Rosuvastatin Attenuates acute nephrotoxicity through modulation of oxidative stress in Sprague Dawley rats

Huda Abdulbaki Rasheed, Hayder M. Al-kuraishy, Ali I. Al-Gareeb

Abstract
Objectives: To assess the reno-protective effect of rosuvastatin on gentamicin-induced nephrotoxicity in rats.

Methods: The prospective experimental study was conducted at the College of Medicine, Mustansiriya University, Baghdad, Iraq, from March to July, 2018, and comprised Sprague Dawley male rats aged 3-4 months and weighing 200-400g each. The rats were divided into 3 equal groups which were treated for 14 days. Group 1 was treated with distilled water plus normal saline, Group 2 with distilled water plus gentamicin, and Group 3 with rosuvastatin plus gentamicin. Parameters measured were blood urea, serum creatinine, serum malondialdehyde, superoxide dismutase, glutathione reductase, neutrophil gelatinase associated lipocalin, kidney injury molecule-1, interleukin-18 and Cystatin-c. SPSS 20 was used for data analysis.

Results: Of the 30 rats, there were 10 (33.3%) in each of the three groups. Rosuvastatin produced significant reno-protective effect through reduction of blood urea, kidney injury molecule-1 and interleukin-18 (p<0.01) compared to the gentamicin group.

Conclusion: Rosuvastatin was found to be a reno-protective against gentamicin-induced nephrotoxicity through modulation of pro-inflammatory and oxidative/anti-oxidant pathways.

Keywords: Gentamicin, Rosuvastatin, Nephroprotective effect, Nephrotoxicity. (JPMA 69: S-98 (Suppl. 3); 2019)

Introduction
Gentamicin is a bactericidal antibiotic used for treatment of different bacterial infections. It is mostly used for the treatment of urinary tract infection (UTI), pneumonia, bone infection, meningitis and pelvic inflammatory diseases.1,2 Gentamicin gets selectively deposited in the epithelial cells of proximal renal tubules, leading to acute nephrotoxicity.3 The selective accumulation of gentamicin at renal tubules is due to the presence of megalin and cubilin which are membrane endocytic proteins at proximal renal tubules that are involved in the transport of cations such as aminoglycosides and xenobiotics. This accumulation alters the function of epithelial cells of proximal renal tubules via the alteration of extra-cellular calcium sensing receptors, leading to necrosis and cell death.4 Chronic or high dose of gentamicin initiates free radical generations and induction of oxidative stress. Furthermore, gentamicin at proximal renal tubules activates mitochondrial superoxide anions causing generation of free radicals. Besides, gentamicin stimulates the mitochondrial respiratory chain that generates free radicals.5 Previous studies documented that gentamicin therapy leads to significant reduction in the activity of glutathione, superoxide dismutase (SOD) and other endogenous anti-oxidants at renal proximal tubular cells that contribute in part in gentamicin-induced nephrotoxicity. Therefore, anti-oxidant agents carry reno-protective effects and reduce nephrotoxicity via the reduction of tissue damage and oxidative stress.6 Therapy with reduced glutathione fails in the prevention of gentamicin-induced nephrotoxicity despite the reduction of lipid peroxidation and increment in the renal glutathione activity as gentamicin produces dose-dependent effects.7 Rosuvastatin is a synthetic lipid-lowering agent which is a competitive inhibitor of hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase enzyme. Rosuvastatin is a rate-limiting enzyme in the conversion of HMG-CoA to mevalonate, reduces the level of low-density lipoprotein (LDL) and triglycerides (TG), and it increases the level of high-density lipoprotein (HDL). Also, rosuvastatin has significant reno-protective effect due to its anti-oxidant and free radical scavenging effects.6,8,9 The current study was planned to evaluate the reno-protective effect of rosuvastatin in gentamicin-induced nephrotoxicity in rats.

Materials and Methods
The prospective experimental placebo-controlled
The study was conducted at the Department of Pharmacology, College of Medicine, Mustansiriya University, Baghdad, Iraq, from March to July, 2018, and comprised Sprague Dawley male rats aged 3-4 months and weighing 200-400g each. The animals were obtained from the Iraqi National Centre of Cancer and Genetic Researches, University of Baghdad. The animals were isolated as 3 rats in each cage and placed at suitable room temperature under artificial 12/12hr light-dark cycle. They were kept for one week for acclimatisation without any intervention and with free access to normal chow pellets and water ad libitum. Permission for the study was obtained from the institutional ethics committee in accordance with the Guide to the Care and Use of Laboratory Animal.10 The study protocol for the induction of nephrotoxicity was in line with literature.11

Group 1 rats were treated with distilled water 5 ml/kg orally for 14 days, and on days 6-14 they received an intra-peritoneal injection of normal saline 5 ml/kg daily. Group 2 rats treated with distilled water 5 ml/kg orally for 14 days, and on days 6-14 they received intra-peritoneal injection of gentamicin 100 mg/kg. Group 3 rats were treated with rosuvastatin 10 mg/kg orally for 14 days, and on days 6-14 they received an intra-peritoneal injection of gentamicin 100 mg/kg at an interval of 1h.

Estimated glomerular filtration rate (eGFR) was measured according to Schwartz formula, 
\[ eGFR = \frac{k \times \text{height (cm)}}{\text{serum creatinine (mg/dl)}} \]

The next step was the assessment of anthropometric variables. Length was measured by graduated tape from nose to the anus (naso-anal length in cm). Body mass index (BMI) was calculated as body weight in grams divided by the square of length in cm; BMI=BW/(grams/length (cm))2.13

On day 15, rat decapitation was done under chloroform anaesthesia. The blood sample was centrifuged for 10 minutes at 5000rpm at room temperature. The isolated samples were kept at -20°C till assessment.

Among biochemical variables, blood urea and serum creatinine were estimated by using an auto-analyzer (ILab-300-Biomerieux Diagnostic, Milano, Italy) and they were expressed as mg/dL. Serum malondialdehyde (MDA), SOD, glutathione reductase (GSH), neutrophil gelatinase associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), interleukin-18 (IL-18) and Cystatin-c (Cyst-c) were measured using enzyme-linked immunosorbent assay (ELISA) according to the instructions given on the kit the the manufacturer (Myo-bio source, USA).

Data analysis was done using SPSS 20, and it was presented as mean± standard deviation (SD). The variables were tested using unpaired student t-test to compare the control and the treated groups. The levels of significance was set at p<0.05.

Results

Of the 37 rats assessed, 30(81%) comprised the final sample, with 10(33.3%) in each of the three groups (Figure-1).

Throughout gentamicin-induced nephrotoxicity, blood urea and serum creatinine increased significantly while eGFR decreased significantly in the gentamicin group compared to the control group (p<0.05). There were non-significant increase in the MDA serum levels and non-significant decrease in SOD and GSH levels in the gentamicin group compared to the control group (p>0.05). Also, there was significant increase in IL-18, KIM-1 and NGAL levels compared to the control group (Table-1).

Blood urea increased from 56.87±9.33mg/dL in the gentamicin group compared to 40.25±8.95mg/dL in the rosuvastatin group (p=0.002), while serum creatinine got reduced non-significantly (p>0.05). IL-18 and KIM-1 were reduced in the rosuvastatin group compared to the gentamicin group, as they were 242.63±21.78pg/mL compared to 494.36±69.32pg/mL, and 154.58±33.59pg/mL compared to 354.98±46.38 pg/mL.

Rosuvastatin showed non-significant reduction of Cyst-c levels compared to the gentamicin group, as they were 73.78±16.29pg/mL compared to 354.98±46.38 pg/mL.

Table 1:: Effect of gentamicin on the biochemical and inflammatory biomarkers in gentamicin-induced nephrotoxicity.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control (n=10)</th>
<th>Gentamicin (n=10)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood urea (mg/dL)</td>
<td>41.83±7.46</td>
<td>56.87±9.33</td>
<td>0.007*</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>0.70±0.14</td>
<td>1.08±0.40</td>
<td>0.04*</td>
</tr>
<tr>
<td>Estimated GFR (ml/min/1.73)</td>
<td>16.89±4.21</td>
<td>11.19±5.16</td>
<td>0.04*</td>
</tr>
<tr>
<td>MDA(ng/mL)</td>
<td>289.85±44.18</td>
<td>408.11±145.8</td>
<td>0.08</td>
</tr>
<tr>
<td>SOD(pg/mL)</td>
<td>48.12±32.92</td>
<td>26.39±16.86</td>
<td>0.13</td>
</tr>
<tr>
<td>GSH(uS/mL)</td>
<td>15.94±2.39</td>
<td>13.89±2.94</td>
<td>0.18</td>
</tr>
<tr>
<td>IL-18(pg/mL)</td>
<td>29.79±3.27</td>
<td>494.36±69.32</td>
<td>0.0001*</td>
</tr>
<tr>
<td>KIM-1(pg/mL)</td>
<td>73.78±16.29</td>
<td>354.98±46.38</td>
<td>0.0001*</td>
</tr>
<tr>
<td>NGAL(pg/mL)</td>
<td>15.78±3.07</td>
<td>20.04±2.88</td>
<td>0.02*</td>
</tr>
</tbody>
</table>

*p<0.05; unpaired t-test; BMI: body mass index; GFR: glomerular filtration rate; MDA: malondialdehyde; SOD: superoxide dismutase; GSH: glutathione reductase; IL-18: interleukin-18; KIM-1: kidney injury molecule-1; NGAL: neutrophil gelatinase associated lipocalin.
serum levels in gentamicin-induced nephrotoxicity (Figure-2).

**Discussion**

The present study definitely demonstrated that gentamicin was brilliant to induced experimental nephrotoxicity in rats via significant elevation of blood urea and serum creatinine. These findings corresponded with a recent study. It has been established that generation of free radicals and induction of oxidative stress are the main pathways of gentamicin-induced nephrotoxicity. Overproduction of reactive oxygen species is linked with depletion of proximal renal tubules' anti-oxidant potential which is subsequently developed into lipid peroxidation and tubular damages. Therefore, serum level of MDA is elevated while SOD and GSH levels are reduced in different models of gentamicin-induced nephrotoxicity. Despite these findings, gentamicin in the present study increased MDA serum levels, reduced SOD and GSH non-significantly which might be due to insufficient gentamicin dose, small sample size or short duration of the study.

The present study also showed significant elevation in IL-18 serum levels in gentamicin-induced nephrotoxicity which corresponds with a recent study that pointed to the benefit of IL-18 serum levels in the prediction of nephrotoxicity and acute kidney injury (AKI). IL-18 serum level is elevated prior to the elevation of blood urea and serum creatinine as it is highly sensitive and specific. The present study also illustrated significant effect of gentamicin in the elevation of KIM-1 and NGAL as both sera level are sensitive and specific biomarkers and are correlated with gentamicin-induced nephrotoxicity within seven days. However, the
increment in those biomarkers is time- and dose-dependent due to the progressive gene expression of KIM-1 and NGAL.\(^\text{18}\)

Moreover, the present study illustrated significant elevation in Cyst-c serum level in gentamicin-induced nephrotoxicity compared to the controls due to glomerular damage. Peng et al. study confirmed that elevation of serum Cyst-c indicated glomerular damage while elevation of urinary Cyst-c indicated renal tubular damage.\(^\text{19}\)

The present study recognised that rosuvastatin had renoprotective effect through reduction of blood urea significantly, as supported by a study that confirmed the renoprotective effect of rosuvastatin against amikacin-induced nephrotoxicity.\(^\text{20}\) Nevertheless, rosuvastatin produced insignificant amelioration of serum creatinine and eGFR which might be due to the short duration of the study. Rosuvastatin has potent anti-oxidant, free radical scavenging and anti-inflammatory effects. The anti-inflammatory effect of rosuvastatin was well revealed through significant reduction of IL-18 compared to the gentamicin group which was in line with a study that discussed the immunological effect of rosuvastatin and inhibition of IL-18 by rosuvastatin pharmacotherapy.\(^\text{21}\) In spite of these facts, rosuvastatin in the present study was unsuccessful in producing the anti-oxidant effect and reduction of MDA which is the biomarker of lipid peroxidation and oxidative stress. The non-significant effect might be due to small dose and/or short duration of the study. A study illustrated non-significant effect of rosuvastatin and atorvastatin on the oxidative stress in thioacetamide-induced liver cirrhosis.\(^\text{22}\)

The renoprotective effects of rosuvastatin are linked to different pleiotropic effects that affect renal vasculature via augmentation of endothelium-dependent mechanism, anti-platelet effects, inhibition of vasoconstrictor endothelin-1 and reduction of renal inflammation.\(^\text{23}\) Similarly, a study accounted that rosuvastatin played a role in the prevention of renal injury through the attenuation of tubule-interstitial fat deposition via inhibition of matrix metallopeptidase 9 (MMP-9) and MMP-2 which participate in the renal inflammation and AKI.\(^\text{24}\)

Gentamicin and other aminoglycosides are selectively accumulated at proximal convoluted tubules via megalin receptors.\(^\text{3}\) Rosuvastatin inhibits megalin-dependent endocytosis so it attenuates gentamicin-induced nephrotoxicity.\(^\text{25}\) Furthermore, a study established that hydrophilic statins, including rosuvastatin and pravastatin, are more effective in the reduction of lipid accumulations at renal tubules than lipophilic statins, including atorvastatin and pitavastatin.\(^\text{26}\) Despite borderline results, rosuvastatin has nephro-protective given that tubular effect regarding KIM-1 was more than glomerular effect regarding NGAL.

**Conclusion**

Rosuvastatin led to significant renoprotective effect against gentamicin-induced nephrotoxicity through modulation of pro-inflammatory and oxidative/anti-oxidant pathways.

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**Conflicts of Interest:** None.

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**References**


