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LABORATORY STUDY

Atorvastatin Prevents Gentamicin-Induced Renal Damage in Rats through the Inhibition of p38-MAPK and NF- κ B Pathways

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Background and aims. Gentamicin (GM) is still considered to be an important antibiotic against life-threatening, gram-negative bacterial infections despite its known nephrotoxic effects. We aimed to evaluate the potential protective effect of atorvastatin (ATO) against GM-induced nephrotoxicity in rats. **Materials and methods.** The rats were randomly divided into five groups of six animals each: control, GM (100 mg/kg/day), ATO (10 mg/kg/day), GM + ATO, and GM + Vehicle. Kidney function tests, tissue oxidative stress parameters, and histopathological and immunohistochemical studies clarified GM nephrotoxicity. **Results.** GM caused a marked reduction in renal functions and increased oxidative stress parameters. Histopathological examination revealed tubular necrosis especially in the renal cortex in GM rats. On immunohistochemical evaluation, GM rat showed more intense expressions of mitogen-activated protein kinase (MAPK), nuclear factor kappa B (NF- κ B), and inducible nitric oxide synthase (iNOS) compared with control. Kidney function tests and tissue oxidative stress parameters were normalized in the GM + ATO group. Histopathological and immunohistochemical pictures were also greatly

ameliorated. **Conclusions.** ATO acts in the kidney as a potent scavenger of free radicals to prevent the toxic effects of GM via the inhibition of MAPK and NF- κ B signaling pathways and iNOS expression.

Keywords inducible nitric oxide synthase (iNOS), gentamicin, nephrotoxicity, nuclear factor kappa B (NF- κ B), p38-MAPK (mitogen-activated protein kinase), statin

INTRODUCTION

Gentamicin (GM) is widely used aminoglycoside antibiotic for the treatment of life-threatening gram-negative bacterial infections. However, its clinical usefulness is limited by the development of nephrotoxicity, which is characterized by tubular necrosis, without morphological changes in glomerular structures.^[1,2] Despite the introduction of less nephrotoxic antibiotics against gram-negative microorganisms, GM is still used because of its low cost and effectiveness against resistant β -lactam positive microorganisms.^[3]

The exact mechanism of GM-induced nephrotoxicity is not clearly understood. However, reactive oxygen species (ROS) are considered to be one of the important mediators of GM nephrotoxicity.^[4–6] In vitro and in vivo studies have shown that GM enhances the generation of ROS metabolites, which may cause cellular injury and

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necrosis via several mechanisms, including peroxidation of membrane lipids, protein oxidation, and DNA damage. It has also been reported that GM acts as an iron chelator, and that the iron-GM complex is a potent catalyst of oxygen derived radical formation.^[7,8] In addition to causing oxidative stress, GM also activates phospholipases and alters the lysosomal membrane.^[9] On the other hand, recent studies have shown that redox-sensitive transcription factors, mitogen-activated protein kinase (MAPK) and nuclear factor-kappa B (NF- κ B), are involved in nephrotoxicity caused by GM.^[10–12]

MAPK is an important mediator involved in the intracellular network of interaction proteins that transduce extracellular stimuli to intracellular responses.^[13] Three distinct MAPK pathways have been described: extracellular signal-regulated kinase (ERK), c-Jun-N-terminal kinase (JNK), and p38-MAPK.^[14] p38-MAPK is a ubiquitous, highly conserved protein kinase that plays an important role in the inflammatory response and in the apoptosis process.^[15,16] p38-MAPK is activated by cytokines and cellular stress, and its activation results in the increased production of inflammatory cytokine genes, including interleukin-1 β and tumor necrosis factor- α (TNF- α).^[16,17] p38-MAPK has been suggested to mediate lipopolysaccharide (LPS) or cytokine-induced iNOS expression in *in vitro* experiments.^[18,19] It has also been reported that p38-MAPK may be involved in NF- κ B activation.^[20]

NF- κ B is a ubiquitous transcriptional factor of the inducible expression of many genes, including iNOS, which encodes proteins involved in the modulation of inflammatory and host defense processes.^[21] The NF- κ B family includes p50, p52, Rel A (p65), Rel B, c-Rel, v-Rel, and dorsal and Dif proteins.^[22] Normally, these are sequestered in the cytoplasm of cells through its binding with its inhibitors, p105 and inhibitor κ B (I κ B)-like proteins.^[22] The activation of NF- κ B by external stimuli such as cytokines or ROS causes the degradation of its inhibitor I κ B-alpha or proteolytic cleavage of p105. Free NF- κ B dimers translocate to nucleus and activate the target genes, such as iNOS.^[23–25]

The role of nitric oxide (NO) in the pathophysiology of GM-induced rat nephrotoxicity has been studied. It has been reported that activation of inducible NOS (iNOS) aggravates renal injury in GM-associated rat nephrotoxicity.^[26]

3-Hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, referred to as statins, are widely used in the treatment of hypercholesterolemia,^[27] and their anti-oxidative properties may be of value in the fight against oxidative DNA damage. Statins have been shown to reduce lipoprotein oxidation and ameliorate free radical injury, and atorvastatin (ATO) possesses significant anti-oxidant activity against OH and peroxy radicals. Furthermore, metabolites of ATO reduce lipoprotein oxidation in

a number of oxidative systems.^[28] Statins also exert anti-inflammatory and antithrombotic effects. It has been reported that these effects of statins are not directly related to their cholesterol-lowering activity.^[29–31] Statins upregulate endothelial NOS (eNOS) and down regulates iNOS expression, independent of cholesterol levels.^[16] Recent studies have reported that MAPK and NF- κ B can be activated by oxidative stress and lipid peroxidation,^[32,33] and the activation of MAPK and NF- κ B might be inhibited by statins.^[34,35]

Therefore, the aim of the present study was to investigate whether ATO could prevent against GM-induced nephrotoxicity in rats.

MATERIALS AND METHODS

Drugs

Gentamicin (GM) was purchased from Bilim Pharmaceuticals, and atorvastatin (ATO) was obtained from Sanovel Corp., both of which are located in Istanbul, Turkey.

Animals

Male Wistar Albino rats (340–350 g) were housed in clean plastic cages in a temperature and humidity-controlled facility with a constant 12 h light/dark cycle with free access to food and water. The Institutional Animal Care and Use Committee approved the use of animals, and the experimental protocol and animals were treated in accordance with the Guide for the Care and Use of Laboratory Animals of Research Council.

Experimental Procedure

After a quarantine period of seven days, the rats were randomly divided into five groups, all of which consisted of six animals. GM was injected intraperitoneally (i.p.) at the dose of 100 mg/kg/day for ten consecutive days. The dose of GM was selected according to previous studies that demonstrated significant renal toxicity.^[36] ATO suspended in 0.5% carboxymethylcellulose (CMC) was administered via nasogastric gavage route at the dose of 10 mg/kg/day for ten consecutive days. The dose of ATO was selected based on the result of recent studies where the antioxidant and anti-inflammatory action of this agent was apparent.^[37,38]

Group 1 rats served as control and received a single dose i.p. injection of 1 mL saline. Group 2 rats were injected with GM alone, group 3 rats were treated ATO

alone, and group 4 rats were treated with GM + ATO. ATO was administered 30 minutes after injection of GM. Group 5 received GM + CMC (the ATO vehicle).

The urine produced by animals in 24 h in metabolic cages was collected. The urine volume of each animal was measured, and urine samples were used for the determination of urinary creatinine levels.

Tissue Samples

At 24 h after the administration of last doses of GM and ATO, rats were anesthetized by i.p. injection of ketamine, and blood samples were also taken from the vena cava to assess the serum levels of urea and creatinine concentrations.

Then, all rats were sacrificed with a high dose ketamine. Kidneys were reached with an abdomen middle line cut, and then quickly removed, cortex separated from medulla and surrounding tissues, and washed twice with cold saline solution. Fresh cortical samples of the kidneys were stored at -80°C to analyze malondialdehyde (MDA), an end product of lipid peroxidation, GSH (glutathione), and nonenzymatic antioxidant, and total nitrite, a stable product of nitric oxide (NO), was evaluated as a means of oxidative stress. Another sample of the kidney was stored in formol solution for histopathological and immunohistochemical examination. Paraffinized tissue samples were examined for tubular necrosis on light microscopy and inducible nitric oxide synthase (iNOS), p38-MAPK, and NF- κ B activities using immunohistochemistry.

Determination of Kidney Function

As indicators of kidney function, serum creatinine and BUN levels were measured. Creatinine clearance was calculated by multiplying urinary creatinine by 24 h urinary flow, divided by plasma creatinine. All biochemical variables were determined using an autoanalyzer (Olympus Instruments, Tokyo, Japan).

Measurement of Tissue Lipid Peroxidation Level

Frozen kidney samples homogenized in Teflon-glass homogenizer with a buffer containing 1.5% potassium chloride to obtain 1:10 (w/v) whole homogenate. Malondialdehyde (MDA), which formed as an end product of the peroxidation of lipids, served as an index of the intensity of oxidative stress. MDA, referred to as thiobarbituric acid reactive substance, was measured with thiobarbituric acid at 532 nm in a spectrophotometer, as described previously.^[39] The MDA level was expressed as nmol/g wet tissue.

Measurement of Tissue Glutathione Level

Reduced glutathione (GSH) was estimated by the method of Moron et al.,^[40] where the color developed was read at 412 nm. Protein concentrations in all samples were measured using the method of Lowry et al.^[41] Results were reported as $\mu\text{mol/g}$ wet tissue.

Measurement of Tissue Nitric Oxide (NO) Level

Total nitrite (NOx) was quantified by the Griess reaction^[42] after incubating the supernatant with *Escherichia coli* nitrate reductase to convert NO_3 to NO_2 . Griess reagent (1 mL 1% sulfanilamide, 0.1% naphthyl-ethylene-diamine hydrochloride, and 2.5% phosphoric acid; Sigma Chemical Co., St. Louis, Missouri, USA) was then added to 1 mL of supernatant. The absorbance was read at 545 nm after a 30-minute incubation. The absorbance was compared with the standard graph of NaNO_2 , obtained from the reduction of NaNO_3 (1–100 $\mu\text{mol/L}$). The accuracy of the assay was checked in two ways; the inter- and intra-assay coefficients of variation were 7.52% and 4.61%, respectively. To check conversion of nitrate to nitrite (recovery rate), predetermined amounts of nitrate were added to control plasma samples; these samples were deproteinized and reduced as above. The results were expressed as nmol/g wet tissue.

Histopathological Examinations

Histopathological evaluation was made in kidney tissues. Paraffin embedded specimens was cut into 6 μm thickness sections and stained with Hematoxylin-Eosin for light microscope examination (Olympus, BH-2, Tokyo, Japan). All sections of kidney samples were examined for characteristic histological changes including tubular epithelial alterations (vacuolization, degeneration, and cell desquamation) and cortical interstitial congestion. The kidney sections were analyzed semi-quantitatively using the technique of Houghton et al.^[43] The lesions were graded as follows:

- 0: normal;
- 1: areas of focal granula-vacuolar epithelial cell degeneration and granular debris in the tubular lumen, with or without evidence of tubular epithelial cell desquamation in small foci (<1% of the tubule population involved by desquamation);
- 2: tubular epithelial necrosis and desquamation easily seen but involving less than half of the cortical tubules;

- 3: more than half of proximal tubules showing desquamation and necrosis but involved tubules easily found; and
- 4: complete or almost complete proximal tubular necrosis.

Immunohistochemical Study

For immunohistochemical evaluation, specimens were processed for light microscopy and sections incubated at 60 °C overnight and then de-waxed in xylene for 30 minutes. After rehydrating in a decreasing series of ethanol, sections were washed with distilled water and PBS for 10 minutes. Sections were then treated with 2% trypsin in 50 mM Tris buffer (pH 7.5) at 37°C for 15 minutes and washed with PBS.

Sections were delineated with a Dako pen (Dako, Glostrup, Denmark) and incubated in a solution of 3% H₂O₂ for 15 min to inhibit endogenous peroxidase activity. Then, sections were incubated with NF- κ B/p65 (Rel A) Ab-1 (R-B-1638-R7, Neomarkers, Labvision, Fremont, California, USA), MAPK/p38 (Vector Laboratories, Burlingame, California, USA), and iNOS Ab-1 (R-B-1605-R7, Neomarkers) antibodies. The Ultra-vision (Labvision) horseradish peroxidase /3-amino-9-ethylcarbazole staining protocol was used at this stage.

Sections prepared for each case were examined by light microscopy. Sections of rat lung were used as the control for immunohistochemical staining specificity, according to data provided by the antibody manufacturer.

Sections prepared for each case were examined by light microscopy. Positive and negative controls were conducted in parallel with NF- κ B and iNOS stained sections. Staining of sections with commercially available antibodies served as the positive control. Negative controls included staining tissue sections with omission of the primary antibody.

According to the diffuseness of the staining, sections were graded as 0 = no staining, 1 = staining < 25%; 2 = staining between 25% and 50%; 3 = staining between 50% and 75%; or 4 = staining > 75%. According to staining intensity, sections were graded as 0 = no staining; 1 = weak but detectable staining; 2, distinct staining; and 3 = intense staining.^[44] Immunohistochemical values were obtained by adding the diffuseness and intensity scores.

Statistical Analyses

Statistical analyses of the histopathologic and immunohistochemical evaluation of the groups were carried out by the chi-square test, and analyses of the other data were compared by one-way ANOVA followed by Tukey multiple

comparison tests. Results of all groups were shown as mean values \pm standard deviation (SD). $p < 0.05$ was accepted as statistically significant value.

RESULTS

The biochemical, histopathological, and immunohistochemical results were similar for control and ATO groups, and for GM + ATO and GM + Vehicle groups. Therefore, we decided to consider them without distinction and report only the control and GM groups, respectively.

The Effect of ATO on GM-Induced Kidney Dysfunction

The effects of ATO on GM-induced kidney dysfunction are summarized in Table 1. GM caused a marked reduction in renal functions, as characterized by significant increases in serum BUN and creatinine levels with a concomitant decrease in calculated creatinine clearance values ($p < 0.05$). In all groups, the urinary volumes were found to be similar.

Administration of ATO to GM-treated rats markedly prevented GM-induced increases in serum BUN and creatinine levels as well as the reduction in calculated creatinine clearance values ($p < 0.05$).

The Effect of ATO on Oxidative Stress Parameters

GM administration caused a marked reduction in GSH level in the kidney tissues of rats treated with GM alone ($p < 0.001$), and treatment with ATO significantly elevated the GSH levels ($p < 0.001$; see Table 2).

The renal MDA and NO levels were found to be significantly higher in only GM-treated rats than those in the control group ($p < 0.001$), and treatment with ATO significantly prevented the elevation of MDA and NO levels (see Table 2).

The Effect of ATO on Histologic Changes and Grade of Tubular Necrosis in Kidney Tissues

Light microscope evaluation of kidneys in control group showed normal morphology of renal parenchyma with well-defined glomeruli and tubules (score 0, see Figure 1A). Morphological changes including tubular epithelial degeneration, vacuolization, cell desquamation, and necrosis were clearly observed in the GM-treated rats (grade of tubular necrosis score: 3–4; see Table 3 and

Table 1

The laboratory findings showing kidney function of control, gentamicin (GM), atorvastatin (ATO), GM + ATO, and GM + Vehicle rats, and the effect of ATO treatment on these parameters

Variable	Groups				
	Control	GM	ATO	GM + ATO	GM + Vehicle
Serum creatinine (mg/dL)	0.48 ± 0.01	1.96 ± 0.3*	0.50 ± 0.04	0.64 ± 0.13 [†]	2.13 ± 0.2
Serum BUN (mg/dL)	42.4 ± 2.1	120 ± 12.4*	39.5 ± 1.3	49.3 ± 0.7 [†]	112.8 ± 9.4
Urine volume (mL/24 h)	11.4 ± 1.54	10.9 ± 2.13	11.34 ± 1.78	10.7 ± 1.84	11.3 ± 1.49
Creatinine clearance (ml/min)	0.52 ± 0.04	0.042 ± 0.003 [‡]	0.49 ± 0.01	0.48 ± 0.06 [§]	0.044 ± 0.008

Values are expressed as mean ± SD for six rats in each group.

*Significantly different from control ($p < 0.001$).

[†]Significantly different from gentamicin group ($p < 0.001$).

[‡]Significantly different from control ($p < 0.05$).

[§]Significantly different from GM group ($p < 0.05$).

Abbreviation: BUN: blood urea nitrogen.

Table 2

Effect of atorvastatin (ATO) (10 mg/kg/day) on the levels of glutathione (GSH), malondialdehyde (MDA), and nitric oxide (NO) of rats treated with gentamicine (GM) (100 mg/kg/day)

Variable	Control	GM	ATO	GM + ATO	GM + Vehicle
GSH (μmol/g wet tissue)	1.94 ± 0.4	0.60 ± 0.1*	1.98 ± 0.3	1.6 ± 0.3 [†]	0.58 ± 0.2
MDA (nmol/g wet tissue)	36.5 ± 6.4	97 ± 16.4*	38.8 ± 5.3	47.1 ± 5.6 [†]	95 ± 13.7
NO (nmol/g wet tissue)	35.3 ± 6.1	102 ± 24.1*	36.4 ± 7.8	43.5 ± 7.5 [†]	107 ± 21.3

Values are expressed as mean ± SD for six rats in each group.

*Significantly different from control ($p < 0.001$).

[†]Significantly different from gentamicin group ($p < 0.001$).

Abbreviations: NO = nitric oxide, MDA = malondialdehyde, GSH = reduced glutathione.

Figure 1B). However, in rats treated with GM + ATO, despite the presence of mild tubular degeneration and epithelial vacuolization in the proximal tubules, cellular desquamation was minimal and glomeruli maintained a better morphology (grade of tubular necrosis: 0–1) compared with the GM group (see Table 3 and Figure 1C).

The Effect of ATO on Immunohistochemical Changes in Kidney Samples

On immunohistochemical evaluation, there were more intense expressions of iNOS, p38-MAPK, and p65-NF-κB in rats treated with GM alone compared with control (see Table 3 and Figures 2A–2H). The administration of ATO to rats treated with GM reduced the expressions of iNOS, p38-MAPK, and p65-NF-κB. Although there were poor or slight expressions of iNOS, p38, and p65 in GM + ATO and control groups, there was no significant difference between these two groups ($p > 0.05$; see Table 3).

DISCUSSION

GM-induced nephrotoxicity is characterized by a reduction in renal function as reflected by an increase in serum creatinine and urea levels with a concomitant decrease in glomerular filtration rate.^[45] In the present study, serum levels of creatinine and BUN were significantly higher in the GM-treated rats when compared with the control group. The result of this study confirmed that GM, at a dose of 100 mg/kg/day, produces nephrotoxicity as evidenced by the reduction of glomerular filtration rate, which is shown by the increase in serum creatinine. The increases in serum creatinine and BUN levels induced by GM were significantly blocked by ATO. The protective effect of ATO on creatinine and BUN levels can be attributed to its antioxidant effect because it has been found that ROS may be involved in the impairment of glomerular filtration rate.^[45]

Although the exact mechanism of GM-induced nephrotoxicity is not well understood, there is much in vivo and

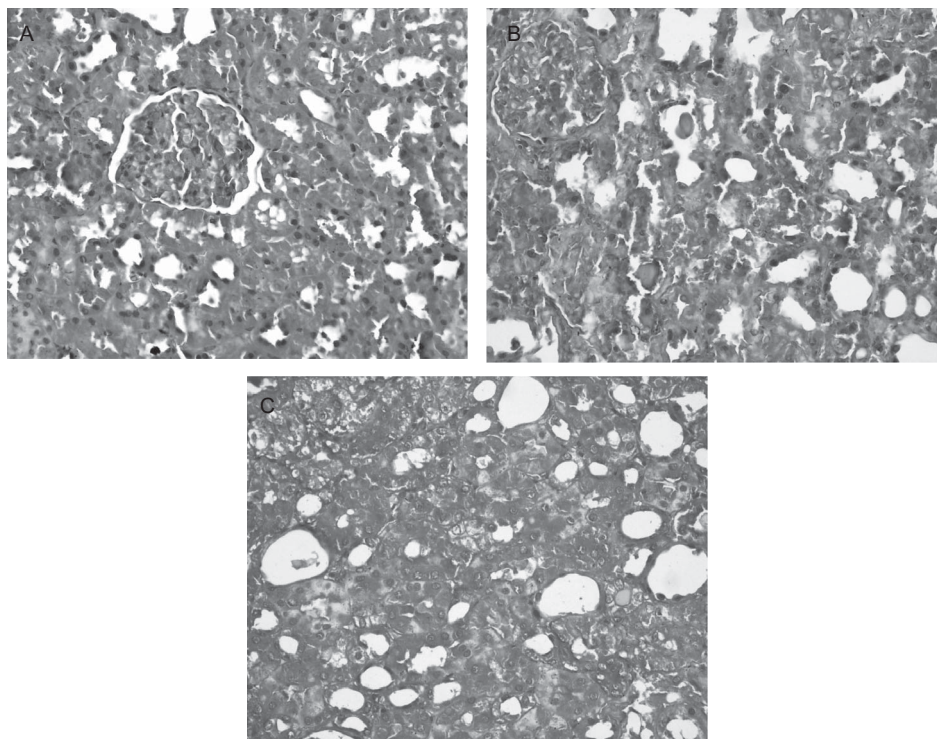


Figure 1. Kidney morphology in (a) a control rat, (b) a rat treated with GM alone, and (c) a rat treated with GM + ATO (Haematoxylen & Eosin \times 400).

in vitro evidence that suggests that GM-induced ROS are essential mediators of its nephrotoxic effects.^[45,46] Therefore, the administration of several agents with antioxidant activity has been successfully used to prevent or ameliorate GM nephrotoxicity.^[47-50] Here we measured the MDA, the end-product of lipid peroxidation, GSH, and total nitrite, a stable product of nitric oxide (NO), as a means of oxidative stress. GM-induced nephrotoxicity manifested by a significantly decreased in kidney MDA and NO levels, and GSH content significantly decreased in the kidney tissue. ATO administration caused significant decreases in lipid peroxidation and NO generation, and promoted increases in GSH content in the kidney. These findings strongly indicate that ATO is important in protecting the kidney from GM-induced injury through improvement in oxidant status. In addition to these biochemical parameters, we evaluated the expression of redox sensitive transcription factors, NF- κ B and MAPK, as well as iNOS expression by immunohistochemistry. Reverse transcriptase-polymerase chain reaction or Western blotting analyses are functional assays by which to measure the actual activity of MAPK and iNOS. Western blotting provides a more quantitative way of measuring iNOS and NF- κ B p65 subunit activity. Therefore, it may be a limitation of this study that Western blotting analyses were not

performed. However, there are a number of studies in the literature that have made use of immunohistochemical grading of iNOS and MAPK to evaluate their activity.^[51]

Increasing evidence suggests that statins have not only cholesterol-lowering effect but also have anti-inflammatory, antioxidant, as well as NF- κ B, MAPK and iNOS inhibitor properties, which are not directly related to their cholesterol-lowering activity.^[52] In the clinical medicine, the statins are used at the dose range of 20–80 mg/day for the treatment of hypercholesterolemia. Data of preliminary studies showed that ATO at the dose we used in this study did not change the serum cholesterol levels of the animals compared to control values. Thus, attenuation of the oxidative stress parameters in this study may reflect the direct anti-oxidant activity of the drugs that are independent of their cholesterol-lowering activity. Therefore, plasma lipid levels were not studied in this study.

In ours and other previous works, it has been shown that p38-MAPK and NF- κ B pathways are involved in gentamicin nephrotoxicity.^[10,53,54] In resting cells, NF- κ B is inactive in cytoplasm through its binding with its inhibitors, p105 and I κ B-like proteins.^[55] We think that increased ROS secondary to GM therapy in rat kidney causes the degradation of its inhibitor I κ B-alpha or proteolytic cleavage of p105, and free NF- κ B dimers

Table 3
The grade of necrosis and immunohistochemical staining score in control, gentamicine (GM), atorvastatin (ATO), GM + ATO, and GM + Vehicle groups

Grade of necrosis	Groups				
	Control	GM	ATO	GM + ATO	GM+Vehicle
0	5	0	4	3	0
1	1	0	2	2	0
2	0	0	0	1	0
3	0	2	0	0	3
4	0	4	0	0	3
Immunohistochemical staining score					
iNOS					
0	—	—	—	—	—
1	5	—	4	—	—
2	1	—	2	—	—
3	—	—	—	5	—
4	—	—	—	1	—
5	—	—	—	—	—
6	—	3	—	—	2
7	—	3	—	—	4
p65					
0	—	—	—	—	—
1	2	—	3	—	—
2	4	—	3	—	—
3	—	—	—	2	—
4	—	—	—	4	—
5	—	—	—	—	—
6	—	1	—	—	1
7	—	5	—	—	5
p38					
0	—	—	—	—	—
1	4	—	5	—	—
2	2	—	1	—	—
3	—	—	—	1	—
4	—	—	—	5	—
5	—	—	—	—	—
6	—	1	—	—	3
7	—	5	—	—	3

translocates to nucleus and activates the target genes, such as iNOS. Beneficial effects of selective iNOS blockade in GM-induced nephrotoxicity have been investigated and found that inhibition of iNOS may prevent GM-induced nephrotoxicity, whereas non-selective inhibition of NOS aggravates it.^[56] Although endothelial NO may have a beneficial role as a vasodilator by inducing an increase in renal blood flow and in glomerular filtration in these animals, excessive NO production can lead to cytotoxic injury. Peroxynitrite anion formation, protein tyrosine nitration, and hydroxyl radical production may

be responsible from the evolution of the renal injury induced by GM.^[57] This finding supports our hypothesis and results.

It has been reported that selective p38-MAPK inhibitors can block the production of inflammatory molecules, reducing the apoptotic cell death and ameliorating the acute renal injury observed in some animal models of renal disease, such as anti-GBM glomerulonephritis and ischemia/reperfusion.^[58,59] Furthermore, there is evidence that the production of TNF post-renal injury is triggered by the locally produced ROS, which activate NF- κ B

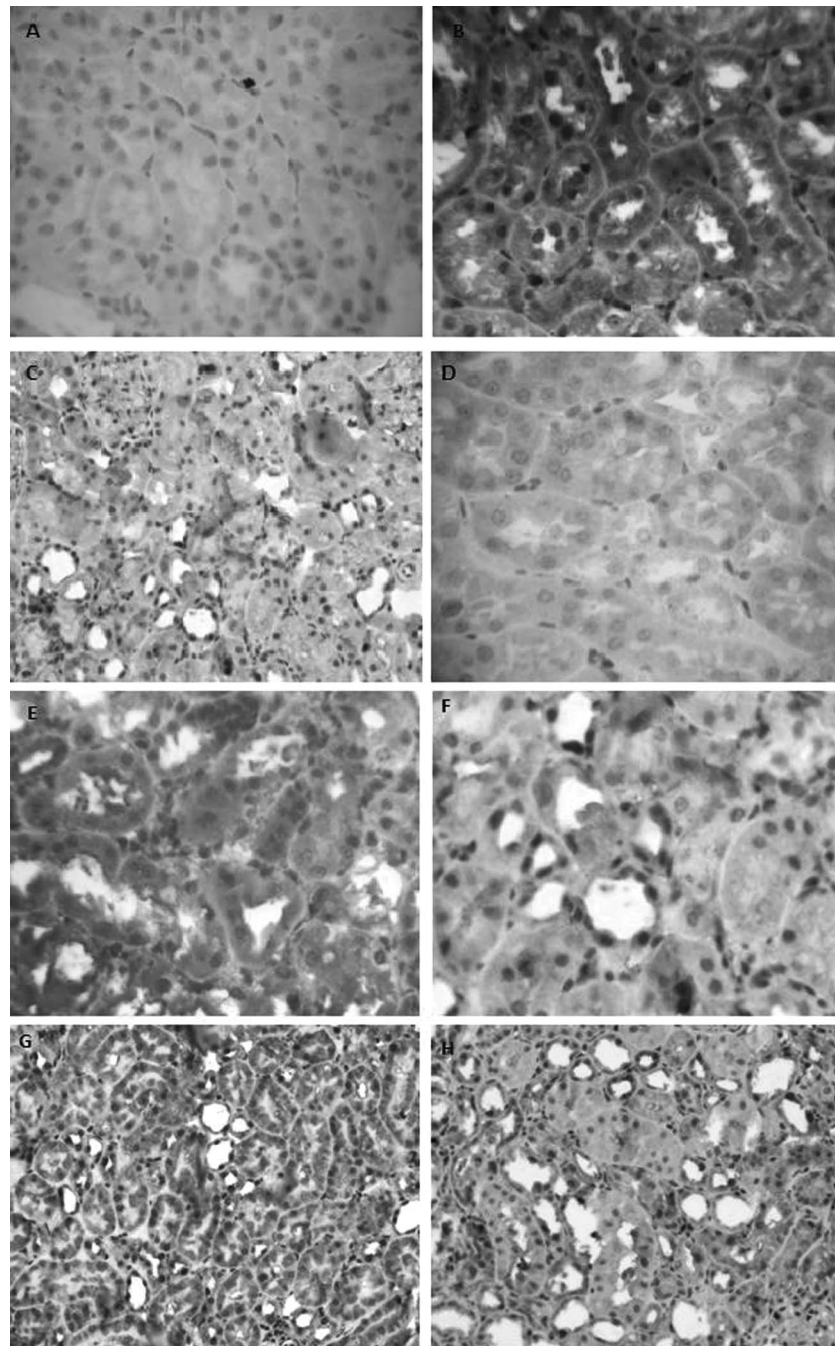


Figure 2. Immunohistochemical staining showing iNOS, p38/MAPK, and p65/NF- κ B expression: (a) focal poor staining with iNOS in proximal tubular cells in control ($\times 400$), (b) diffuse iNOS staining in proximal tubular cells in gentamicin group ($\times 400$), (c) focal mild iNOS staining in proximal tubular cells in gentamicin+atorvastatin ($\times 400$), (d) low MAPK/p38 positivity in proximal tubular cells in control group ($\times 400$), (e) Diffuse, intensive MAPK/p38 positivity in proximal tubular cells in gentamicin group ($\times 400$), (f) low MAPK/p38 positivity in proximal tubular cells in gentamicin+atorvastatin group ($\times 400$), (g) diffuse, intensive NF κ B/p65 staining proximal tubular cells in gentamicin group ($\times 400$), and (h) low NF κ B/p65 staining in proximal tubular cells in gentamicin + atorvastatin group ($\times 400$).

through p38-MAPK.^[60] Activation of p38-MAPK can also induce NF- κ B activation and subsequent transcription of inflammatory cytokines.^[48] TNF- α and ROS also activates NF- κ B.^[53] In GM nephrotoxicity, we can suggest that increased p38-MAPK activity further activates NF- κ B and, as a result, inflammatory and apoptosis-related gene expression and finally tubular necrosis and nephrotoxicity. There is evidence indicating that HMG-CoA reductase inhibitors reduce I κ B kinase activity induced by oxidative stress in monocytes and vascular smooth muscle cells^[61] and inhibits carboxymethyllysine-induced monocyte chemoattractant protein-1 expression in podocytes via prevention of signaling events.^[62] The same mechanism may be effective in GM nephrotoxicity and inhibition of I κ B kinase activity by statin prevents translocation of free NF- κ B dimers to nucleus and kidney injury.

GM treatment provokes acute tubular necrosis and acute renal failure in about 30% of high-risk patients.^[45] Animal models of aminoglycoside nephrotoxicity also present residual areas of interstitial fibrosis in the renal cortex and progressive tubular injury.^[63,64] In the present study, there was tubular cell necrosis, focal areas of denuded basement membrane, intraluminal casts, diffuse interstitial edema, and interstitial inflammatory cell infiltrates, as described in literature in GM-treated rats.^[65] On the other hand, the tubules from rats of the GM + ATO group were nearly normal in histological appearance except for a slight desquamation and atrophy of the tubular epithelial cells. The protective effect of statins have not been reported in GM-induced nephrotoxicity, but it is reported that simvastatin (statin) attenuates cisplatin-induced kidney and liver damage via prevention of lipid peroxidation and tissue fibrosis, preservation of antioxidant glutathione, and suppression neutrophil infiltration.^[66] We can say that similar mechanisms are responsible from beneficial effect of statin in GM toxicity in rats.

Our data may help to further understand the molecular mechanisms of statins in GM nephrotoxicity. Statins attenuates GM nephrotoxicity presumably antioxidant as well as p38-MAPK, NF- κ B, and iNOS inhibitor properties. Statins also can be used in clinical practice in patients needing GM therapy because of severe gram-negative infection, especially in high-risk groups for GM toxicity. Further animal and clinical studies are needed to confirm our suggestion.

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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