Investigate the Effect of Fluvastatin and Pravastatin on Gentamicin-induced Acute Kidney Injury in Sprague Dawley Rats

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Abstract

Gentamicin is widely used antibiotics for gram-negative bacterial infections. Even, it is known to causes acute kidney injury (AKI). The present study was designed to investigate the effect of fluvastatin and pravastatin (20 mg/kg; p.o.) against gentamicin (100 mg/kg; i.p.) associated AKI. The rats were subjected to the administration of statins and gentamicin for 9 consecutive days. On the 9th day, rats body weight was recorded, then blood sample and renal tissues were collected for the assessment of renal biomarkers i.e. serum creatinine and urea. Thereafter, kidney and body weight ratio was calculated. Further, the histopathological evaluation also carried out to assess the renoprotective effect of statins. The administration of statins ameliorated the gentamicin-induced AKI. This effect was evidenced by attenuation of gentamicin-induced biomarker levels and histopathological changes. Hence, statins may be useful for the management of gentamicin-induced AKI owing to its pleiotropic actions.

Keywords: Acute Kidney Injury; Creatinine; Gentamicin; Nephrotoxicity; Statins

Abbreviations

AKI: Acute Kidney Injury; HDL: High-Density Lipoprotein; GFR: Glomerular Filtration Rate; LDL-C: Low-Density Lipoprotein-C; RNS: Reactive Nitrogen Species; TNF-α: Tumor Necrosis Factor Alpha; ROS: Reactive Oxygen Species; GFR: Glomerular Filtration Rate; SD: Sprague Dawley; CMC: Carboxy Methyl Cellulose; KW/BW: Kidney Weight and Body Weight; KDOQI: Kidney Disease Outcomes Quality Initiative

Introduction

Statins are mainly used for the reduction of plasma cholesterol levels. Fluvastatin and pravastatin have potential pleiotropic (multiple) actions in the body along with the reduction of plasma cholesterol via β-hydroxy-β-methylglutaryl-Coenzyme A (HMG CoA) reductase inhibition [1]. In addition to that, high plasma cholesterol also enhances the risk level of kidney injury via reduction of high-density lipoprotein (HDL, “good cholesterol”) and reduced glomerular filtration rate (GFR) [2,3]. Furthermore, the administration of gentamicin significantly elevates the plasma total cholesterol and plasma triglyceride concentration [4,5]. In addition, high dietary cholesterol also exacerbated the gentamicin associated nephrotoxicity in rabbits [6,7]. This literature report is evidenced that, cholesterol has a connection to worsen the renal function [8]. Further, high dietary cholesterol with gentamicin treatments is shown rising the severity level to
the renal dysfunction [6]. Hence, statin therapy is expected to ameliorate the cholesterol level along with the regulation of direct and cholesterol-mediated risk factor of AKI [3]. Fluvastatin and pravastatin are widely used medicines for the treatment of high cholesterol and cholesterol associated organ toxicity [3,9]. However, fluvastatin is also documented to produce more efficient action to the reduction of low-density lipoprotein-C (LDL-C) than pravastatin [10,11].

Gentamicin is one of the wide spectrum (aminoglycoside) antibiotics and it has been used for the treatment of gram-negative bacterial infections. It is also active against Staphylococcus and Enterococcus organisms [12]. Hence, it remains a first-line antibiotic for various severe bacterial infections. The major clinical advantage of gentamicin has lower resistance and inexpensive [13]. In contrast, at a higher dose and/or chronic usage of gentamicin induces the damage of the vestibular neuron and renal tissue [12,14]. The damage of renal tissue is mainly due to the inhibition of protein synthesis of host (renal) cells [15]. Furthermore, it also accelerates the synthesis of reactive oxygen species (ROS) and reactive nitrogen species (RNS); reduction of antioxidant defence system; activation of inflammatory molecules like tumor necrosis factor alpha (TNF-α); and contraction of mesangial cells which leads to reduction of renal blood flow, glomerular filtration rate (GFR) and renal dysfunction [16,17]. Recent review report revealed that, statins can be a choice for the management of acute kidney injury and chronic kidney disease due to its pleiotropic actions, regulation of multiple pathophysiological events, and protection of cells at different levels i.e., to induce the stability of cell membrane potential; regulation of cytosolic ions and proteins; and prevention of mitochondrial and nuclear DNA [3,18]. Similar pathophysiological mechanisms are involved in gentamicin-induced AKI [19-21]. However, the role of fluvastatin in gentamicin-induced AKI has not been explored yet. One study report revealed that pravastatin can inhibit the aminoglycoside (gentamycin) associated cytotoxicity of renal proximal tubular cells [22]. Hence, the role of fluvastatin and pravastatin in gentamicin-induced AKI remains to be investigated. The recent review suggested that statins can be employed as a repurposing agent for renal protection [23]. Though, the experimental and clinical evidence is required to make the successful uses in the health care system. Therefore, the present study was investigated the role of fluvastatin and pravastatin in gentamicin-induced AKI in rats.

Materials and Methods

Animal

Male Sprague Dawley (SD) rats (160 - 200g) were employed in this study. The disease-free animals were procured from University Science Malaysia, Penang, Malaysia. Animals were acclimatized for a period of one week (before starting the experiment) in Central Animal House of AIMST University with controlled temperature (37°C) and humidity (60%). All the animals were allowed to take standard laboratory rodent diet and water at ad libitum. The light cycles were maintained at 12:12 hours of normal light and dark throughout the experimental period. The experimental research protocol was duly approved (Reference number: AUHAEC 1/FOP/2013; Dated: 10/09/2013) by ‘Research and Ethics committee” of AIMST University, Malaysia.

Drugs and chemicals

Creatinine, urea, total cholesterol, and triglyceride kits were procured from Elabscience PVT Ltd, Malaysia. Gentamicin was purchased from Sigma Aldrich, Malaysia. Pravastatin and fluvastatin were procured from Actiza Pharmaceutical Private Limited, Mumbai, India; and Jigs Chemical, Gujarat, India respectively. Rest of the chemicals for cholesterol estimation and histopathology evaluation were used as an analytical grade. About 200 mg of gentamicin was dissolved in 2 ml of normal saline (0.9% w/v of sodium chloride). About 20 mg of fluvastatin and pravastatin were suspended in 5 ml of 0.5% w/v of carboxymethyl cellulose (CMC).

Induction of acute kidney injury

Acute kidney injury was induced by intraperitoneal administration of 0.1 ml of gentamicin (100 mg/kg) in 100g of rat for nine consecutive days, as described by Balakumar, et al [24].

2.4. Experimental protocol

Six groups were employed in this study. Each group consists of 6 SD rats (n = 6). Group 1 (Normal control): Rats were administered with 0.5% of CMC to eliminate the false positive and/or false negative results. Group 2 (Gentamicin): About 0.1 ml of gentamicin sulfate
administered to 100 mg/kg of rat for nine consecutive days via the intraperitoneal route. Group 3 (Fluvastatin per se): About 0.5 ml of fluvastatin was administered to 100 g of normal disease free healthy animal. Group 4 (Pravastatin per se): About 0.5 ml of pravastatin was administered to 100 g of normal disease free healthy animal. Group 5 (Gentamicin + fluvastatin): About 0.5 ml of fluvastatin was administered to gentamicin sulfate treated rat for nine consecutive days via the oral route. Group 6 (Gentamicin + pravastatin): About 0.5 ml of pravastatin administered to gentamicin sulfate treated rat for nine consecutive days via the oral route. Animals were weighed at different time intervals i.e. 0, 5 and 9th days from the day of experiments. On a ninth day, blood sample and kidney tissues were collected. The renal tissue weight was quantified and calculated the ratio of kidney weight and body weight (KW/BW). The serum samples were used for the estimation of creatinine, urea, total cholesterol and triglycerides levels using commercial diagnostic kits.

**Histopathological evaluation**

The histopathological changes of gentamicin were evaluated as a described method of Balakumar., *et al.* (2017) with a slight modification of Shimada., *et al.* [25]. Briefly, renal tissue samples were fixed with 10% formalin fixative solution. About 4 µm thickness cross section of renal tissue was made by microtome device. Staining was done using hematoxylin and eosin. Nerve sections were analyzed qualitatively under a light microscope (450 ×) for axonal degeneration. The scale bar was for 35 µm.

**Statistical analysis**

All the experimental data were expressed as mean ± standard deviation (SD, n = 6). Data obtained from body weight were analyzed statistically via two-way analysis of variance (ANOVA) followed by Bonferroni's post-hoc analysis using Graph pad prism Version-5.0 software. The data of biomarker changes were analysed via one way ANOVA followed by Tukey’s multiple range tests using Sigma Stat Version-3.5 software. A probability value of *P* < 0.05 was considered to be statistically significant.

**Results**

**Effect of fluvastatin and pravastatin in body weight and KW/BW changes**

Administration of gentamicin (100 mg/kg; *i.p.*) was shown significant (*P* < 0.05) decrease the levels of body weight and the ratio of kidney weight and body weight (KW/BW) levels. Whereas, the fluvastatin *per se* and pravastatin *per se* treated animal did not show any changes in body weight and KW/BW ratio when compared to normal control. The treatment of fluvastatin (20 mg/kg; *i.p.*) and pravastatin (20 mg/kg; *i.p.*) were shown significant improvement against gentamicin associated nephrotoxicity via attenuation of body weight and KW/BW changes. However, fluvastatin was shown more effective changes than pravastatin treatment (Table 1).

<table>
<thead>
<tr>
<th>Groups</th>
<th>0 day</th>
<th>5th day</th>
<th>9th day</th>
<th>KW/BW (mg/µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>137.9 ± 1.5</td>
<td>160.5 ± 1.8</td>
<td>184.6 ± 3.1</td>
<td>3.4 ± 0.4</td>
</tr>
<tr>
<td>Fluvastatin per se</td>
<td>132.5 ± 2.1</td>
<td>162.5 ± 1.5</td>
<td>183.2 ± 2.3</td>
<td>3.2 ± 0.6</td>
</tr>
<tr>
<td>Pravastatin per se</td>
<td>137.3 ± 1.9</td>
<td>161.8 ± 1.7</td>
<td>181.3 ± 1.8</td>
<td>2.9 ± 0.3</td>
</tr>
<tr>
<td>Gentamicin (100)</td>
<td>139.4 ± 2.7</td>
<td>147.6 ± 1.4*</td>
<td>151.3 ± 2.4*</td>
<td>1.2 ± 0.7*</td>
</tr>
<tr>
<td>Gentamicin + fluvastatin (20)</td>
<td>140.8 ± 2.4</td>
<td>159.3 ± 1.3*</td>
<td>180.7 ± 1.7*</td>
<td>3.3 ± 0.5*</td>
</tr>
<tr>
<td>Gentamicin (100) + pravastatin (20)</td>
<td>138.9 ± 2.3</td>
<td>157.8 ± 1.1*</td>
<td>179.2 ± 2.1*</td>
<td>3.0 ± 0.6*</td>
</tr>
</tbody>
</table>

*Table 1: Effect of fluvastatin and pravastatin in body weight and KW/BW changes.

Digits in parenthesis indicate dose in mg/kg. Data were expressed as mean ± SD, n = 6 rats per group. *P* < 0.05 Vs normal control group.

#P < 0.05 Vs gentamicin control group. Abbreviation: KW/BW, the ratio of kidney weight and body weight.

**Effect of fluvastatin and pravastatin in serum biomarker changes**

Administration of gentamicin (100 mg/kg; *i.p.*) shown significant (*p* < 0.05) decrease in serum urea and creatinine levels. Whereas, the fluvastatin *per se* and pravastatin *per se* treated animal did not show any changes in above biomarkers when compared to normal control. The treatment of fluvastatin (20 mg/kg; *i.p.*) and pravastatin (20 mg/kg; *i.p.*) were shown significant improvement in gentamicin-induced serum biomarker changes. However, fluvastatin was shown more effective changes than pravastatin treatment (Figure 1).

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Figure 1: Effect of fluvastatin and pravastatin in serum biomarker changes. Figure 1a indicated the effect of statins in serum urea concentration and figure 1b, indicating the effect of statins in serum creatinine concentration. Digits in parenthesis indicate dose in mg/kg. Data were expressed as mean ± SD, n = 6 rats per group. *P < 0.05 Vs normal control group. #P < 0.05 Vs gentamicin control group.

Effect of fluvastatin and pravastatin in serum lipid biomarker changes

Administration of gentamicin (100 mg/kg; i.p.) shown significant (P < 0.05) increase the serum total cholesterol and triglycerides levels. Whereas, the fluvastatin per se and pravastatin per se treated animal did not show any changes in above biomarkers when compared to normal control. The treatment of fluvastatin (20 mg/kg; i.p.) and pravastatin (20 mg/kg; i.p.) were shown significant reduction of gentamicin-induced lipid biomarkers. However, fluvastatin was shown more effective changes than pravastatin treatment (Figure 2).

Figure 2: Effect of fluvastatin and pravastatin in serum lipid biomarker changes. Figure 2a indicated the effect of statins on serum cholesterol level and figure 2b indicated the effect of statins on serum triglyceride level. Digits in parenthesis indicate dose in mg/kg. Data were expressed as mean ± SD, n = 6 rats per group. *P < 0.05 Vs normal control group. #P < 0.05 Vs gentamicin control group.

Effect of fluvastatin and pravastatin in histopathological changes

The administration of gentamicin (100 mg/kg; i.p.) showed significant histopathological changes i.e. degeneration of glomerular cell lining, tubular necrosis, and infiltration of inflammatory cells. Whereas, the fluvastatin per se and pravastatin per se treated animal did not show any major changes in above histological changes. The treatment of fluvastatin (20 mg/kg; i.p.) and pravastatin (20 mg/kg; i.p.) were shown prevention of gentamicin-induced histopathological changes (Figure 3).

Figure 3: Effect of fluvastatin and pravastatin in histopathological changes. Figure 3a-3f, were shown a transverse section of the kidney of normal, fluvastatin per se, pravastatin per se, gentamicin, fluvastatin, and pravastatin-treated groups respectively. In the figure, thin arrow showed degeneration of glomerular cell lining, bold arrow showed tubular necrosis and arrowhead showed infiltration of inflammatory cells. In figure 3d, was shown the severity of gentamicin-induced AKI. Figure 3e and 3f showed attenuation of fluvastatin and pravastatin against the gentamicin associated pathological changes of renal tissue respectively. Microscopic examinations were performed under 450 × light microscopy, scale bar 35 μm.

Discussion

The present research data showed the administration of gentamicin (100 mg/kg; i.p.) produced the significant changes of body weight, KW/BW ratio, serum urea, creatinine, cholesterol, triglyceride, and histopathological changes. It indicates that gentamicin potentially exacerbates the AKI, whereas the fluvastatin per se and pravastatin per se treated did not show any alteration of all in the above changes. Furthermore, the treatment of pleiotropic agents i.e. fluvastatin (20 mg/kg; i.p.) and pravastatin (20 mg/kg; i.p.) showed significant ameliorative effect against the gentamicin-induced AKI. It is a first study report of fluvastatin and pravastatin impact in gentamicin associated AKI.

Gentamicin causes the nephrotoxicity by multiple pathophysiological actions like biosynthesis of free radicals i.e. reactive oxygen species (ROS) and reactive nitrogen species (RNS) [15]; alteration of endogenous antioxidant molecules (glutathione, lipoic acid, bilirubin and ferritin) [26]; defence enzymes (superoxide dismutase, catalase and glutathione peroxidase); generation of inflammatory molecules (TNF-α, and interleukin-1); absence of anti-inflammatory molecules (interleukin-6 and interleukin-6) [27] and so on. The net effect of gentamicin induces cellular and molecular changes to enhance the contraction of mesangial cells of the renal tissue and it further declines the level of renal blood flow and glomerular filtration rate (GFR) [28]. In addition to that, it also alters the normal protein synthesis pattern of host cells. Further, the level of gentamicin associated AKI are experimentally indicates as raise in the level of urea and creatinine

levels in the bloodstream [29]. Further, gentamicin also alters the level of total cholesterol and triglyceride levels [30]. The similar results were observed in the present study. Gentamicin associated AKI also reflected in histopathological parameter changes i.e. degeneration of glomerular cell lining, tubular necrosis and infiltration of inflammatory cells. Numerous reports are revealed that, gentamicin is major culprit in the progression of AKI due to its potential modulator action of renal cell function and systemic alteration of cholesterol metabolic pathways [31,32]. Statins competitively inhibit the HMG-CoA reductase enzyme and it is the rate-limiting enzyme of cholesterol synthesis pathway [33]. Further, it also regulates the biology of T-cell expression and complements cascade protein expression [34]. The early regulation of these proteins is beneficial for the protection of various vital organs [35]. There are debates employed for the recommendation of statin therapy in renal dysfunction, because, the statin identified as rhabdomyolysis causing agent [3].

However, hydrophilic statins like pravastatin and fluvastatin are rarely causing the myalgia and rhabdomyolysis; whereas simvastatin is a lipophilic agent and it causes the muscular adverse effects and rhabdomyolysis [9,36]. Furthermore, the current update of Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines suggests that, lowering of LDL-C with newer statins useful for a large number of kidney injured patients with exception of dialysis and diabetic patients [3,37]. Moreover, fluvastatin and pravastatin have not induced the elevation of serum urea and creatinine levels in a normal subject [38-40]. Even fluvastatin also ameliorates the endotoxin and hemorrhagic shock renal injury [39,41]. The present histopathological study also revealed that fluvastatin and pravastatin are attenuated gentamicin-induced kidney injury.

Conclusion

Hence, fluvastatin and pravastatin can be recommended to prevent the nephrotoxic condition of renal injury. However, more extensive studies are required to prove their possible molecular mechanism in different pathological condition and chronic kidney disease with diabetic and hypercholesterolemic conditions.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

Bibliography


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