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α-Tocopherol Reduces Morphological Changes and Oxidative Stress during Gentamicin-Induced Acute Renal Failure

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We studied the effect of α-tocopherol on gentamicin-induced morphological and functional changes in the kidneys of Wistar rats. Special attention was paid to the ability of α-tocopherol administered in combination with gentamicin to correct ultrastructural changes in the glomerular basal membrane and tubules. Combined treatment with α-tocopherol (100 mg/kg) and gentamicin (100 mg/kg) led to correction of histopathological and biochemical changes and oxidative injury to the kidneys induced by this antibiotic.

Key Words: *α-tocopherol; gentamicin; glomerular basal membrane; Wistar rats*

Gentamicin is extensively used in clinical practice for the treatment of Gram-negative infections. However, this antibiotic exhibits nephrotoxicity in 10-25% patients [6]. Nephrotoxicity of gentamicin is related to oxidative and nitrosative stress in the renal cortex [3]. The increased generation of ROS leads to damage and necrosis of renal cells due to membrane LPO, protein denaturation, DNA injury, and cytochrome C release from mitochondria [7,9]. α-Tocopherol (vitamin E) is an endogenous antioxidant that attenuates cell damage caused by oxidative stress and apoptosis [1,4].

This work was designed to study the effect of α-tocopherol on gentamicin-induced morphological and functional changes in the kidneys of rats. Special attention was paid to the ability of α-tocopherol administered in combination with gentamicin to correct ultrastructural changes in the glomerular membranes and tubules.

MATERIALS AND METHODS

Experiments were performed on 24 Wistar rats weighing 250 g. The animals were housed in polycarbonate cages under controlled conditions (12/12 h light/dark cycle, 20±2°C) and had free access to water and food. The study was approved by the Ethics Committee of the Faculty of Medicine at the University of Nis (No. 01-2625-7).

The animals were divided into 3 groups (8 rats per group) and received gentamicin (Galenika AD) in a dose of 100 mg/kg for 8 days (group 1), or α-tocopherol (100 mg/kg, Sigma-Aldrich) in combination with gentamicin (100 mg/kg) for 8 days (group 2), or physiological saline in a dose of 1 ml/kg (control). On day 9, the animals were narcotized with 80 mg/kg ketamine (Ketamidor 10%, Richter Pharma AG) and sacrificed.

The concentrations of urea and creatinine in blood samples were measured on a biochemical analyzer (Biosystems A25) at the Laboratory of the Nephrology Clinics in the Nis Clinical Center. For histopathological analysis of the renal tissue, the tubular basal membrane and glomerular tubules were stained with silver methenamine (Jones' method). The preparations were examined under a Leica DM 2000 LED microscope. For evaluation of the intensity of oxidative stress, the renal tissue was minced and homogenized in ice-cold water using a homogenizer (IKA Works de Brasil Ltda Taquara, RJ 22,713-00). Protein concentration was measured by the Lowry method using bovine serum as the standard. The intensity of LPO was evaluated by the content of MDA and TBA-reactive products in renal tissues measured spectrophotometrically (Ohkawa me-

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thod). MDA concentration was expressed in nmol/mg protein (molecular extinction coefficient of MDA 1.56×10^{-5} mol×cm^{-1}). Catalase activity was evaluated spectrophotometrically by the Goth method: spectrophotometric assay of hydrogen peroxide based on formation of its stable yellow-collored complex with ammonium molybdate that was measured at 405 nm. Enzyme activity was expressed in catalytic units per 1 g protein (cU/g). Structural changes in the glomeruli, glomerular basement membrane (GBM), proximal and distal tubules, and nuclei of renal intestinal cell were evaluated quantitatively by the morphometric method.

Morphometry was conducted with a Leica DMR microscope and ImageJ software. Special calibration of a micrometer (1:100) and optical density calibration were performed before each measurement. Morphometry of the glomeruli and tubules was performed at 10-fold magnification after hematoxylin and eosin staining. Interstitial cell nuclei were examined at ×40. The glomeruli were analyzed by the following morphometric parameters: thickness of GBM, cellularity, area, optical density, perimeter, Feret diameter, and roundness. Morphometry of renal tubules included measurement of the surface and nucleus/cytoplasm (NC) ratio. The area and roundness of renal interstitial cell nuclei were estimated morphometrically.

The results of biochemical and morphological studies were analyzed by one-way ANOVA and post-hoc Tukey test with GraphPad Prism 5.03 software. The data are presented as the means and standard deviations.

RESULTS

Administration of gentamicin in a dose of 100 mg/kg was followed by the development of acute renal failure. A histopathological study of the renal tissue showed that glomeruli are significantly enlarged on day 1 (Table 1). GBM of the capillary was diffusely thickened. Coagulation necrosis and dark inclusions were seen in the cytoplasm of epithelial cells in the proximal tubules (Fig. 1). In group 2, the glomeruli were only slightly enlarged, GBM in capillaries was thickened only in some glomerular segments (Table 1). Hyaline cylinders without signs of coagulation necrosis were found in some proximal tubules (Fig. 1). Histopathological study of the renal tissue from control rats showed normal histological structure.

In group 1 rats, the concentrations of blood serum creatinine (310.85±23.46 μmol/liter) and urea (29.57±2.88 μmol/liter) were much higher ($p<0.001$) than in control controls (43.22±3.67 and 6.90±1.74 μmol/liter, respectively) and in group 2 animals (81.49±6.82 and 9.99±2.37 μmol/liter, respectively). Increased levels of urea and creatinine are associated with impaired glomerular filtration, which results in retention of nitrogen products. These data are consistent with the results of our previous experiments [10,11]. Administration of α-tocopherol was followed by a decrease in the content of urea and creatinine and produced a cytoprotective effect due to inhibition of ROS [8,14]. The protective effect of α-tocopherol is probably associated with its ability to reduce the degree of oxidative damage to DNA [2].

The results of blood biochemical analyses were shown to correlate with the data obtained in histopathological and morphometric studies (Table 1). Gentamicin potentiates generation of ROS, which can lead to cell damage and death. These changes are mediated by various mechanisms, especially by LPO. These processes are followed by destabilization of the cell membrane, which results in cell swelling and necrosis [7]. Analysis of MDA content showed that the intensity of LPO, the process that can cause cell damage and death [12], was maximum in group 1 animals ($p<0.001$, Fig. 2). In group 1 rats, catalase

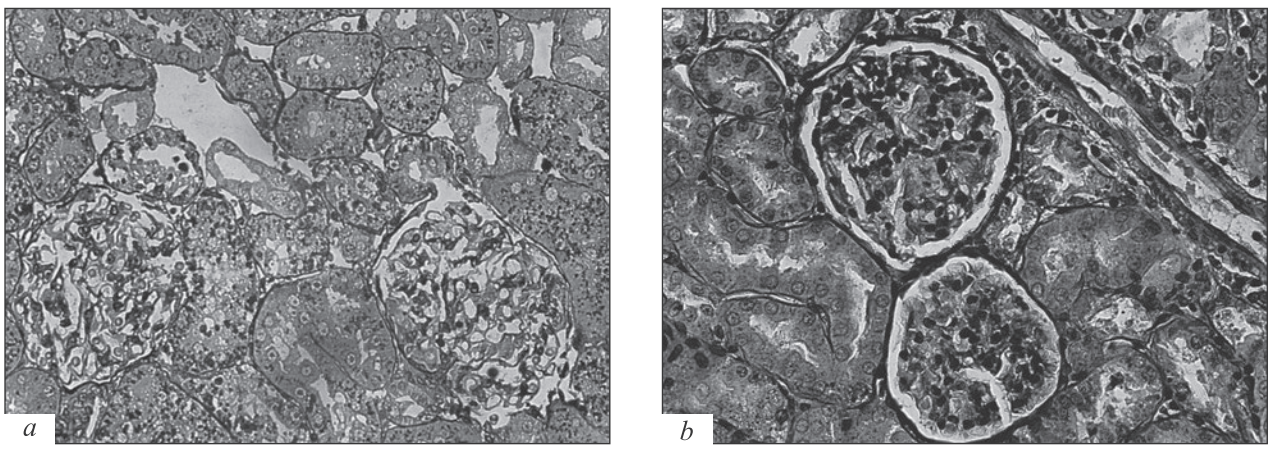

Fig. 2. Parameters of oxidative stress in rat renal tissue. *$p<0.05$, **$p<0.01$, and ***$p<0.001$ in comparison with the control; $^{+}p<0.01$ and $^{++}p<0.001$ in comparison with group 1.

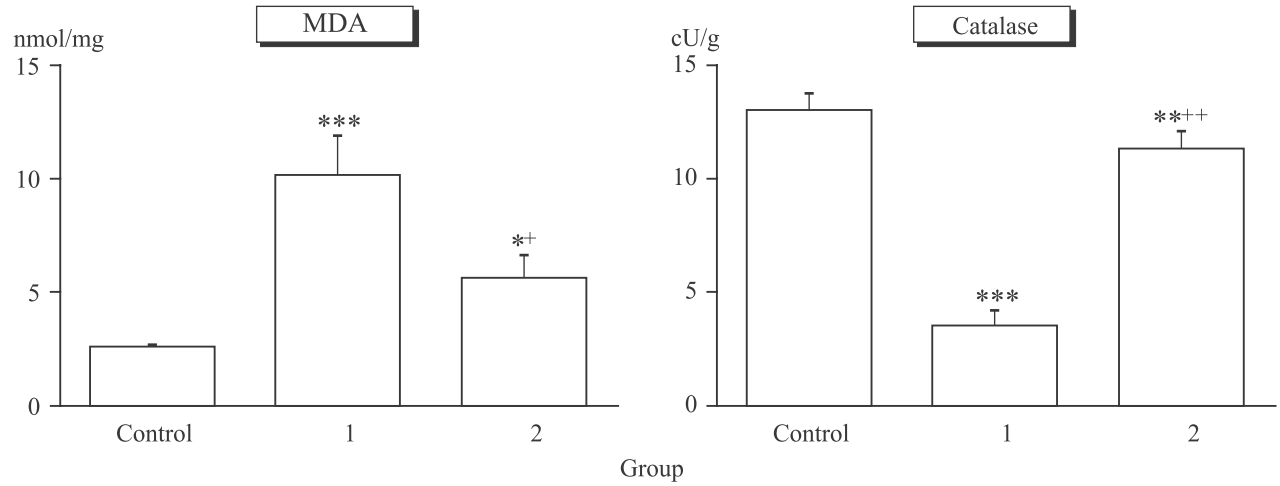

Fig. 1. Histopathological signs in the renal tissue in rats receiving gentamicin (*a*) or gentamicin+α-tocopherol (*b*), ×400.

activity was considerably lower than in the control ($p<0.001$, Fig. 2), while in group 2 animals receiving combined treatment with α-tocopherol and gentamicin, this parameter was higher than in group 1 specimens ($p<0.01$, Fig. 2). Therefore, pretreatment with α-tocopherol increases antioxidant status of the kidneys. Morphometry showed that optical density of epithelial cell nuclei in the proximal and distal tubules of group 1 rats was much lower than in group 2 animals and in controls. These data indicate that gentamicin damaged the nuclei of tubular epithelial cells (Table 1). Morphometry of cell nuclei in the proximal and distal tubules showed that optical density of these structures in group 2 rats was much higher than in group 1. These findings attest to the protective effect of α-tocopherol on nuclear chromatin, which is consistent with published data [13].

Morphometry of the proximal and distal tubules revealed a significant decrease in the epithelial surface and increase in the NC ratio in gentamicin-receiving rats of group 1 (in comparison with other groups, Table 1). Published data show that gentamicin causes proliferation and apoptosis in glomerular mesangial cells [5]. Cell counting showed high degree of apoptosis on the model of gentamicin nephrotoxicity (Table 1). Morphometry of renal interstitial cells revealed preva-

TABLE 1. Morphometric Parameters of the Glomeruli, Proximal and Distal Tubules, and Nuclei of Renal Interstitial Cells in Rats of the Control and Treatment Groups

Parameter	Control group	Group 1 (gentamicin)	Group 2 (gentamicin+α-tocopherol)
GBM thickness, μ	0.555±0.028	0.815±0.051***	0.598±0.034+++
Area of glomeruli, μ²	7122.0±380.3	10030.0±741.4***	7979.0±340.7**+++
Perimeter of glomeruli, μ	313.900±9.760	366.7±15.85***	319.700±8.150+++
Feret diameter of glomeruli, μ	112.500±5.478	127.9±6.575***	113.5±4.446+++
Cellularity, cells/μ²	0.0074±0.0015	0.0051±0.0009***	0.0072±0.0006++
Area of proximal tubules, μ²	1471.0±134.2	1204.00±96.91***	1429.0±145.1+++
NC ratio of proximal tubules	0.476±0.116	0.607±0.102*	0.490±0.108+
Area of distal tubules, μ²	1068.0±177.6	1221.0±236.8	1139.0±243.9
NC ratio of distal tubules	0.550±0.105	0.635±0.136	0.638±0.151
Optical density of nuclei in proximal tubules	0.51±0.09	0.41±0.06*	0.48±0.08+
Optical density of nuclei in distal tubules	0.49±0.11	0.40±0.12*	0.46±0.07+
Roundness of nuclei in renal interstitial cells	0.788±0.070	0.935±0.009***	0.845±0.073+
Area of nuclei in renal interstitial cells, μ²	33.01±1.55	16.88±0.94***	28.71±3.20**+++

Note. *$p<0.05$, **$p<0.01$, and ***$p<0.001$ in comparison with the control; +$p<0.05$, ++$p<0.01$, and +++$p<0.001$ in comparison with group 1.

lence of small cells with round nuclei (Table 1), which probably results from significant peritubular infiltration with leukocytes under conditions of gentamicin nephrotoxicity. Administration of α-tocopherol had a correcting effect on gentamicin-induced histological and morphometric changes. Our results illustrate the protective activity of this vitamin, which is related to its antioxidant properties. We conclude that a combined treatment with α-tocopherol and gentamicin has a correcting effect on histological and biochemical changes and oxidative damage to the kidneys induced by this antibiotic. These data indicate that α-tocopherol can be used as an antioxidant, when the long-term therapeutic treatment with gentamicin is required.

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