | Group 3 G+C80(n=6) |
|---|---|---|---|
| **Mean HP score** | 0 | 10.66 | 12.33 |
| **Standard deviation (SD)** | | 0.81 | 1.21 |
| **Standard error of mean (SEM)** | | 0.33 | 0.49 |

C= Control, G=Gentamicin, C=Ciprofloxacin,

observed in 50% of rabbits of group 3, receiving combination of gentamicin and ciprofloxacin (table 3).

## DISCUSSION

Conventionally, aminoglycoside nephrotoxicity has been considered to result mainly from tubular damage. Both lethal and sub-lethal alterations in tubular cells handicap reabsorption and, in severe cases, may lead to a significant tubular obstruction[14]. 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[15,16].

The present study was designed to observe the histopathological changes in gentamicin induced nephrotoxicity and document any possible protective or aggravating effects of fluoroquinolone (ciprofloxacin) when Co administered with gentamicin.

Regarding nephrotoxic effects of gentamicin, data of present study shows significant *p* value (< 0.01) in the group given gentamicin alone when compared with a control group which was given a normal saline only. These results are in concordance with another study where treatment with gentamicin for five days induced moderate to severe histological damage ($p<0.001$) with dominant tubular necrosis extended to distal parts of proximal tubules and epithelial cell dissociation with cast formation, loss of brush border in large parts of proximal tubules and tubular obstruction[17].

To assess the histopathological changes were combination of drugs was used, the group that received only gentamicin was taken as control group and group 3 was compared with it. In comparison with this control, marked histopathological changes i.e. higher grades of tubular necrosis were seen in groups with

**Table-2: Comparison of all groups by ANOVA.**

| Comparison | Mean difference | Q | *p*-value |
|---|---|---|---|
| Control vs Gentamicin | -10.66 | 24.54 | ** $p<0.01$ |
| Control vs Gentamicin + Cipro 80 | -12.33 | 28.37 | ** $p<0.01$ |
| Gentamicin vs Gentamicin+ Cipro 80 | -1.667 | 3.835 | ** $p<0.01$ |

**significant Dunnett Multiple Comparisons Test
Q = degree of confidence

**Table 3: Grades and severity of histopathological parameters in groups.**

| Necrosis | Group 1(C) | Group 2(G) | Group 3 (G+C80) | *p*-value |
|---|---|---|---|---|
| Grade 0 | 6 (100%) | 0 | 0 | $p<0.01$ |
| Grade 1 | 0 | 3(50%) | 0 | $p<0.01$ |
| Grade 2 | 0 | 3(50%) | 3(50%) | $p<0.01$ |
| Grade 3 | 0 | 0 | 3(50%) | $p<0.01$ |
| Grade 4 | 0 | 0 | 0 | |
| **Congestion** | | | | |
| Mild | 0 | 5 (83.3%) | 4 (66.7%) | $p<0.01$ |
| Moderate | 0 | 1(16.7%) | 2(33.3%) | $p<0.01$ |
| Severe | 0 | 0 | 0 | |
| **Inflammatory infiltrate** | | | | |
| Mild | 0 | 6 (100%) | 3(50%) | $p<0.01$ |
| Moderate | 0 | 0 | 3(50%) | $p<0.01$ |
| Severe | 0 | 0 | 0 | |

ciprofloxacin, gentamicin combination and having significant *p*-values of <0.01. Although gentamicin alone administration for fourteen days also resulted in a significant increase in histopathological changes but when ciprofloxacin was concurrently administered with gentamicin, there was further aggravation of tissue damage.

The present study recognized the fact that combination of ciprofloxacin with gentamicin does not attenuate the gentamicin induced nephrotoxicity rather enhances it. There are many studies carried out to document the possible nephrotoxic effects of fluoroquinolones alone and demonstrated that acute renal failure developed in patients within a few days of starting ciprofloxacin hydrochloride therapy[18]. However, renal function improved shortly after withdrawal of the drug in all the patients. Because ciprofloxacin is used extensively by the urological community, it is important to be aware of this potential complication of therapy by ciprofloxacin[19].

While studying the histopathological changes like necrosis, desquamation of tubular epithelial cells in renal cortex, congestion, presence of inflammatory infiltrate, casts, basement membrane thickening and other degenerative changes in renal glomeruli and tubules one can precisely determine the renal parenchymal changes inflicted by various drugs. Evaluating through comparison, the attenuation and aggravation of these histopathological changes by combining various drugs or compounds with gentamicin, we hope to find an ideal combination of drugs which can be combined safely with gentamicin.

## CONCLUSION

Combined administration of ciprofloxacin and gentamicin in rabbits enhances adverse histopathological effects of gentamicin induced nephrotoxicity. Ciprofloxacin in combination with gentamicin revealed relatively higher grades of tubular cell necrosis and glomerular changes histopathologically.

## RECOMMENDATIONS

Patients on gentamicin therapy may require a renal biopsy to evaluate histological deleterious effects of gentamicin and ciprofloxacin. It is recommended in those cases only where there is ambiguity regarding the cause of renal failure.

It is advisable that the physician must exercise caution while prescribing a combination of aminoglycosides and ciprofloxacin, as this is not a safe arrangement with reference to the enhancement of nephrotoxicity.

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