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MELATONIN REDUCES LIPOPOLYSACCHARIDE-INDUCED KIDNEY DAMAGE IN RATS

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Lipopolysaccharide (LPS) is a constituent of Gram-negative bacterial cell walls, thus LPS injection has been widely used as a model of experimental acute kidney injury associated with sepsis. LPS-induced sepsis is caused by excessive secretion of pro-inflammatory mediators and reactive oxygen species (ROS). The neurohormone melatonin, which is mainly secreted by the pineal gland, regulates the circadian rhythm, has an anti-inflammatory and immunoregulatory role. Melatonin and its metabolites have been shown to scavenge various free radicals and reactive oxygen intermediates. The aim of this study was to evaluate the effect of melatonin in preventing endotoxemia-induced kidney damage caused by LPS, by analysing the concentration of urea and creatinine in the blood serum of rats. Twenty-eight Wistar albino rats were randomly divided into four groups (n = 7 per group) as follows: 1) Control group, 2) MLT group (50 mg/kg, per os), 3) LPS group (10 mg/kg, i.p.) and 4) LPS + MLT group (10 mg/kg + 50 mg/kg). Serum levels of creatinine and urea were significantly higher ($p<0.05$) in the LPS-treated animals compared with values in the control group. Co-application of LPS and MLT significantly reduced an increase in serum creatinine and urea levels ($p<0.05$). It can be concluded that oral administration of melatonin significantly alleviates LPS-induced acute nephrotoxicity in rats. It is likely that the beneficial effects of melatonin are related to its known antioxidant effects on kidney tissue, and possibly to some other less known/studied effects. *Acta Medica Medianae 2023;62(1):15-20.*

Key words: *melatonin, lipopolysaccharide, kidney, urea, creatinine*

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Introduction

Acute kidney injury (AKI) is a major public health hazard that adversely affects patient health and results in approximately 1.4 million deaths per year (1). AKI is a very common complication caused by lipopolysaccharide (LPS) (2). In this case, hemodynamical changes occur, reflecting the changes in kidney tissue blood flow and glomerular filtration rate (3). Endotoxemia associated with sepsis has been shown to result in approximately 50% mortality in ICU (4). Sepsis is a dynamic and very complex clinical syndrome caused by the host's systemic inflammatory response to infection, with numerous complications (5). Sepsis is characterized by fever, tachycardia, tachypnea, changes in the number of leukocytes, increased synthesis of acute phase proteins, metabolic disorders, damage to the endothelium, development of shock and multiple organ dysfunction syndrome (6). Sepsis is one of the main causes of AKI and accounts for almost 26%–50% of all AKI in developed countries (7). According to a 2018 WHO report, more than 30 million people worldwide are affected by sepsis every year (8).

It is well known that the injection of LPS which is a molecule part of a Gram-negative bacterial cell wall could be used as a model of experimental AKI associated with sepsis (9). LPS has been proven to be one of the most important sources of sepsis, which can contribute to the "cytokine storm", intense oxidative stress, renal hypoperfusion, low blood pressure and impaired

renal function (10). Lipopolysaccharide-induced sepsis of Gram-negative bacteria is caused by excessive secretion of pro-inflammatory mediators, reactive oxygen and reactive nitrogen species (ROS and RNS, respectively). Inflammation and intense oxidative stress play the most important role in the pathogenesis of sepsis-related AKI (11). ROS production can damage the glomeruli and tubules in the kidneys (12).

The neurohormone melatonin, which is mainly secreted by the pineal gland, has important molecular/biochemical roles when released into the blood (13). Melatonin regulates the circadian rhythm, has an anti-inflammatory and immunoregulatory role, and is also an oncostatic agent (14). Melatonin and its metabolites have been shown to scavenge various ROS and intermediates generated by ROS both *in vitro* and *in vivo*, which may explain its protective effects against LPS toxicity (15). Melatonin has been proven to remove toxins such as hydrogen peroxide, hydroxyl radical, nitric oxide (NO), peroxynitrite anion, hypochlorous acid, singlet oxygen, superoxide anion, and its antioxidant properties are manifested by stimulating superoxide dismutase (SOD), glutathione metabolizing enzymes, as well as by the inhibition of nitric oxide synthase (NOS) (16).

Aim of the study

The aim of this study was to evaluate the effect of melatonin in preventing endotoxemia-induced AKI caused by LPS, through the analysis of the concentration of urea and creatinine in the blood serum of rats.

Materials and methods

Animals

The experimental protocol was approved by the Local Animal Ethics Review Committee of the Faculty of Medicine, University of Niš. All experimental procedures were performed in accordance with the ethical regulations of the European Community guidelines for laboratory animals, as well as those given by the laws of the Republic of Serbia (No. 323–07–08988/2022–05). Experiments were performed on healthy, male Wistar rats (2-2,5 months old) obtained from the Vivarium of the Institute of Biomedical Research, Faculty of Medicine in Niš. The animals were housed under standard housing conditions, and were allowed *ad libitum* food and water.

Experimental design

Twenty-eight Wistar rats (weighing from 200 to 250 g) were randomly allocated into four groups (7 animals per group) as follows: 1) Control group, 2) MLT group, 3) LPS group and 4) LPS + MLT group. Septic shock in rats was induced by the application of LPS (obtained from Escherichia coli serotype O111:B4 (Sigma, St. Louis, MO, USA)) in a single dose of 10 mg/kg (17). Melatonin (MLT) (Sigma, St. Louis, MO, USA) solutions were prepared before its administration at a dose of 50 mg/kg (18). Rats were treated as follows: Control group - 8 % ethanol in saline at a dose of 10 ml/kg by oral gavage, MLT group - single 50 mg/kg dose of MLT administered by oral gavage, LPS group - single intraperitoneal (i.p.) injection of LPS at a dose of 10 mg/kg, LPS + MLT group - single 50 mg/kg dose of MLT (per os), followed by a single 10 mg/kg dose of LPS (i.p.). Twelve hours after treatment, the animals were sacrificed with an overdose of ketamine (general anesthetic, Richter Pharma AG - Wells, Austria), after which a blood sample was taken for further biochemical analysis.

Blood biochemical analysis

Serum samples from rats were prepared by centrifugation (3,000 rpm for 10 min) and analyzed for the blood biochemistry kidney markers (creatinine and blood urea levels), using an automated biochemical analyzer (Olympus AU680).

Statistical analysis

All quantitative values are expressed as mean ± standard deviation (SD). Statistical differences between two groups were examined by one-way ANOVA followed by Tuckey's post hoc test using SPSS version 17.0. Probability values (p) less than 0.05 were considered to be statistically significant.

Results

As shown in Figure 1 and 2, serum levels of creatinine (55.2 ± 2.9 vs 38.2 ± 2.1, $p<0.001$) and urea (14.31 ± 1.76 vs 7.83 ± 0.99, $p<0.001$) were significantly elevated in the LPS-treated animals compared with the control group. In the group treated with combined application of LPS and MLT (50 mg/kg), significant reduction of serum creatinine (45.60 ± 2.80; $p<0.05$) and urea (11.95 ± 2.61; $p<0.05$) were noted, however, they were still found to be higher than in the control group.

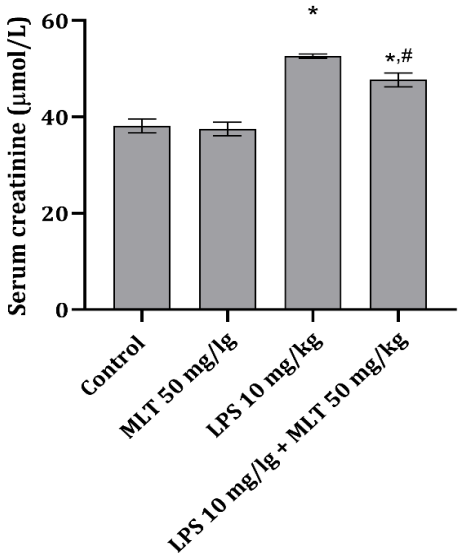

Figure 1. Serum levels of creatinine in rats belonging to different experimental groups; Data are given as mean values ± SD (n=7); Comparison was performed using One Way ANOVA followed by Tuckeys post hoc test; *p<0.001 vs control, #p<0.001 vs LPS-treated animals.

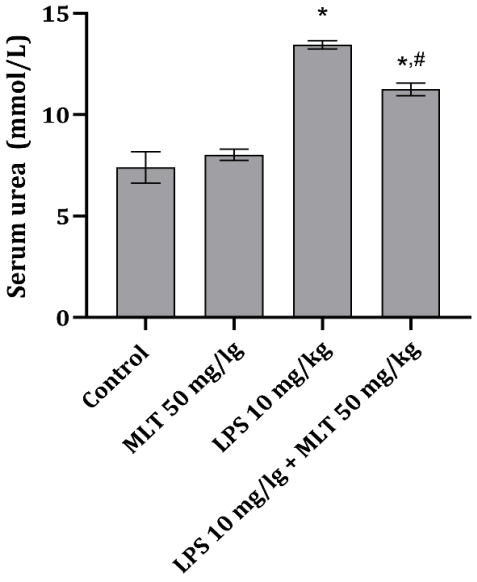

Figure 2. Serum levels of urea in rats belonging to different experimental groups; Data are given as mean values ± SD (n=7); Comparison was performed using One Way ANOVA followed by Tuckeys post hoc test; *p<0.001 vs control, #p<0.001 vs LPS-treated animals.

Discussion

Severe septic conditions are clinically accompanied by azotemia and oliguria, and the renal damage itself can vary from minimal proteinuria to AKI. Decreased diuresis during sepsis is most often a consequence of hypotension. Along with hypotension, the most common pathogenetic mechanisms of AKI are hypovolemia and renal vasoconstriction. In addition to oliguria and azotemia, AKI is also accompanied by hyperkalemia and acidosis, decreased sodium content in urine, elevated levels of urea and creatinine, in the case of primarily prerenal AKI, and in the case of an acute tubular necrosis we could expect an increase in the activity of creatine kinase (CPK) and the presence of cylindrical cysts (19). Lipopolysaccharide (LPS) is an important pro-inflammatory factor, capable

of causing endotoxemia with sepsis, as well as multiple organ dysfunction. Several models of AKI and sepsis in experimental animals have been successfully established by intraperitoneal or intravenous injection of LPS (20).

It has been proven that LPS causes a major disturbance of renal tissue perfusion, apoptosis and renal insufficiency. In this model of AKI, vascular endothelial cell injury (21), neutrophil migration (22), or intravascular coagulation (23) play a significant role in small blood vessel mechanical obstruction leading to AKI and further renal failure.

Serum creatinine and urea levels are important markers of kidney tissue damage (24). A significant increase in serum creatinine and urea levels in LPS-treated rats suggests possible impairment of nephron structural integrity and renal tissue dysfunction (25). Pre-treatment with MLT was found to significantly reduce creatinine and urea levels in serum as well, possibly through the maintenance of cell membrane integrity (26).

Melatonin was revealed to possess significant nephroprotective potential in the present study. Melatonin is widely distributed in the body and can penetrate into every subcellular compartment of the kidney, and to inactivate free radicals. The neurohormone MLT, which can be found in vegetables, fruits, herbs, etc. (27), exhibits a wide range of antioxidant and anti-inflammatory activities, which have been clinically tested in adults and even in premature infants (28).

In both *in vitro* and *in vivo* experimental models, MLT was proven to possess protective effects against oxidative damage caused by various toxic agents in kidney and sepsis-damaged kidneys (28). Melatonin is widely spread, with almost no side effects, readily available and active after oral administration, and it is commonly used in humans to treat insomnia (29).

In this research, by analyzing non-protein nitrogenous compounds (urea and creatinine), we proved that MLT has a protective effect during LPS-induced nephrotoxicity. It was proven that in addition to its antioxidant capacity in kidney tissue MLT is able to neutralize various forms of ROS and to stimulate antioxidant enzymes (SOD and glutathione peroxidase) (28). Several experiments showed that MLT protects against kidney damage (30,31), which is accompanied by a normalization of reduced glutathione (GSH) concentration. Melatonin was shown to reduce tubular necrosis and protect renal function in a model of renal tubular injury, mediated by free radicals (32).

It has been proven that MLT prevents damage to the mitochondria and inhibits an increased expression of iNOS, which is induced by endotoxemia with bacterial LPS. Melatonin prevents multiple organ dysfunction (kidney, liver, lung) and circulatory failure during endotoxemia, protects mitochondria from damage in experimental sepsis, and reduces sepsis mortality. Therefore, it should be kept in mind that in septic patients, the use of MT has a significant role in preserving the mitochondrial energy level in cells (33).

Conclusion

It can be concluded that a single oral administration of melatonin significantly alleviates lipopolysaccharide-induced acute nephrotoxicity in rats by reducing urea and creatinine levels. It is likely that the beneficial effects of melatonin are related to its known antioxidant effects on kidney tissue and to some of its less known mechanisms of action.

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MELATONIN SPREČAVA OŠTEĆENJE BUBREGA IZAZVANO LIPOPOLISAHARIDOM KOD PACOVA

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Lipopolisaharid (LPS) je sastavni deo ćelijskih zidova gram -negativnih bakterija, tako da je LPS injekcija široko primenjena kao model eksperimentalne akutne bubrežne insuficijencije povezane sa sepsom. Sepsa indukovana LPS-om uzrokovana je prekomernim lučenjem proinflamatornih medijatora i reaktivnih vrsta kiseonika (ROS). Neurohormon melatonin, koji uglavnom luči epifiza, reguliše cirkadijalni ritam, ima antiinflamatornu i imunoregulatornu ulogu. Pokazalo se da melatonin i njegovi metaboliti uklanjaju različite slobodne radikale i intermedijere reaktivnog kiseonika. Cilj ovog istraživanja bio je da se proceni efekat melatonina u prevenciji endoksemije izazvane oštećenjem bubrega izazvanog LPS -om, analizom nivoa uree i kreatinina u krvnom serumu pacova. Dvadeset osam Wistar albino pacova nasumično je podeljeno u četiri grupe (n = 7 po grupi) na sledeći način: 1) Kontrolna grupa, 2) MLT grupa (50 mg/kg, oralno), 3) LPS grupa (10 mg/kg, i.p.) i 4) LPS + MLT grupa (10 mg/kg + 50 mg/kg). Nivoi kreatinina i uree u serumu ($p < 0,05$) bili su značajno viši kod životinja tretiranih LPS-om u poređenju životinjama iz kontrolne grupe. Zajednički tretman životinja sa sepsom indukovanom LPS-om i MLT značajno je smanjio visok nivo serumskog kreatinina i uree ($p < 0,05$). Može se zaključiti da oralna primena melatonina značajno ublažava akutnu nefrotoksičnost izazvanu LPS-om kod pacova. Verovatno je da su korisni efekti melatonina povezani sa njegovim poznatim antioksidativnim efektima na bubrežno tkivo, a potencijalno i sa nekim drugim manje poznatim mehanizmima dejstva. *Acta Medica Medianae 2023;62(1):15-20.*

Ključne reči: *melatonin, lipopolisaharid, bubreg, urea, kreatinin*