Levofloxacin and Gentamicin Induced Nephrotoxicity

ORIGINAL ARTICLES

DETERMINATION OF THE EFFECTS OF LEVOFLOXACIN ON GENTAMICIN INDUCED NEPHROTOXICITY IN RABBITS: A COMPARATIVE STUDY

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ABSTRACT

Objective: To determine the effects of levofloxacin on gentamicin induced nephrotoxicity in rabbits.

Study Design: Comparative experimental study.

Place and Duration of Study: The animal house of Army Medical College, Rawalpindi, and the pathology department of Army Medical College, Rawalpindi, from July 2009 to January 2010.

Material and Methods: The effects of levofloxacin on gentamicin-induced nephrotoxicity were evaluated in rabbits. Twenty four rabbits were used in this study which were randomly divided into four groups (n= 6 in each group). Six animals were injected for 15 days with saline (NaCl; 0.9%), six with gentamicin alone at doses of 20 mg/ kg of body weight/ 12 h (intramuscularly), six with combination of gentamicin (20 mg/ kg/ 12 h) with low therapeutic doses of levofloxacin (30 mg/ kg/ 24 h) and the last six were treated with gentamicin and high therapeutic doses of levofloxacin (50 mg/ kg/ 24 h). Levofloxacin was given by intraperitoneal route.

Results: Gentamicin induced nephrotoxicity was evaluated by histopathological and serum analysis. The extent of nephrotoxicity was significantly increased when gentamicin was given in combination with levofloxacin both in low and high doses.

Conclusion: Levofloxacin enhances gentamicin induced nephrotoxicity and extent of this nephrotoxicity increased with increasing dose of levofloxacin.

Keywords: Gentamicin, Histopathology, Levofloxacin, Nephrotoxicity, Serum analysis.

INTRODUCTION

Kidney is the target organ for numerous environmental toxins and therapeutic agents like antibiotics. One of the most important side effects of antibiotics is acute or chronic renal failure. Wide range of toxicity has been reported with commonly rates of 5-14% for nephrotoxicity.

Although aminoglycosides are being used for the treatment of several gram negative bacterial infections, but consumption of large quantities may cause nephrotoxicity. Gentamicin is probably one of the most commonly used aminoglycosides for the treatment of gram negative infections. Like all other aminoglycosides, gentamicin is essentially eliminated by glomerular filtration. Gentamicin gets accumulated in proximal tubules and leads to the production of free radicals in these tubules. However the pathological mechanisms involved in gentamicin induced nephrotoxicity include induction of oxidative stress, apoptosis, necrosis, up regulation of transforming growth factor B (TGF-B), elevation of endothelin1 and increase in monocyte/macrophage infiltration.

Levofloxacin is a synthetic broad spectrum antimicrobial belonging to the class of fluoroquinolones. Levofloxacin has an extended spectrum of activity compared with older-generation fluoroquinolones (ciprofloxacin, ofloxacin), with improved activity against gram-positive bacteria and excellent activity against gram-negative bacteria and atypical organisms. It is primarily excreted by the kidneys. The effects on kidney that have been reported rarely with levofloxacin include mild interstitial nephritis, occult blood in urine, decreased urine function and crystalluria.

Levofloxacin has proven efficacy against Pseudomonas aeruginosa infections and is also in use in combination with gentamicin for treatment of pseudomonal infections. As levofloxacin and gentamicin are used synergistically, it is imperative that effects of
levofloxacin on gentamicin induced nephrotoxicity should be evaluated.

**MATERIAL AND METHODS**

This study was a comparative experimental study which was carried out in the animal house of Army Medical College and analysis was done in the pathology department of Army Medical College Rawalpindi. Duration of study was six months.

Gentamicin preparations commercialized for clinical applications were used for the current study. Injection gentamicin (containing 80 mg of gentamicin sulfate in 2ml solution) prepared by Reckettte and Colman Pharmaceuticals, Karachi, Pakistan were purchased from local market.

Pure salt of levofloxacin was generously donated by Amson Pharmaceuticals, Rawalpindi, Pakistan. From this salt solution solutions were prepared for intraperitoneal injections. All 36 animals were divided randomly into four groups, with six rabbits in each. Gentamicin was administered intramuscularly, whereas levofloxacin was administered by intraperitoneal route.

Gentamicin was given at twelve hour interval at 8 a.m and at 8 p.m while levofloxacin was given at 24 hour interval for a period of fourteen days according to the following regimen:

Group 1 (G-1) served as control and received 0.5 ml of isotonic saline twice daily.

Group 2 (G-2) received gentamicin sulfate 40 mg/kg/day, nephrotoxic dose.

Group 3 (G-3) received combination of gentamicin 40 mg/kg/day and levofloxacin 30 mg/kg/day.

Group 4 (G-4) received gentamicin 40 mg/kg/day and levofloxacin 50 mg/kg/day.

Blood was collected twice during the study period from the marginal ear veins. First samples were taken at the start of study and second after 15 days. The collected blood was allowed to clot at room temperature and then centrifuged at 3000 rpm for 15 minutes. Serum was separated with the help of an automatic micropipette and stored in a clean and dry serum storage vial at -20°C for estimation of

<table>
<thead>
<tr>
<th>TESTS</th>
<th>G-1 Day 0</th>
<th>G-1 Day 15</th>
<th>G-2 Day 0</th>
<th>G-2 Day 15</th>
<th>G-3 Day 0</th>
<th>G-3 Day 15</th>
<th>G-4 Day 0</th>
<th>G-4 Day 15</th>
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<tr>
<td>BUN mmol/l</td>
<td>7.3</td>
<td>7.2</td>
<td>15.92</td>
<td>5.88</td>
<td>27.32</td>
<td>7.33</td>
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<td>±0.82 ±0.7</td>
<td>±0.52 ±0.3</td>
<td>±0.81 ±0.84</td>
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<td>p&lt;0.001</td>
<td>p&lt;0.01</td>
<td>p&lt;0.001</td>
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<tr>
<td>S. Creatinine µmol/l</td>
<td>89.67</td>
<td>92.5</td>
<td>139.83</td>
<td>87.83</td>
<td>161.5</td>
<td>85.83</td>
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<td>±6 ±5.66 ±6.72</td>
<td>±13.20 ±4.51</td>
<td>±15.07 ±6.10</td>
<td>±29.09</td>
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<td>p&lt;0.01</td>
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<td>S. Sodium mmol/l</td>
<td>138</td>
<td>137.5</td>
<td>138.17</td>
<td>136.33</td>
<td>135</td>
<td>139.33</td>
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<td>±1.86 ±1.07 ±1.75</td>
<td>±3.98 ±2.06</td>
<td>±1.68 ±1.09</td>
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<td>p=NS</td>
<td>p=NS</td>
<td>p&lt;0.04</td>
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<td>S. Potassium mmol/l</td>
<td>6.17</td>
<td>6</td>
<td>5.63</td>
<td>6.03</td>
<td>6.1</td>
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<td>±0.17 ±0.15 ±0.3</td>
<td>±0.31 ±0.17</td>
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<td>p=NS&lt;0.04</td>
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<tr>
<td>S. Calcium mmol/l</td>
<td>3.18</td>
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<td>2.86</td>
<td>3.22</td>
<td>2.8</td>
<td>3.15</td>
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<td>±0.05 ±0.07 ±0.07</td>
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<td>p=NS</td>
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urea, creatinine and electrolytes (sodium, potassium and calcium) later on.

All the animals were sacrificed 24 hours after the last dose of the drug. The abdomen was opened by a longitudinal incision immediately after death. Both the kidneys were dissected out and removed after cutting the ureters and renal vessels. Capsules were stripped off gently. Kidney specimens were sliced sagitally and placed in 10% formaline for 24 hours. The kidney tissue then processed for paraffin embedding. Approximately 3-5µm thick sections were taken with a rotary microtome. Sections were mounted on glass slides and stained with Hematoxylin and Eosin stain (H. & E. stain) for routine histopathological study under light microscope with special attention to proximal tubules.

Serum creatinine and BUN levels were measured by automated enzymatic assays. Serum creatinine was measured by Jaffe reaction and BUN levels by urease kinetic method. Serum sodium and potassium were measured by ion selective electrode method and serum calcium by CPC method.

Histopathological abnormalities were scored on plastic sections at X400 magnification. Each slide was coded. Sections were taken from three different regions of renal cortex for each rabbit. The lesions in renal cortex were scored: 0= No cell necrosis, 1= Mild, only single cell necrosis in sparse tubules, 2= Moderate, more than one cell involved in sparse tubules, 3= Marked, tubules exhibiting total necrosis in almost every power field, 4= Massive total necrosis.

The standard error of means was calculated on computer using Microsoft Office Excel 2007. In order to find the significance of the difference between two observations Student’s t test was used. The difference between two observations was considered significant if the ‘p’ value was less than 0.05. The results of histopathology were analyzed by using the ‘Chi Square Test’. The difference between the two observations was considered significant if the ‘p’ value was less than 0.05.

RESULTS

After 15 days of treatment, a significant difference was observed between the groups. Gentamicin given for 15 days at 40 mg/kg/day induced a significant change in serum creatinine levels and BUN compared with control animals, their p values were 0.001 and 0.01 respectively. Concomitant administration of levofloxacin with gentamicin both in low and high doses significantly raised serum creatinine and BUN levels, although both parameters were still significantly higher in the rabbits receiving combination of gentamicin and high doses of levofloxacin (p < 0.001 for both parameters).
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Serum sodium, potassium and calcium levels were statistically almost unchanged in animals treated with gentamicin alone and with gentamicin-levofloxacin (low doses) combination on day15. But a significant change in serum sodium (p < 0.04) and serum potassium levels (p < 0.01) was observed in animals treated with combination of gentamicin and high doses of levofloxacin as compared with control. Data is shown in the table.

After 15 days of treatment with gentamicin alone, renal proximal tubular cells showed patchy necrosis, grade 2 necrosis (figure-1). While treatment with gentamicin plus low doses of levofloxacin resulted in slightly increased focal necrosis of proximal tubules but overall assessment was also that of grade 2 (figure 2 left). In this group infiltration of interstitium with different inflammatory cells including some eosinophils (showing type III hypersensitivity reaction) was also observed, particularly in the surroundings of necrotic tubules. As the dose of levofloxacin was increased element of necrosis and inflammatory component both were augmented, but the tubular necrosis was still in grade 2 with more marked inflammatory infiltration of interstitium (figure 2 right). By applying chi square test ‘p’ value was not significant.

DISCUSSION

This study strongly suggests that levofloxacin enhances the gentamicin-induced nephrotoxicity. This assumption is based on various criteria such as the rise in serum creatinine and BUN levels and more severe histopathological lesions in the renal cortex of the animals treated with gentamicin-levofloxacin combination (although p value is insignificant) compared with the animals treated with gentamicin alone. Levofloxacin is one of the safest and best tolerated fluoroquinolone over a wide range of daily doses16. It is not primarily nephotoxic, but it has nephrotoxic potential especially in old age and in immuno compromised patients17.

Our present work was based upon the hypothesis that fluoroquinolone can attenuate gentamicin induced nephrotoxicity in rats8. Protection against aminoglycoside induced nephrotoxicity has been demonstrated for many antibiotics or chemical compounds. Actually, several antimicrobials including ceftriaxone, ticarcillin, carbenicillin, latamoxef and daptomycin18,19, decrease gentamicin induced nephrotoxicity. This protection occurs with or without a reduction in the uptake of gentamicin, suggesting different mechanisms of protection.

Even though such combinations of gentamicin and levofloxacin are not additive or synergistic in vitro8, they are often used clinically, like the combination of gentamicin and antipseudomonal fluoroquinolones (ciprofloxacin and levofloxacin) in the treatment of Pseudomona aeruginosa infections20. Besides gentamicin persists within the renal parenchyma for many months after their use, and since they are often used as step down therapy following intravenous therapy, the chances of exposing the kidneys to these combinations are high.

CONCLUSION

It can be concluded from present work that caution must be exercised when using gentamicin with levofloxacin due to enhancement of nephrotoxic potential. It is also concluded that nephrotoxic potential enhances with increasing the dose of levofloxacin.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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