

Journal of Nephrothology



Losartan and magnesium sulfate administration reduce gentamicin-induced nephrotoxicity in rat model

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ARTICLE INFO

Article type:
Original Article

Article history:
Received: 14 April 2018
Accepted: 2 August 2018
Published online: 20 August 2018

Keywords:
Gentamicin
Nephrotoxicity
Magnesium sulfate
Losartan
Renal function
Renin angiotensin system

ABSTRACT

Background: Nephrotoxicity is the most known side effect of gentamicin. In addition, renin angiotensin system (RAS) plays an important role in the pathogenesis of renal injury and nephrotoxicity. Hypomagnesaemia is other complication of gentamicin. Previous studies reported that magnesium plays an important role in cell enzymatic functions, reducing lipid peroxidation.

Objectives: We investigated the role of losartan and magnesium sulfate (MgSO₄) on gentamicin nephrotoxicity.

Materials and Methods: In this study, rats randomly assigned to five groups. The first group, received saline, the second group received gentamicin 80 mg/kg/d, intraperitoneally (ip), and the third group, received a regular dose of losartan, 10 mg/kg/d + gentamicin 80 mg/kg/d. The fourth group received MgSO₄, 80 mg/kg/d + gentamicin 80 mg/kg/d. The fifth group obtained a continuous dose of gentamicin 80 mg/kg/d + losartan 10 mg/kg/d + MgSO₄ 80 mg/kg/d simultaneously. Nine days after administration of drugs, blood samples were collected from the heart. The level of urea, creatinine (Cr), malondialdehyde (MDA) and nitrite were measured in the animal serum and homogenized kidney tissue.

Results: Gentamicin increased serum urea and Cr levels. The administration of losartan and MgSO₄ lonely and combination of them, significantly reduced the levels of serum urea and Cr. Losartan alone and combination of losartan and MgSO₄ compared with gentamicin, significantly decreased kidney MDA level too. Decrease of kidney nitrite level by gentamicin was compensated by the administration of losartan, MgSO₄ alone or their combination. Additionally, losartan and MgSO₄ alone and their combination together significantly reduced renal damage.

Conclusions: The results of this study indicated that administration of losartan and MgSO₄ individually and their combination decreased kidney nephrotoxicity and improved renal function. This effect is probably related to the improvement of antioxidant status and renal blood flow.

Implication for health policy/practice/research/medical education:

In an experimental study we found that administration of losartan and magnesium sulfate individually and in their combination reduced kidney nephrotoxicity and improved renal function. This effect is probably related to the improvement of antioxidant status and renal blood flow.

Please cite this paper as: Kourkinejad Gharaei F, Safari T, Niazi AA, Zeynali Bujani M. Losartan and magnesium sulfate administration reduce gentamicin-induced nephrotoxicity in rat model. J Nephrothol. 2019;8(x):x-x. DOI: 10.15171/jnp.2019.xx.

1. Background

Aminoglycoside antibiotics, including gentamicin, are widely used to treat gram-negative infections. The most

known side effect of these drugs is nephrotoxicity. Ten percent to 20% of people treated with gentamicin show this condition (1,2). Previous studies have reported that

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gentamicin is mainly accumulated in renal cortex (3), then leading to tubular necrosis, particularly in the proximal tubule (4). On the other hand, many studies have shown that reactive oxygen species (ROS) are responsible for gentamicin induced nephrotoxicity (4-6). In fact, gentamicin effects on mitochondria and respiratory tract, causing ROS and inducing renal damage (7). In this regard Lopez-Nova and colleagues have shown that gentamicin by increasing intracellular calcium, contracted mesangial cells and reduce glomerular filtration (7). In addition, the contraction of mesangial cells is associated with the activation of renin angiotensin system (RAS) and the release of many vasoconstrictors (8-10). RAS plays an important role in the pathogenesis of renal injury, and many studies have shown the involvement of this system in different models of nephrotoxicity (11,12). Recent studies have highlighted the protective role of RAS inhibitors on diabetic nephropathy and drug nephrotoxicity (13-15). Losartan, as an selective angiotensin II type 1 (AT1) receptor antagonist, plays a major role in eliminating angiotensin-induced effects and improving renal function (16).

Increasing urinary excretion of magnesium sulfate ($MgSO_4$) and thus reducing its serum level is one of the gentamicin treatment options (17). Magnesium plays an important role in cell enzymatic functions as an intracellular cation. In fact, this cation reduces the level of malondialdehyde (MDA) in tubular epithelial cells by inhibiting lipid peroxidation (18). In this regard, Razumjoo and colleagues report that continuous use of $MgSO_4$ reduces nephrotoxicity caused by cisplatin (19).

2. Objectives

Considering the antioxidant effects of losartan and $MgSO_4$ in this study, we investigated the probable role of losartan and $MgSO_4$ on gentamicin nephrotoxicity.

3. Materials and Methods

3.1. Animals

In this research, 40 male Wistar rats, weighting 180-250 g were used from the animal center of Zahedan University of Medical Sciences. These rats were housed at a temperature of 23–25°C. They had free access to water and rat chow. They were also acclimatized to their diet for at least one week prior to the experiment. Moreover, the experimental procedure of the research was approved by the Zahedan University Medical Sciences Ethics Committee.

3.2. Drugs

Gentamicin was purchased from Caspian Company in

Iran, losartan was obtained from Daroo Pakhsh Drug Company (Tehran, Iran) and $MgSO_4$ and chloral hydrate from Sigma (St. Louis, MO, USA).

3.3. Experimental protocol

The animals were randomly assigned to five groups. The first group (saline), including 7 rats, received saline for 9 days. The second group (n=8) received gentamicin (GM) 80 mg/kg/d, intraperitoneally (ip) for 9 days (20). Similarly, the third group, including 8 rats, received a regular dose of losartan (10 mg/kg/d); ip (21) + gentamicin (80 mg/kg/d) for 9 days (GM+L). The fourth group (GM+ Mg) received $MgSO_4$ (80 mg/kg/d), ip (22) + gentamicin (80 mg/kg/d) for 9 days (n=8). The fifth group (GM+ L+ Mg), consisting of 9 animals, obtained a continuous dose of gentamicin (80 mg/kg/d) + losartan (10 mg/kg/d) + $MgSO_4$ (80 mg/kg/d) simultaneously for 9 days.

Then on the 10th day, blood samples were taken from the heart of each animal. The serum levels of urea, creatinine (Cr), MDA and nitrite were measured. During the study, the animals' weights were measured and recorded on a daily basis. Additionally, kidney nitrite and MDA levels were measured in the homogenized tissue.

3.4. Measurements

The levels of serum Cr and urea were determined using quantitative diagnostic kits (Pars Azmoon, Iran). The level of nitrite (stable NO metabolite) in serum and supernatant was measured using a colorimetric assay kit (Promega Corporation, USA) (Griess reaction). MDA serum level and the homogenized tissue supernatant were calculated based on the manual methodology(23).

3.5. Histopathological procedures

The kidneys were fixed in 10% formalin solution and then embedded in paraffin for staining. The tissue sections were stained with hematoxylin and eosin and examined. The assessment was conducted by two pathologists blindly. Based on the intensity of tubular lesions (hyaline cast, debris, vacuolization, flattening and degeneration of tubular cells, and dilatation of tubular lumen), kidney tissue damage score (KTDS) was graded from 1 to 5, while considering a zero score for natural tubes without any damage.

3.6. Ethical issues

This project was approved by Ethics Committee of Zahedan University of Medical. Prior to the experiment, the protocols were confirmed to be in accordance with the guidelines of Animal Ethics Committee of

Zahedan University of Medical Sciences. The study was supported by Zahedan University of Medical Sciences (IR.WEBZAUMS.REC.1395.94).

3.7. Statistical analysis

Data are expressed as mean \pm SEM. The levels of urea, Cr, MDA, nitrite and kidney weights were analyzed by one-way analysis of variance (ANOVA) followed by the Tukey test. The groups were compared by the Kruskal-Wallis or Mann-Whitney U tests with regard to the kidney tissue damage score (KTDS). *P* values less than 0.05 were considered statistically significant using SPSS version 16 for the data analysis.

4. Results

4.1. Effect of gentamicin on kidney weight

Evaluation of kidney weight (g)/100 g body weight in different groups does not show any significant difference between groups (Saline 0.39 ± 0.00 , GM 0.50 ± 0.01 , GM+L 0.51 ± 0.02 , GM+ Mg 0.51 ± 0.08 , GM+L+Mg 0.44 ± 0.07).

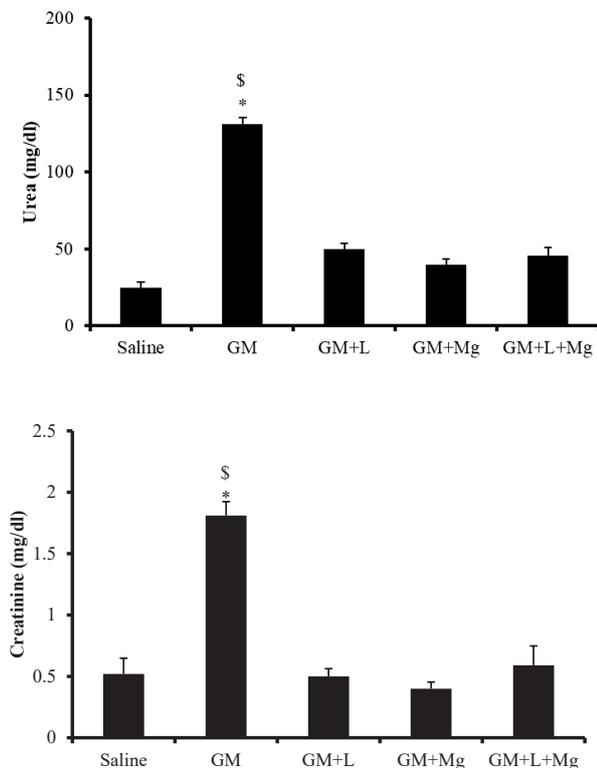


Figure 1. Serum level of urea (above) and Creatinine (below). The groups received saline, gentamicin 80 mg/kg/d (GM), gentamicin + losartan 10 mg/kg/d (GM + L), gentamicin + MgSO₄ 80 mg/kg/d (GM + Mg) and gentamicin + losartan + MgSO₄ (GM + L + Mg) for 9 days. The symbols indicate significant difference; * from saline group, § from GM + L group, GM +Mg group or GM +L+ Mg groups (*P* < 0.05).

4.2. Effect of gentamicin on urea and Cr levels

The results of this study showed that the use of gentamicin for 9 days significantly increased serum urea and Cr levels (*P* < 0.05), which confirms renal damage (Figure 1). It should be noted that treatment with losartan, MgSO₄ individually and combination of them significantly reduced the level of these two variables (*P* < 0.05).

4.3. Effect of gentamicin on MDA and nitrite levels

The mean of MDA level indicates that gentamicin has increased serum and kidney levels. It has been clear that lipid peroxidation increased after gentamicin administration. Additionally, it has been noted that administration losartan and MgSO₄ individually did not effect on serum level of MDA, but combination losartan and MgSO₄ decreased serum MDA level. On the other hand, in comparison with the gentamicin group kidney MDA level decreased after losartan administration individually and in combination with MgSO₄ (Figures 2 and 3).

The results of serum nitrite level showed that gentamicin did not change the serum level of this variable. However, losartan alone increased its serum level compared with the gentamicin group (*P* < 0.05). MgSO₄ alone and also with losartan did not affect serum nitrite. However, the results of renal tissue nitrite show a significant decrease,

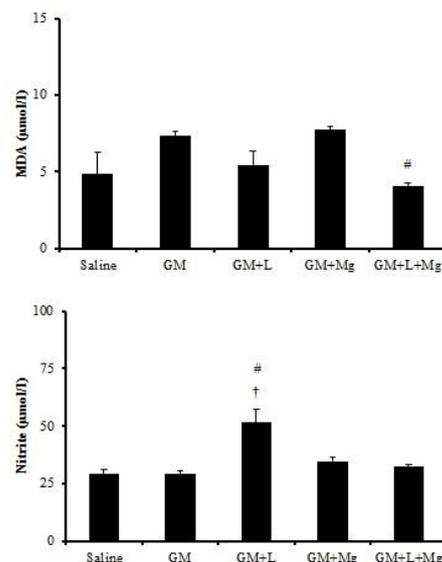


Figure 2. Serum level of MDA (above) and nitrite (below). The groups received saline, gentamicin 80 mg/kg/d (GM), gentamicin + losartan 10 mg/kg/d (GM + L), gentamicin + MgSO₄ 80 mg/kg/d (GM + Mg) and gentamicin + losartan + MgSO₄ (GM + L + Mg) for 9 days. The symbols indicate significant difference; # from GM group, † from GM + L + Mg group and GM + Mg group (*P* < 0.05), MDA stands for malondialdehyde.

in gentamicin group when compared with the saline group ($P < 0.05$). Administration of losartan, $MgSO_4$ individually or their combination had led to a significant increase in the level of nitrite in kidney tissue ($P < 0.05$) (Figures 2 and 3).

4.4. Effect of gentamicin on KTDS

The results of KTDSs (Figures 4 and 5) show severe renal injury in the gentamicin group compared to the saline recipient group ($P < 0.05$). On the other hand, losartan and $MgSO_4$ alone and their combination together significantly reduced renal damage ($P < 0.05$).

5. Discussion

Gentamicin is administered as an aminoglycoside antibiotic for the treatment of gram-negative infections. Nephrotoxicity is the most important of its side effect. The most important finding of this study was that losartan and $MgSO_4$ reduced gentamicin-induced nephrotoxicity. Several studies have reported that gentamicin treatment

is associated with renal toxicity and shows an increase in blood urea nitrogen (BUN) and Cr levels (24). In the present study, gentamicin-induced nephrotoxicity, which was confirmed by increasing the levels of urea and Cr that also confirmed by histological studies. The nephrotoxicity of this drug is associated with its accumulation in the renal cortex and its affinity and kinetics (25). Based on morphological studies, the membrane of proximal tubule cells is attached and then gentamicin is transferred into the cells by pinocytosis and induces renal injury (25). As already mentioned, the induction of ROS formation in the renal toxicity of gentamicin is involved (4,7). MDA, as a lipid peroxidation product, produces polyunsaturated fatty acids, which acts as a substrate for free radicals. Interaction between cationic drugs such as gentamicin and anionic phospholipids is considered as the first step in inducing nephrotoxicity of this drug (3,7). Another study has shown that gentamicin acts as an iron chelator and can release iron from the cortical mitochondria. Then the iron – gentamicin complex act as a strong catalyst for formation of ROS (8, 26). In addition, a study by Lopez-Novoa et al (7) showed that reducing glomerular filtration mediated by mesangial cells is likely to interfere with the activation of the RAS system and the production of angiotensin II and a large number of vasoconstrictors. Based on evidence, angiotensin II mediates the formation of ROS especially superoxide in renal tubule and smooth muscle cells (27,28) by AT1 receptor (29,30). Meanwhile, there are reports that angiotensin II also produces ROS through phospholipase D (31).

Increasing excretion of magnesium from urine following gentamicin administration reduces its serum level and

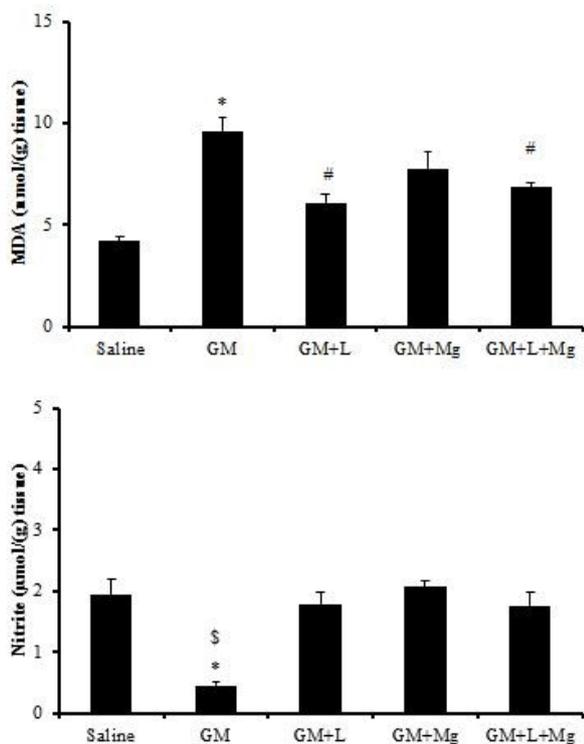


Figure 3. Kidney level of MDA (above) and nitrite (below). The groups received saline, gentamicin 80 mg/kg/d (GM), gentamicin + losartan 10 mg/kg/d (GM +L), gentamicin + $MgSO_4$ 80 mg/kg/d (GM +Mg) and gentamicin + losartan + $MgSO_4$ (GM + L +Mg) for 9 days. The symbols indicate significant difference; * from saline group, # from GM group, \$ from GM + L group, GM +Mg group or GM +L+ Mg groups ($P < 0.05$), MDA stands for malondialdehyde.

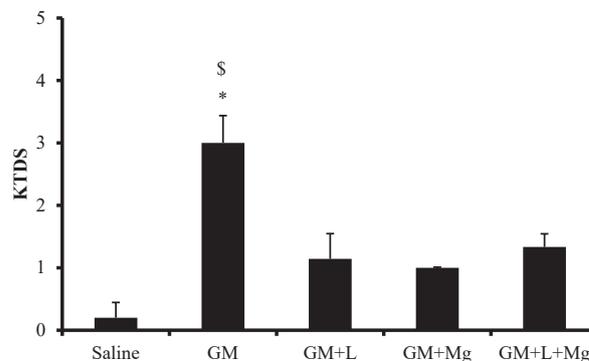


Figure 4. Kidney tissue damage score (KTDS). The groups received saline, gentamicin 80 mg/kg/d (GM), GM+ losartan 10 mg/kg/d (GM +L), gentamicin + $MgSO_4$ 80 mg/kg/d (GM +Mg) and gentamicin + losartan + $MgSO_4$ (GM + L +Mg) for 9 days. The symbols indicate significant difference; * from saline group, \$ from GM+L, GM + Mg or GM +L+ Mg groups ($P < 0.05$).

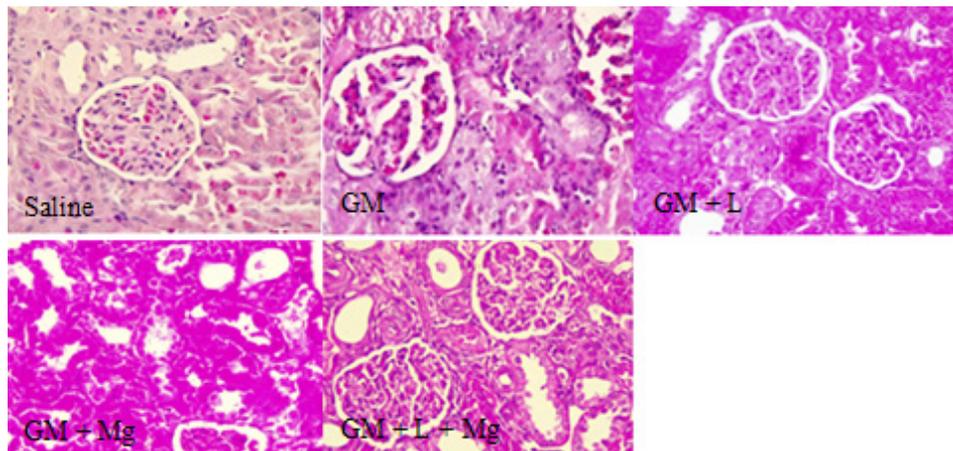


Figure 5. The pathology images ($\times 400$) of kidney tissue. The groups received saline, gentamicin 80 mg/kg/d (GM), gentamicin + losartan 10 mg/kg/d (GM +L), gentamicin + MgSO_4 80 mg/kg/d (GM + Mg) and gentamicin + losartan + MgSO_4 (GM + L +Mg) for 9 days.

possibly increases gentamicin accumulation in the renal cortex and increases its associated damage (32).

In the present study, treatment with losartan, and also the combination of losartan and MgSO_4 , reduced lipid peroxidation and reduced serum and kidney levels of the MDA. The oxidative stress biomarkers induced by gentamicin are reduced by losartan. On the other hand, AT1 receptor blockage is an important mechanism involved in the nephroprotective effects of losartan (33,34). In the same way Haghghi et al referred to the nephroprotective effects of losartan on the cisplatin-induced nephrotoxicity (21). Another study on candesartan, as an AT1receptor blocker, has shown that it reduces the nephrotoxicity of cyclosporine. It has been suggested that losartan, by binding to the AT1 receptor, reduces the production of free radicals (13). Ibrahim et al have shown that telmisartan, as another angiotensin antagonist, has the same effect as captopril on doxorubicin-induced nephrotoxicity, pointing out that these effects are at least partly due to the antioxidant and anti-inflammatory effects of this drug (35). Our study showed that the protective effects of losartan and magnesium are due to an increase in the level of nitrite in the kidney, probably due to AT1 receptor block and an increase in nitric oxide level due to stimulation of AT2 receptor (36). In this regard, Alaa et al, reported that losartan increases the amount of nitric oxide compared to valsartan, which probably has a role in improving blood pressure, renal blood flow and kidney function. Additionally, Siragy reported that stimulation of AT2 receptor causes increased nitric oxide in kidney, which contributes to the improvement of the function of this member (37).

According to the present study, we suggest studying the

effects of other mediators of the RAS, especially the role of the vasodilator arm (AT₂ receptor and Mas) on the gentamicin nephrotoxicity, by experimental or clinical investigations.

6. Conclusions

The results of the present study showed that administration of losartan and MgSO_4 individually and in combination reduced kidney nephrotoxicity and improved renal function. This effect is probably related to the improvement of antioxidant status renal blood flow.

Authors' contribution

TS and FK designed, conducted, supervised and analyzed the research and prepared the first draft of the manuscript. FK, AN and MZ participated in the performance of the research and collected the data. TS and FK participated in the writing and editing of the paper.

Conflicts of interest

The authors declared no competing interests.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

Funding/Support

The study was supported by the Deputy of Research & Technology Development at Zahedan University of Medical Sciences (#7822).

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