RENOPROTECTIVE EFFECT OF MELATONIN IN CONDITIONS OF ACUTE KIDNEY INJURY AND ALTERED PINEAL GLAND ACTIVITY

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Abstract:

INTRODUCTION: Melatonin is a promising therapeutic agent due to its multiple beneficial effects, wide availability and relatively high safety. As melatonin acts as a chronobiotic agent, its adequate production by the pineal gland allows for adaptation to environmental changes, while disturbances in melatonin secretion are associated with health disorders. The renoprotective effect of exogenous melatonin was established on different experimental models of acute kidney injury (AKI), while the influence of the altered pineal gland activity on the efficacy of melatonin treatment has not been investigated.

OBJECTIVES: The aim of this research was to study the renoprotective potential of melatonin in conditions of aminoglycoside-induced AKI against the background of pineal hypo- and hyperfunction.

METHODS: Nonlinear mature white rats (n=40) were randomly divided into 5 groups. Animals from the I (Control), and II (AKI) group were kept under the natural light regimen. Pineal hypofunction was simulated in rats from the III group by maintenance under conditions of constant light at 500 lux (24.00 light : 0.00 darkness) for 7 days. Pineal hyperfunction was simulated in rats from the IV group by maintenance under conditions of constant darkness (0.00 light : 24.00 darkness). Toxic AKI (II-IV groups) was induced by daily administration of gentamicin at a dose of 80 mg/kg for 6 days. Animals from the III-IV groups were injected daily with melatonin at a dose of 5 mg/kg. 24 h after the last injection biochemical and histological examination was performed. For the statistical analysis SPSS 17.0 software was used.

RESULTS: Nephrotoxicity of gentamicin caused significant ($p<0.05$) functional changes and structural alterations to the rat kidneys. Treatment with melatonin in conditions of gentamicin-induced kidney injury significantly limited the degree of damage to renal tissue and prevented a critical reduction in kidney function, confirming a protective effect of melatonin. At the same time, significant ($p<0.05$) differences between the indices of the III and IV group allow us to state, that treatment with exogenous melatonin on the background of endogenous melatonin deficiency was less effective in comparison to the administration of melatonin in conditions of pineal hyperfunction.

CONCLUSION: Melatonin ameliorates gentamicin-induced kidney injury by the limitation of histopathological changes in kidney tissue and the preservation of kidney function. Pre-existing deficiency of endogenous melatonin decreases the resistance of kidneys to the damaging action of the toxin and lessens the protective effect of the exogenous melatonin. Alternatively, in rats with increased pineal gland activity and melatonin production, co-treatment with exogenous melatonin more effectively protects the kidney from gentamicin-induced structural and functional changes and prevents the development of renal failure.

Introduction

The pineal hormone melatonin is considered to be the main regulator of circadian rhythms, neuroendocrine functions, and ageing, possessing various biological and pharmacological effects. As it stated by Ekmekcioglu (2006), melatonin receptors have been found in both central and peripheral tissues including heart, arteries, adrenal gland, kidney, liver, lung, intestines, ovaries, uterus, breast, prostate, skin, and lymphocytes. Consequently, besides the regulation of sleep-wake rhythms, synchronization by melatonin of peripheral oscillators allows individual adaptation to periodic internal and external environmental changes, while disturbed circadian rhythms are associated with sleep disorders and impaired health (Tordjman, 2017).

Multiple effects of melatonin make it a potential therapeutic agent. According to Eghbal (2016), Reiter (2017) and Tavakoli (2014), melatonin, due to its free-radical scavenging activity and ability to potentiate the antioxidant system is a highly important antioxidant. Several researchers (Bonnefont-Rousselot, 2010; Espino, 2018; Esrefoglu, 2017; Ničković, 2018; Reiter, 2017) reported a therapeutic effect of melatonin in various pathologies related to oxidative stress. Besides, numerous studies (Majidinia, 2017; Pacini, 2016; Reiter, 2018; Tordjman, 2017) report beneficial immunostimulatory, anti-inflammatory, anti-apoptotic, cytoprotective, oncostatic, and anti-ageing effects of melatonin.

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The renoprotective effect of melatonin and its ability to restore kidney function and structure in rats has been shown in different experimental models of ischemia-reperfusion and toxic AKI (Bai, 2016; Fadda, 2018; Kilic, 2013; Onk, 2016; Tavakoli, 2014), including aminoglycoside-induced nephrotoxicity (Dudka, 2018; Kapic, 2014; Lee, 2012). Moreover, some researchers (Andersen, 2015; Sharman, 2016) also consider melatonin to be a natural, widely available, and relatively safe product.

According to Tordjman (2017), pineal activity and subsequent melatonin synthesis and secretion are enhanced by darkness and inhibited by light. Although melatonin was found to protect against aminoglycoside-induced AKI, its renoprotective effect in conditions of different functional states of the pineal gland has not been studied.

Thus, the aim of this research was to study the renoprotective potential of melatonin in conditions of aminoglycoside-induced AKI against the background of pineal hypo- and hyperfunction.

Material and Methods

Experiments were conducted on nonlinear mature white rats (n=40) weighing 150-200 g, maintained in the vivarium conditions at constant temperature and humidity, free access to water and food (full value fodder for the laboratory animals). Animals were randomly divided into 5 groups (n=8). Animals from the I (Control), and II (AKI) group were kept under the natural light regimen (12 h light : 12 h darkness). Pineal hypofunction was simulated in rats from the III group by maintenance under conditions of constant light at 500 lux (24.00 light : 0.00 darkness) for 7 days. Pineal hyperfunction was simulated in rats from the IV group by maintenance in conditions of constant darkness (0.00 light : 24.00 darkness) for 7 days. Toxic AKI (II-IV groups) was induced by daily intramuscular administration of 4% gentamicin sulphate (Galychpharm JSC, Ukraine) at a dose of 80 mg/kg for 6 days, starting from the 8th day of the experiment. Animals from the III-IV groups were daily injected with melatonin (Sigma Aldrich, USA) at a dose of 5 mg/kg, intraperitoneally, 1 h after every gentamicin injection. Animals were sacrificed 24 h after the last injection, while blood, urine and kidneys were sampled 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).

Kidney function was assessed by diuresis, creatinine clearance, urine protein excretion, fractional excretion of sodium, and plasma potassium level. Plasma and urine creatinine levels were determined using the Jaffe reaction; sodium and potassium levels – using an electronic flame photometry method; urine protein content – using the sulfosalicylic acid precipitation test.

The kidneys of rats were fixated in 10% formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin (H&E). The preparations were analyzed using light microscopy and photographed. Documentation of the pathological processes was performed by the computer morphometry of objects in histological preparations using the computer software "VideoTest-Razmer 5.0".

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±m). Estimation of the differences between the samples was conducted using a parametric Student's t-test and a nonparametric Mann-Whitney U test. The values $p < 0.05$ were considered significant.

Results and Discussion

According to Pierson-Marchandise (2017) and Randjelovic (2017), aminoglycoside antibiotic gentamicin remains among the leading causes of drug-induced nephrotoxicity due to its extensive use in treating severe infections. On the other hand, gentamicin nephropathy is a well-studied and popular experimental model for nephrotoxicity. As Randjelovic (2017) points out, the central aspect of gentamicin nephrotoxicity is direct tubular cytotoxicity, resulting in mitochondrial dysfunction, induction of cellular apoptosis, necrosis, inflammation, and oxidative stress, with consequent glomerular and vascular damage.

In our research, the nephrotoxic effect of gentamicin caused functional changes and structural alterations of the rat kidneys. An almost 2-fold reduction of diuresis and a 3-fold decrease in creatinine clearance confirmed the development of AKI as a result of direct tubular damage as well as the activation of tubuloglomerular negative feedback. Significant oliguria caused an increase in plasma creatinine level

and retention azotemia, which was accompanied by aciduria and hypokalemia as a result of insufficient tubular reabsorption and loss of potassium and sodium ions with urine. Pronounced proteinuria reflected both tubular and glomerular damage to nephrons (Table 1).
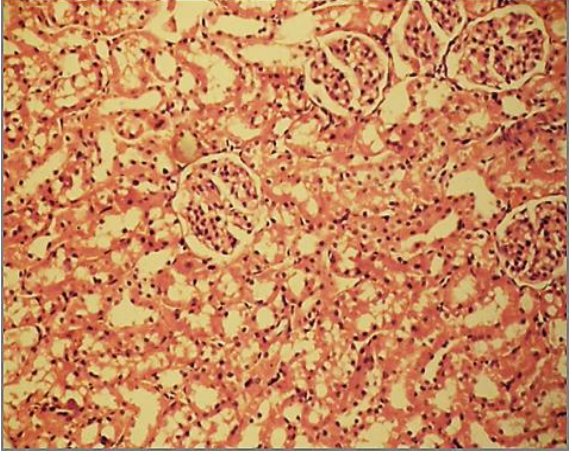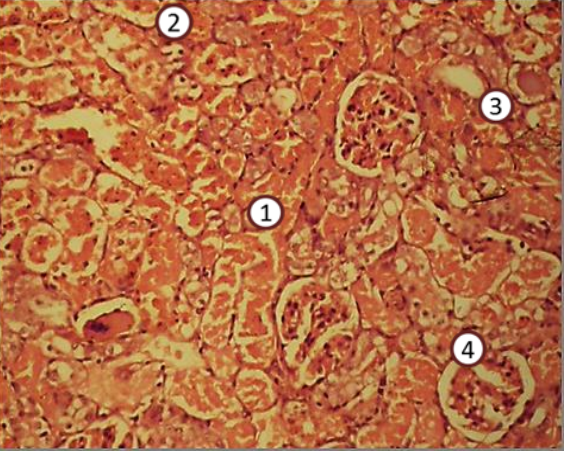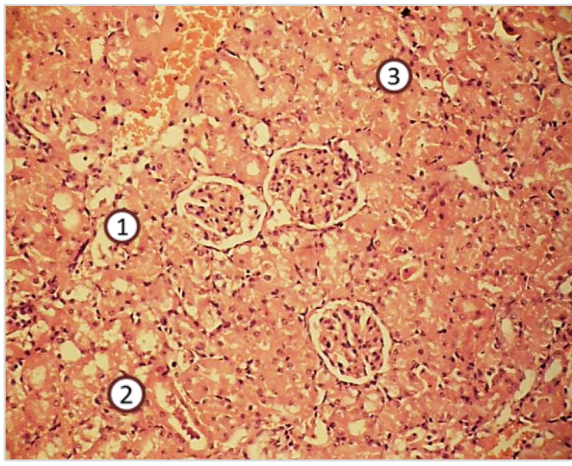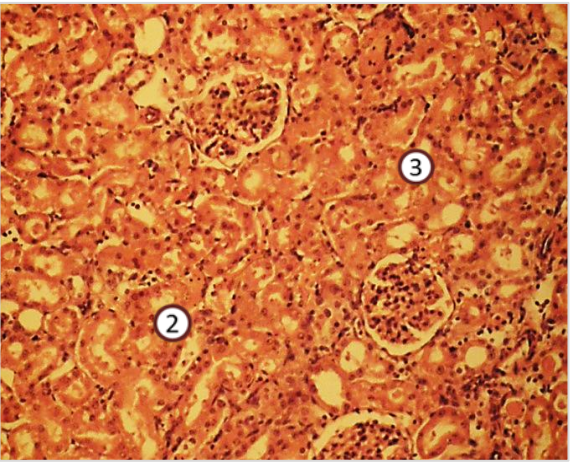
Table 1: Influence of melatonin (5 mg/kg) on the kidney function of rats with gentamicin-induced AKI on the background of pineal hypo- and hyperfunction (M±m)				
Index	Control (I group)	AKI (II group)	Pineal hypofunction + AKI + Melatonin (III group)	Pineal hyperfunction + AKI + Melatonin (IV group)
Diuresis, ml/2 h	4.650.19	2.460.19 $p_1<0.05$*	3.090.11 $p_2<0.05$	3.730.16 $p_2<0.05$, $p_3<0.05$
Plasma creatinine, µmol/l	59.673.92	155.094.97 $p_1<0.05$	121.207.28 $p_2<0.05$	100.474.16 $p_2<0.05$, $p_3<0.05$
Creatinine clearance, ml/min	54.797.85	17.541.94 $p_1<0.05$	25.092.27 $p_2<0.05$	37.321.64 $p_2<0.05$, $p_3<0.05$
Urine protein, g/l	0.0180.002	0.0930.007 $p_1<0.05$	0.0720.003 $p_2<0.05$	0.0430.003 $p_2<0.05$, $p_3<0.05$
Fractional Na$^+$ excretion, %	0.670.07	3.430.72 $p_1<0.05$	2.02±0.18 $p_2<0.05$	1.120.11 $p_2<0.05$, $p_3<0.05$
Plasma P$^+$, µmol/l	5.390.27	4.360.26 $p_1<0.05$	5.110.35 $p_2<0.05$	5.320.25 $p_2<0.05$
Urine pH	7.140.04	6.290.12 $p_1<0.05$	6.890.02 $p_2<0.05$	7.060.05 $p_2<0.05$
*Significant difference compared to I group (p_1), II group (p_2), III group (p_3)				
Source: Authors				

Compared to the kidneys of rats from the intact control group (Fig. 1), histopathological examination of the rat kidney from II (AKI) group (Fig. 2) revealed a severe impairment of kidney tissue structure, caused by the toxic influence of gentamicin. In the absence of cells without pathological changes, there was necrosis 27±5% of cortical tubular epithelial cells with deformation, swelling, and atrophy of some glomeruli, dilation of the tubular lumen, and deposition of hyaline casts. In the renal cortex, 7±2% of epithelial cells exhibited signs of degeneration in a form of hydropic vacuolization, the remaining epitheliocytes (76%) were in a state of reversible hydropic swelling.

As stated above, inhibition of pineal activity by constant light and the consequent reduction of melatonin synthesis leads to disturbances in organism adaptation and, as a result, decreased resistance to environmental changes and damaging factors. Alternatively, stimulation of pineal activity and endogenous melatonin secretion results in increased ability to withstand pathological factors.

It is shown (see Table 1), that treatment with melatonin on the background of gentamicin use significantly limited the degree of damage to renal tissue and prevented a critical reduction in kidney function, which corresponds to the results of other authors (Kapic, 2014; Lee, 2012; Ozbek, 2000). The rats treated with melatonin (group III and IV) had lower levels of plasma creatinine, urine protein, and a degree of sodium excretion, along with higher diuresis and urine pH, which suggest a protective effect of melatonin. At the same time, significant differences between the III and IV group allow us to state that treatment with melatonin in the background of endogenous melatonin deficiency was less effective in comparison to the administration of melatonin in conditions of pineal hyperfunction.

Histological analysis of rat kidneys confirmed the obtained biochemical results. Melatonin treatment significantly limited the severity and prevalence of pathomorphological changes in the kidneys of rats. In the kidneys of rats from the III group (Fig. 3) glomeruli were of a normal structure and size, areas of cortical tubular necrosis were localized to 8%, reversible hydropic swelling was extended to 84±2% of proximal tubular cells, with 8±1% of cells in a state of hydropic vacuolization, and isolated hyaline casts. In the kidneys of rats treated with melatonin in the background of increased activity of pineal gland (Fig. 4), histopathological changes were the mildest: at the absence of necrosis, 70% of cells had signs of degeneration, and approximately 30% of the cells had no signs of damage. The results of the morphological examination confirm the more pronounced protective effect of melatonin in animals from the IV group comparing to the III group.

Figure 1: Photomicrograph of kidney section (cortex) of intact rats (Control group). H&E. x100.	Figure 2: Photomicrograph of kidney section (cortex) of rats with gentamicin-induced AKI (II group). Epithelial necrosis (1), epithelial degeneration (2), hyaline casts (3), dilation of Bowman's space (4). H&E. x100.
Source: Authors	Source: Authors

Figure 3: Photomicrograph of kidney section (cortex) of rats with gentamicin-induced AKI on the background of pineal hypofunction and administration of melatonin (III group). Epithelial necrosis (1), epithelial degeneration (2), hyaline casts (3). H&E. x100.	Figure 4: Photomicrograph of kidney section (cortex) of rats with gentamicin-induced AKI on the background of pineal hyperfunction and administration of melatonin (IV group). Epithelial degeneration (2), hyaline casts (3). H&E. x100.
Source: Authors	Source: Authors

Conclusion

The results of our research show the ameliorative effect of melatonin in gentamicin-induced AKI, which is verified by the limitation of histopathological changes in kidneys and preservation of kidney function. The identified effects of melatonin correspond to the criteria of renoprotection in conditions of toxic AKI development. Pre-existing deficiency of endogenous melatonin decreases the resistance of kidneys to the damaging action of the toxin and lessens the protective effect of the exogenous melatonin. Alternatively, in rats with increased pineal gland activity and melatonin production, co-treatment with melatonin more effectively protects the kidney from gentamicin-induced structural and functional changes and prevents the development of renal failure.

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