Morphofunctional changes in kidneys of rats with gentamicin-induced acute kidney injury and use of melatonin

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Introduction
Aminoglycoside antibiotic gentamicin is widely used for the treatment and prophylaxis of Gram-negative bacterial infections, though development of nephrotoxicity in 20-30% of patients significantly limits its use [2, 17, 20, 23]. Toxic influence of gentamicin on kidneys results from its accumulation in kidney cortex, where its concentration exceeds by more than 100-fold its serum level. Due to its proximal tubular reabsorption gentamicin is accumulated in lysosomes of cells, inhibiting phospholipase and sphingomyelinase and leading to lysosomal phospholipidosis, accumulation of myeloid particles and cellular necrosis [2, 4]. It is also stated the interaction between gentamicin and prostaglandins, leading to decrease in glomerular filtration rate (GFR) [6]. It is verified that local oxidative stress in tubular cells plays a central role in pathogenesis of aminoglycosides toxicity [1, 4, 6, ...]

Aminoglycosides are effective antibiotics, but their accumulation in kidney cortex causes nephrotoxic effects in 20-30% of patients, which significantly limits their use. For this reason, search for the new therapies aimed at prevention of gentamicin-induced acute kidney injury (AKI) is highly relevant. Thus, the objective of our research was to study the functional and histopathological changes in kidneys of rats with gentamicin-induced AKI, and estimate the renoprotective potential of pineal hormone melatonin, which possesses antioxidant, anti-inflammatory and immunomodulatory effects. The study was conducted on 24 non-linear male rats. Gentamicin-induced AKI was modeled by daily administration of 4% gentamicin sulphate (80 mg/kg) for 6 days. Melatonin (Sigma Aldrich, USA) was injected daily at a dose of 5 mg/kg. Functional state of kidneys was assessed by diuresis, creatinine clearance, urine protein excretion, fractional excretion of sodium, and plasma potassium level. Documentation of the pathological processes was performed by the computer morphometry of objects in histological preparations. Statistical analysis of the data was performed using SPSS 17.0 software. Administration of gentamicin resulted in a significant impairment of renal function of experimental animals. A decrease in creatinine clearance by 3.1 times along with a reduction of diuresis by 1.9 times, and an increase in plasma creatinine concentration by 2.6 times was observed. There also was an increase in urine protein level by 5.2 times, an elevation of fractional sodium excretion and a reduction of plasma potassium level. Use of melatonin caused a significant improvement of renal function comparing to model pathology group. Functional disturbances were accompanied with the significant histopathological changes in kidney tissue: necrosis of the 27.0±5.2% epithelial cells of proximal tubules with the signs of hydropic vacuolization (7.0±2.1%) or reversible hydropic swelling (76.0±1.5%) in the rest of cells; swelling or deformation of some glomeruli. In the medulla tubular lumen were dilated and partially filled with hyaline casts, tubular cells had signs of dystrophy. Use of melatonin contributed to the restraint of the histopathological changes, confirmed by the decrease of the prevalence and severity of tubular necrosis (1.2%), dystrophy (64.0±2.3%), and injury of glomeruli. Obtained results verify the significant nephroprotective effect of pineal hormone melatonin, providing a background for the further in-depth study of its renal effects as well as its prospects as a nephroprotector.

Keywords: gentamicin-induced acute kidney injury, histopathological changes, melatonin, nephroprotection.
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7]. Gentamicin increases production of reactive oxygen species in mitochondria, which in turn inhibit respiratory chain activity and ATP production, stimulate release of cytochrome C and other proapoptotic factors, and disturb cellular functions by direct damage to proteins, lipids and nucleic acids, induce contraction of mesangial cells, cause dysfunction of endoplasmic reticulum, take part in development of inflammation. It results in disturbances of transmembrane transport of sodium ions, resulting in swelling and necrosis of cells [4, 17, 21]. Histological examination of experimental animals kidneys and biopsy of human kidneys reveals the swelling of proximal tubular cells, loss of brush border and tubular irregularity, as well as changes in intracellular organelles after one week of gentamicin use at therapeutic doses [3, 21].

Numerous experimental data verify an ability of antioxidants to limit a degree of kidney injury by aminoglycoside antibiotics due to minimization of direct toxic influence of gentamicin, prevention of vasoconstriction and contraction of mesangial cells, as well as anti-inflammatory effect [1, 2, 4, 7, 12, 18, 20]. In this regard we focused our attention on melatonin as a possible remedy of pathogenetic correction of gentamicin-induced acute kidney injury (AKI) due to its antioxidant, anti-inflammatory and immunomodulatory effects [2, 6, 15, 25], which is confirmed by some studies [14, 15, 24]. Thus, use of pineal hormone may be considered as a potential therapeutic method of toxic AKI prevention.

Therefore, the objective of our research was to study the influence of melatonin on the histology and function of rats with gentamicin-induced AKI with estimation of its renoprotective potential.

Materials and methods

The study was conducted on 24 non-linear male rats weighting 130-180 g, maintained in the vivarium conditions with constant temperature and humidity, free access to water and food. Animals were randomly divided into 3 groups (n=8): I group - intact control, II group - gentamicin-induced nephropathy, modeled by daily intramuscular administration of 4% gentamicin sulphate (Galychpharm JSC, Ukraine) at a dose of 80 mg/kg for 6 days [22]. Melatonin (Sigma Aldrich, USA) was injected intraperitoneally at a dose of 5 mg/kg 40 min after every gentamicin injection [15]. Animals were sacrificed 24 h after last injection, while blood, urine samples and kidneys were collected for biochemical and histopathological assessments. All interventions were conducted in accordance with the criteria outlined in the European Union Directive 2010/63/EU “On the protection of animals used for scientific purposes” (2010).

Functional state of kidneys was assessed by diuresis, creatinine clearance, urine protein excretion, fractional excretion of sodium, and plasma potassium level [22]. Plasma and urine creatinine levels were determined using the Jaffe reaction; sodium and potassium levels - using electronic flame photometry method; urine protein content - using the sulfosalicylic acid precipitation test.

The kidneys of rats were fixed in 10% formalin, embedded in paraffin, sectioned at 5 mM and then stained with hematoxylin and eosin. The preparations were evaluated using light microscopy and photographed (Olympus C740UZ photo camera, Japan, LUMAM-R8 microscope, LOMO, Russian Federation). Documentation of the pathological processes was performed by the computer morphometry of objects in histological preparations using computer software “VideoTest - Razmer 5.0” (LLC “VideoTest”, Russian Federation).

Statistical analysis of the data was performed using SPSS 17.0 software. All data are represented as a mean ± standard error of the mean (M±σ). Estimation of the differences between the samples was conducted using parametric Student’s t-test and nonparametric Mann-Whitney U test. The values p<0.05 were considered statistically significant.

Results

Administration of gentamicin consecutively for 6 days resulted in a significant impairment of renal morphofunctional state of experimental animals (Tab. 1). A decrease in creatinine clearance by 3.1 times along with a reduction of diuresis by 1.9 times, and an increase in plasma creatinine concentration by 2.6 times was observed. There also was an increase in urine protein level by 5.2 times, an elevation of fractional sodium excretion and a reduction of plasma potassium level. Use of melatonin caused a significant improvement of renal function comparing to model pathology group.

Histopathological examination of rats with gentamicin nephropathy in comparison with intact control group (Fig. 1) revealed a significant impairment of kidney tissue structure, caused by toxic influence of gentamicin (Fig. 2). In the absence of cells without pathological changes, there is a necrosis 27.0±5.2% of cortical tubular epithelial cells with deformation, swelling and atrophy of some glomeruli (Fig. 2A). In the renal cortex 7.0±2.1% of cortical tubular epithelial cells exhibit signs of hydropic vacuolization, the remaining epitheliocytes (76.0±1.5%) are in a state of reversible hydropic swelling.

In the renal medulla (Fig. 2B) and papilla (Fig. 2C) tubular
cells degeneration, lumen dilation and deposition of hyaline casts are observed.

Melatonin co-administration ameliorated histopathological changes in kidneys. In the renal cortex areas of tubular epithelial necrosis are localized to 1.2%, reversible hydropic swelling is extended to 64.0±2.3% of proximal tubular epitheliocytes, with 4.0±1.6% of cells in a state of hydropic vacuolization, about 30.0% of the cells - without any signs of damage. Glomeruli of a normal structure and size, some with dilation of Bowman's space (Fig. 3A). Hyaline casts are
present in small amounts in the renal cortex, medulla and papilla, there are also isolated hemorrhages (Fig. 3B, 3C).

**Discussion**

Gentamicin-induced nephrotoxicity at the beginning of the development of the pathological process is characterized by the development of oliguric form of renal failure, accompanied by retention azotemia, proteinuria, increased loss of sodium and potassium ions with urine, resulting in hypokalemia, leading to a decrease in glomerular filtration rate (GFR) [16]. The established renal effects of melatonin in toxic species and nitrogen oxides directly lead to a decrease in the maintenance of glomerular filtration may be explained by the decrease in proteinuria and fractional excretion of sodium ions compared to non-treated animals (Table 1). At that, the decrease in proteinuria and fractional excretion of sodium ions leads to an improvement in renal function, which is confirmed (administration 40 min after each injection of gentamicin) does at a dose of 5 mg/kg in the prophylactic treatment regimen of the integrity of cellular membranes and development of morphofunctional disorders [1, 17]. This fact conditions the limitation the severity and prevalence of histopathological changes in the kidneys, indicating the cytoprotective activity of this hormone in relation to nephrons, the ability of the drug to ameliorate the toxic effects of gentamicin and prevent the development of renal failure.

The obtained results substantiate the further in-depth study of the renal effects of melatonin on various experimental models of acute kidney injury, including those considering the chronobiological peculiarities of the hormone action, and the prospects of its use as a nephroprotector in acute kidney injury of different genesis.

**Conclusions**

1. A 6-day consecutive administration of gentamicin at a dose of 5 mg/kg leads to an alteration of the morphofunctional state of rats kidneys, which is manifested by the development of oliguric form of renal failure.

2. Structural organization of the renal tissue in gentamicin-induced acute kidney injury is characterized by the occurrence of histopathological changes in both the glomerular and tubular apparatus of the nephron: necrosis of 27% of the epithelial proximal tubular cells and degenerative changes of various degrees of the remaining cells are observed.

3. According to research results, the protective effect of melatonin at a dose of 5 mg/kg is verified, which is manifested by restriction of nephrons damage and normalization of the structural organization of kidney tissue, as well as the preservation of renal function under the conditions of AKI development [2, 8-12].

The protective effect of melatonin has been realized in the form of vacuolization and hydropic dystrophy, and glomerular damage (Figure 2) were revealed for gentamicin nephropathy [3, 8-12, 14]. It is known that the most important mechanism of gentamicin nephrotoxicity is the hyperproduction of reactive oxygen species, causing damage to proteins, DNA and peroxidation of lipids, with an alteration of the integrity of cellular membranes and development of morphofunctional disorders [1, 17]. This fact conditions numerous research on the effectiveness of prevention of morphofunctional disorders [1, 17]. This fact conditions the limitation the severity and prevalence of histopathological changes in the kidneys, indicating the cytoprotective activity of this hormone in relation to nephrons, the ability of the drug to ameliorate the toxic effects of gentamicin and prevent the development of renal failure.

The obtained results substantiate the further in-depth study of the renal effects of melatonin on various experimental models of acute kidney injury, including those considering the chronobiological peculiarities of the hormone action, and the prospects of its use as a nephroprotector in acute kidney injury of different genesis.

**References**


| Table 1. Influence of melatonin on the state of kidney excretory function in conditions of gentamicin-induced acute kidney injury (M±σ, n=7). |
|-------|----------------|----------------|----------------|
| Index | intact control | AKI | AKI+Melatonin |
| Diuresis, ml/2 h/100 g | 4.649±0.193 | 2.464±0.187* | 3.635±0.101** |
| Plasma creatinine, μmol/l | 59.67±3.92 | 155.±5.00* | 106.4±5.60** |
| Creatinine clearance, ml/min | 54.79±7.85 | 17.54±1.94** | 30.29±2.44** |
| Urine protein, g/l | 0.018±0.002 | 0.093±0.007** | 0.058±0.005** |
| Fractional sodium excretion, % | 0.67±0.069 | 3.426±0.716** | 1.781±0.198** |
| Plasma potassium, μmol/l | 5.393±0.266 | 4.357±0.261** | 5.036±0.127* |

Note: ** - statistical significance comparing to: intact control; (p<0.01); AKI group * - (p<0.05), ** - (p<0.01).
Аміноглікозиди є ефективними антибіотиками, але їх накопичення в кірковій речовині нирки викликає нефротоксичні ефекти. Дідка Є.А., Заморський І.І., Петрюк А.Є., Щудрова Т.С. в своєму дослідженні виявили, що аміноглікозиди стимулюють сігнальну активність костного мозку, що підсилює нейротоксичні ефекти.

**References:**


MORFOPROFUNKЦІЙНІ ЗМІНИ НІРОК ЩУРІВ ПРИ ЗАСТОСУВАННІ МЕЛАТОНІНУ НА ТЛІ РОЗВИТКУ ГЕНТАМАЦИНА-ІНДУКОВАННОГО ГОСТРОГО ПОШКОДЖЕННЯ НІРОК

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Аналізуються ефективні антибіотики, але їх накопичення в кірковій речовині нирки викликає нефротоксичні ефекти у 20-30% пацієнтів, що значно обмежує їх застосування. З цієї причини актуальним є пошук нових профілактичних заходів, спрямованих на збалансування гострого пошкодження нирок (ГПН), спричиненого гентаміцином. Таким чином, метою нашого дослідження було вивчення функціональних і гістологічних змін у нирках щурів при гентаміцин-індукуваному ГПН з огляду на нефропротекторний потенціал північного мелатоніну, який впливає на антиоксидантні та протизапальні ефекти.
MORPHOFUNCTIONAL CHANGES IN KIDNEYS OF RATS WITH GENTAMICIN-INDUCED ACUTE KIDNEY INJURY AND USE OF MELOTONIN

Aminoglycosides are effective antibiotics, but their accumulation in the renal cortex causes nephrotoxic effects in 20-30% of patients, which significantly limits their use. Therefore, the search for new prophylactic drugs capable of preventing gentamicin-induced acute kidney injury (AKI) is relevant. Therefore, the aim of our study was to investigate morphofunctional changes in the kidneys of rats with AKI induced by gentamicin with the evaluation of the nephroprotective potential of the hypophyseal hormone melatonin, which has antioxidant, anti-inflammatory, and immunomodulatory properties. The study was conducted on 24 male rats. Gentamicin-induced AKI was simulated by daily administration of 4% gentamicin sulfate (80 mg/kg) for 6 days. Melatonin (Sigma Aldrich, USA) was administered daily in a dose of 5 mg/kg. The functional state of the kidneys was evaluated by the parameters of urine production, creatinine clearance, protein excretion, fractional excretion of sodium and potassium in the blood, and computer morphometry. The introduction of gentamicin led to a significant deterioration of the renal function in the experimental animals. It was found that the creatinine clearance decreased 3.1 times, urine production decreased 1.9 times, and the concentration of creatinine in the blood increased 2.6 times. There was also an increase in protein excretion in urine 5.2 times, an increase in the fractional excretion of sodium, and a decrease in the concentration of potassium in the blood. It was shown that the use of melatonin significantly improved renal function compared to the model group. It was found that functional disorders were accompanied by significant histopathological changes in the kidney tissue: the proximal tubular epithelial cell necrosis reached 27.0±5.2%, in other cells, there were signs of hydropic vacuolization (7.0±2.1%) or reversible hydropic swelling (76.0±1.5%), there was edema or deformation of some glomeruli. In the medullary substance, the lumen of the tubules was dilated and partially filled with hyaline cylinders, tubular cells with signs of dystrophy. It was shown that the use of melatonin hindered histopathological changes, which was confirmed by a decrease in the extent and severity of tubular necrosis (1.2%), dystrophy (64.0±2.3%) and damage to glomeruli. The obtained results convincingly confirmed the nephroprotective action of the hypophyseal hormone melatonin, which justifies its further in-depth study of real effects, as well as perspectives for its use as a nephroprotective agent.

Key words: gentamicin-induced nephropathy, melatonin, histopathological changes, nephroprotection.