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EFFECTS OF SUPPLEMENTATION WITH VITAMIN E ON GENTAMYCIN-INDUCED ACUTE RENAL FAILURE IN RATS

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Abstract. *A frequent administration of gentamicin in clinical practice has shown its bactericidal activity, and besides being vestibulotoxic it is highly nephrotoxic, which can further result in acute renal insufficiency. The study analyzed 24 Wistar rats, divided into three equal groups. GM group received gentamicin (100 mg/kg), GME group received vitamin E (100 mg/kg) and the same dose of gentamicin as GM rats, while the third group served as the control group and received saline (1 ml/24h) for 8 days. Pathohistological examination of the kidney tissues from GM group rats showed areas of coagulation-type necrosis in a large number of proximal tubules, while their glomeruli were considerably enlarged compared both to control and GME group rats. In GME rats, changes in glomeruli were less visible, while areas of coagulation-type necrosis were not found. Biochemical analysis showed significantly higher values of blood urea and creatinine in GM group rats in comparison to C group and GME group ($p<0.001$). The concentrations of potassium in blood serum was significantly lower in GM group compared to control group ($p<0.01$), whereas the concentration of sodium was lower, however, without statistical significance. The concentrations of AOPP for GM group were significantly higher when compared to C group ($p<0.001$), whereas the values for GME group of rats were statistically significantly lower than AOPP recorded for GM group ($p<0.001$). Our experimental study has shown that gentamicin-induced nephrotoxicity can be significantly reduced by simultaneous administration of vitamin E.*

Key words: *Gentamicin, vitamin E, nephrotoxicity, Wistar rats*

Introduction

A very frequent administration of the aminoglycoside antibiotic gentamicin in the clinical practice has shown its undoubted nephrotoxic effect [1]. Even in low concentrations, gentamicin shows its bactericidal activity, and besides being vestibulotoxic it is highly nephrotoxic, which can further result in acute renal insufficiency (ARI). Numerous experimental models have confirmed the nephrotoxicity induced by gentamicin [2–5], cyclosporine [6, 7], cisplatine [8], adriamicin [9], as well as other toxic chemical substances such as glycerol [10, 11], mercury chloride [12] and others. Nephrotoxicity induced in these experimental models showed pathohistological, ultrastructural and functional renal impairments in the form of tubular desquamation and necrosis and elevated blood urea and serum creatinine. The predilection sites of damage are the renal cortex, i.e. glomeruli and proximal tubules. In the recent years, there have been many studies pointing to the significant role of reactive oxygen species (ROS) in gentamicin-induced nephrotoxicity [13]. In their research, Sha and Schacht [14] showed that aminoglycoside antibiotics can stimulate free radical formation. Therefore, it can be claimed with great certainty that gentamicin induces ooxidative stress. Kidney cells can produce free radicals in glomerular mesangial and endothelial cells and in tubular epithelial cells [15]. Epithelial cells of proximal tubules are very sensitive to the effects of oxygen free radicals as 50% of cells die after being exposed to the effect of H_2O_2 [16]. These free radicals destroy the glomerular basement membrane, impair the tubular function, degrade the collagen and other components of matrix [17]. Because of potential gentamicin nephrotoxicity, in the recent years there has been much research on the administration of protective substances which would prevent or reduce renal alterations, and also prevent the onset and development of renal insuffuciency. Vitamin E and N-acetyl cysteine (NAC) are well known for their antioxidant activities. Vitamin E protects unsaturated fatty acids from oxydation via peroxide and other free radicals. Vitamin E and NAC have shown their protective effects in gentamicin-induced nephrotoxicity [18]. Some studies have demonstrated that vitamin E and vitamin C reduce the lipid peroxidation and increase the activities of antioxidant enzymes in diabetic rat kidneys [19]. Vitamin E pretreatment suppresses oxidative stess and glomerulosclerosis in experimental rats [20]. The administration of single doses of vitamin E has possitive effects on cisplatin-induced nephrotoxicity in rat development [21].

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The aim of the research was to show the protective effects of vitamin E on gentamicin-induced acute renal failure in rats.

Material and Methods

Twenty-four adult Wistar rats, weighing 250–300 g, were used in the present study. The animals were housed at the Institute for Biomedical Research, Faculty of Medicine in Niš. The animals were kept in polycarbonate cages under controlled conditions with the twelve-hour day/night cycle, at the temperature of 20 ° C ± 2 ° C, and the "ad libitum" access to food ("VETFARM"-Beograd) and drinking water. All experimental procedures were approved by Ethical Committee of Medical Faculty in Niš. It was documented under number 01-2625-7.

Experimental protocol

Experimental animals were randomly divided into three equal groups of 8 animals each, one of which was used as a control group. The experimental group of animals or GM group was treated with gentamicin (Galenika AD, Belgrade, Serbia) intraperitoneally in a dose of 100 mg /kg body weight (BW)/24h. The experimental group of animals treated with gentamicin and vitamin E or GME group received oily solution of vitamin E (Pharmamagist, Hungary) intraperitoneally in a dose of 100 mg/kg BW/24h and the application of the same dose of gentamicin as in the first group of rats. The control group of animals received physiological saline solution 1 ml/day intraperitoneally. All groups were treated over a period of 8 consecutive days. Following the last application, that is 9 days after the beginning of the experiment, all animals were anaesthetized using ketamine at a dose of 80 mg/kg (10% Ketamidor, Richter pharma AG, Wien, Austria). Blood samples were taken from the aorta (2ml), and the kidneys were subsequently removed.

Histological analysis

The kidneys were dissected out, washed and fixed in 10% paraformaldehyde (in 0.1M phosphate buffer saline), dehydrated in ascending graded series of alcohol and processed for paraffin embedding. Kidney tissue species were cut at a thickness of 5μm using a HistoRange microtome (model: LKB 2218, LKB-Produkter AB, Bromma, Sweden) and stained with hematoxylin–eosin (HE) for the study of morphological changes in the kidney and PAS (Periodic Acid Schiff) for verifying the content of glycogen, according to conventional staining protocols. For histopathological examination of kidney tissue the microscope (LEICA DM 2000 LED) and digital camera (LEICA DFC 450) were used.

Biochemical analysis

Blood samples were analyzed for markers of kidney function impairment. Urea, creatinine, sodium and potassium concentrations in serum were measured in the laboratory of the Department of Nephrology and Dialysis Clinical Center Niš using an automatic biochemical analyzer (A25 Biosystems, Barcelona, Spain).

Determination of protein oxidation

To determine the concentration of advanced oxidation protein products (AOPP) as a marker of oxidative modified proteins, kidney tissue was minced and homogenized in ice-water with homogenizer (IKA Works de Brasil Ltda Taquara, RJ 22, 713-00). Proteins were measured according to Lowry's method [22] using bovine serum as standard. The concentration of AOPP in the renal homogenates was determined by spectrophotometric method by Witko-Sarsat [23]. This method is based on the reaction of AOPP with potassium iodide in an acidic medium. The color intensity was recorded immediately at 340 nm. The concentrations were expressed in μmol/mg protein.

Statistical analysis

Statistical analysis of the data obtained by biochemical blood analysis were expressed as mean values and standard deviations, and statistical significance was determined by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparison (Graphpad Prism 5.03, San Diego, CA, USA).

Results

Histological analysis

In the GM group of rats, a large number of proximal tubules, especially initial convoluting portions, showed coagulation-type necrosis and apoptosis with cytoplasm vacuolation, desquamation and inflammatory cell infiltration in cells still containing nuclei. Glomeruli in this group were enlarged and paler (Fig. 1) than in the control group of animals (Figs. 2 and 3). In experimental group treated with gentamicin some of glomerular capillaries were infiltrated with neutrophil leukocytes

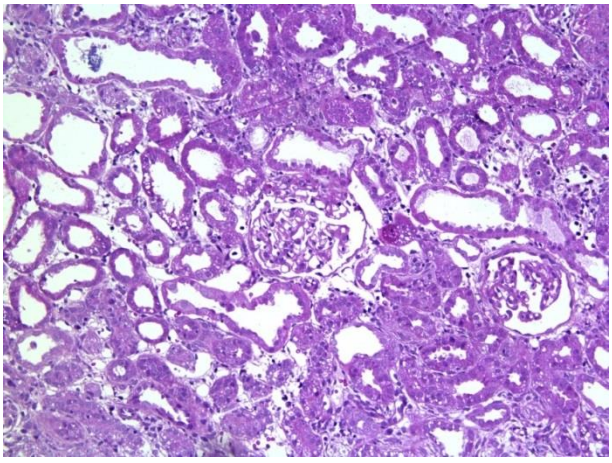

Fig. 1. Histopathological tissue features of renal glomeruli and tubules of gentamicin-treated (GM) group of rats. HE, × 200.

(Fig. 4). The distal tubules were of normal appearance. In the GME group, glomeruli were somewhat enlarged and hyaline cylinders in some proximal tubules were present; the areas of coagulation-type necrosis were not found (Figs. 5 and 6).

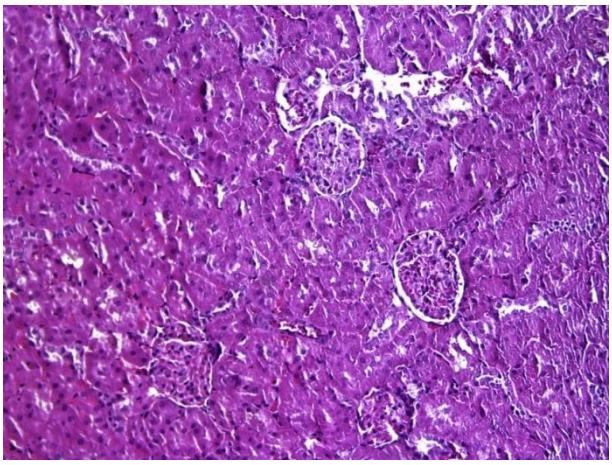

Fig. 2. Histopathological tissue features of renal glomeruli and tubules of control group of rats. HE, × 200.

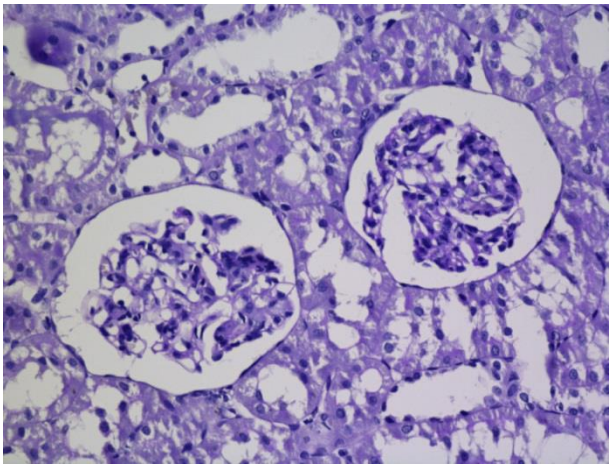

Fig. 3. Histopathological tissue features of renal glomeruli and tubules of control group of rats. PAS, × 400.

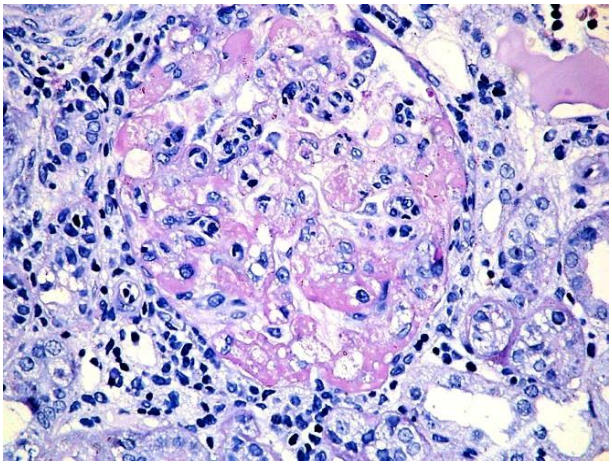

Fig. 4. Histopathological tissue features of renal glomeruli and tubules of gentamicin-treated (GM) group of rats. PAS, × 400.

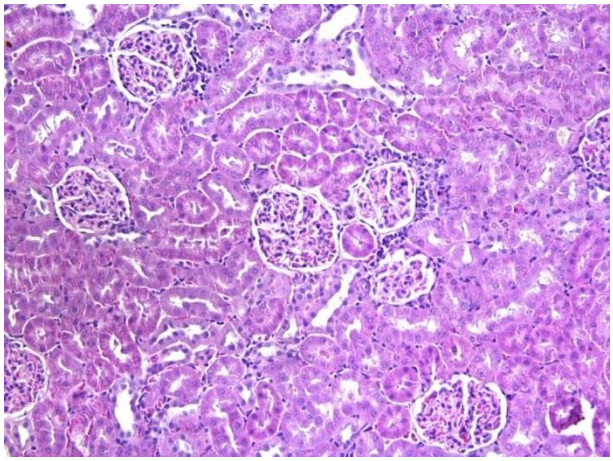

Fig. 5. Histopathological tissue features of renal glomeruli and tubules of gentamicin plus vitamin E-treated (GME) group of rats. HE, × 200.

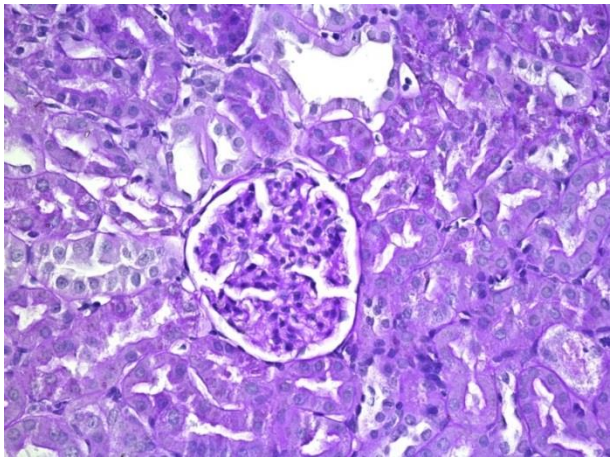

Fig. 6. Histopathological tissue features of renal glomeruli and tubules of gentamicin plus vitamin E-treated (GME) group of rats. PAS, × 400.

Biochemical analysis

Analysis of biochemical parameters showed a significant increase of urea and creatinine serum concentrations in the GM group compared to the C group ($p<0.001$). The concentration of potassium in the blood was significantly decreased in the GM group than concentration in the control group ($p<0.01$), while the concentration of sodium was lower, but not with statistical significance in comparison to the C group. In the GME group, creatinine and urea concentrations were significantly elevated compared to the control group ($p<0.01$), but also these values were significantly decreased compared to the GM group ($p<0.001$). The concentrations of potassium and sodium in the GME-group were not significantly different compared to the other groups (Table 1).

Determination of protein oxidation

Analysis of oxidative stress marker AOPP showed significantly elevated renal AOPP in the GM group than in control group of rats ($p<0.001$). Simultaneous administration of vitamin E with gentamicin reduced

oxidative stress, as evidenced by significantly decreased level of renal AOPP than those in the GM group ($p<0.001$).

Table 1. Biochemical analysis of serum levels of creatinine, urea and electrolytes in the control and experimental groups of rats

Serum concentration	C-group	GM-group	GME-group
Creatinine (μmol/L)	57.03 ±10.89	380.5 ± 29.47[#]	91.4 ± 9.987[*##]
Urea (mmol/L)	6.57 ± 0.577	30.27 ± 6.096[#]	12.73 ± 1.875[*##]
Potassium (mmol/L)	5.513± 0.5668	4.588 ± 0.4486[##]	4.988± 0.4549
Sodium (mmol/L)	143.1 ± 2.031	141.6 ± 2.2	143.6 ± 1.302

[#] $p<0.001$ vs. C-group, [##] $p<0.01$ vs. C-group, [*] $p<0.001$ vs. GM-group

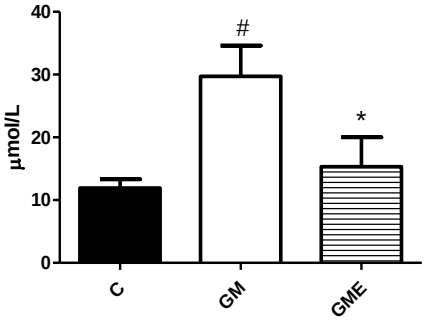

Fig. 7. Renal AOPP values in the control and experimental groups of rats.
# $p<0.001$ vs. C-group, * $p<0.001$ vs. GM-group

Discussion

Due to its strong bactericidal activity, gentamicin is a widely used antibiotic in the management of infections caused by gram-negative microorganisms. However, literature data point to its nephrotoxic effect which has been demonstrated in a large number of experimental studies in which gentamicin acute renal insifficiency was induced [24, 25].

Proximal tubule cells are severely damaged in patients treated with gentamicin or amicacin [26]. Gentamicin binds the cell wall phospholipids, blocking thus the chain reaction of phosphatidylinositol, which impairs the cell integrity [27]. The mechanism of gentamicin nephrotoxicity is complex and has not been elucidated yet. Having been administered, gentamicin reaches the renal cortex, binds the proximal tubule apical membrane, and then, by means of pinocytosis, reaches the epithelial cells of proximal tubules where it interacts with cell organelles, specifically with lysosomes and mitochondria. This further causes lysosomal destabilization, release of lysosomal enzymes and cell damage.

Pathohistological changes, confirmed by light microscopy, in our experimental group of rats treated with gentamicin (GM group) included the enlargement of glomeruli as well as the presence of neutrophil leukocytes in certain glomerular capillaries. The changes in the proximal tubules were dominant and manifested in the form of *coagulation-type necrosis*, cytoplasm vacuolization of tubular epithelial cells with preserved nuclei. The structural changes in the distal tubules were not found. These changes are mostly in keeping with the changes already described by other authors [28, 29]. In the mentioned group of animals (GM group), the biochemical analyses showed the most significant elevation of urea and creatinine levels in the serum as a sign of alterations of kidney, whereas the values of sodium did not statistically differ in all three groups of animals. The values of serum potassium in GM group were statistically significantly reduced in relation to the group of animals treated with gentamicin and vitamin E, as well as the control group. This is quite common having in mind that morphological change in the proximal tubules reduce potassium reabsorption, and consequently increases the urinary excretion of this electrolyte. Matsuda et al. [30] showed that the electrolyte composition of the renal tubular cells in gentamicin nephrotoxicity was different in relation to the necrotic and non-necrotic tubular cells of the proximal tubules. They demonstrated that histological impairment was present only in the proximal tubules, and that the concentrations of sodium and potassium in the necrotic tubular cells were somewhat lower than in the controls, whereas the concentrations of sodium in the non-necrotic cells of proximal tubules were slightly higher in relation to the control group of animals. This indicates that potassium serum levels correlate with the histopathological findings in the proximal tubule cells where gentamicin expresses its main nephrotoxic effect, which is in keeping with our results. The presence of neutrophils in the glomerular capillaries indicates that the administration of gentamicin impaired the renal microcirculation and glomerular hemodynamics. If the changes in the kidneys and glomeruli are primarily due to changed microcirculation and hemodynamics in the capillaries, the removal of these would annul gentamicin effects in renal nephrotoxicity. On the other hand, gentamicin induces oxidative stress, and achieves direct effects by means of ROS which show particular affinity for the endothelial cells of blood vessels. This induces the loss of the architectonics of the endothelial cells' cytoskeleton and organelles, impairs the cell membrane transport mechanism as well as the activity of intracellular enzyme systems. It has been proved that aminoglycoside antibiotics have harmful effects to the kidneys as they produce ROS [14]. At increased concentrations the antioxidant vitamins inhibit pathological states.

Vitamin E is the main endogenous antioxidant which reacting with oxygen radical prevents the chain reaction of free radicals, protecting thus the membrane. However, the endogenous antioxidants reserves, such as vitamin E, gradually decrease in reactions with free radicals [31]. In our experimental group of animals treated with gentamicin and vitamin E, histological and histochemical analyses of glomeruli and proximal tubules demonstrated

slightly increased glomeruli, whereas the changes on the proximal tubule epithelial cells were expressed in the form of vacuolization without any signs of necrosis. In GME group, the values of creatinine and urea were statistically significantly different in relation to the control group ($p<0.01$) and GM group ($p<0.001$) as well. The sodium and potassium serum concentrations in GM group were not statistically significantly different compared to the control group of animals. Similar to our results, the combination of vitamin E and probucol has proved to be efficient for the improvement of the renal function parameters and level of antioxidant enzymes in gentamicin-induced toxicity [32]. In other studies, a synergy between vitamin E and selenium in diminishing renal impairment has been found [3, 25]. Some studies have shown that vitamins C and E in combination provide more efficient antioxidant properties and better effects in gentamicin-induced nephrotoxicity [31]. The results of our study are in keeping with hypotheses that oxidative stress is one of the causes of gentamicin-induced renal impairment. The levels of AOPPs in the kidney homogenate of gentamicin-treated rats were significantly higher when compared with the levels of this marker in control group of animals. Witko-Sarsat et al. [23] have shown that *in vivo* AOPPs levels correlate with creatinine clearance, indicating that AOPPs are an excellent biomarker of chronic renal impairment. That AOPPs are useful biomarkers in acute renal insufficiency has been pointed out by Kimoto et al. [33] when they determined this parameter in cisplatin-induced nephrotoxicity. It is well known that vitamin E acts as an antioxidant within cells, and the mechanisms which contribute to its efficacy involve the suppression of free radicals and improvement in antioxidant system status [34]. In this paper, we confirmed the antioxidant properties of vitamin E, which was supported by statistically significantly lower AOPPs levels in rats simultaneously treated with vitamin E and gentamicin in relation to the group of rats treated with gentamicin alone.

Conclusion

Our experimental study showed that gentamicin-induced nephrotoxicity can be significantly reduced by simultaneous administration of vitamin E as a very significant antioxidant.

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