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Protective effect of melatonin as an antioxidant in the intestine of rats with superior mesenteric arterial occlusion

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ABSTRACT

The superior mesenteric artery is a branch of the aortic artery that supplies blood to the small and some parts of the large intestine. Any obstruction in blood flow and reperfusion causes tissue damage in the intestine. This study aimed to determine the rate of fat peroxidation and tissue protein as an indicator of tissue degradation after ischemia and reperfusion following induction of superior mesenteric artery occlusion in the intestine and to evaluate the protective effect of melatonin as a free radical scavenger and antioxidants in rats. In this study, 36 male Wistar-Albino rats weighing between 80-120 g were equally divided into six groups and received different melatonin doses (10, 20, and 30 mg/kg) intramuscularly. In this regard, Group 1 was the control group, Group 2 was the Sham group (underwent surgery to dissect the upper mesenteric artery and injected the same volume of solvent), Group 3 was Ischemia-reperfusion (IR), Group 4 was IR with melatonin at a dose of 10 mg/kg, Group 5 was IR with melatonin at a dose of 20 mg/kg, and Group 6 was IR with melatonin at a dose of 30 mg/kg. The results showed that the level of tissue malondialdehyde (MDA) was significantly lower in groups 4, 5, and 6 than in group 3 (P <0.05). Tissue protein levels were significantly higher in group 4 than in group 3 (P <0.001). Tissue protein levels in groups 5 and 6 did not significantly differ from group 3 (P = 0.191). Overall, this study showed that melatonin at a dose of 10 mg/kg has an antioxidant effect preventing induced damage due to superior mesenteric artery occlusion.

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Introduction

The superior mesenteric artery is a branch of the aortic artery that supplies blood to the small intestine and part of the large intestine up to two-thirds of the right side of the transverse colon (1). Any obstruction in blood flow and reperfusion of blood flow after removing the obstacle causes tissue damage in the intestine (2, 3). Various conditions and diseases cause ischemia-reperfusion in this artery (4, 5). These include a trapped hernia. Ischemia-reperfusion damage consists of a sequence of chemical events that lead to cell dysfunction and necrosis and consists of two parts (5). Direct damage (which is a mild injury that occurs during the ischemic phase, the tissue and the organ are deprived of blood and they receive insufficient nutrients and oxygen.) and indirect damage (This damage occurs during reperfusion and includes tissue damage that occurs after the ischemic stage and after restoring blood flow. This damage is more severe than the damage caused by ischemia. In this case, free radicals released by re-oxygenating ischemic tissue are responsible for the tissue damage) (6, 7).

The production of free radicals causes Ischemia-reperfusion-induced damage. Free radicals are ionic compounds with one or more unpaired (single) electrons and are highly active (8). Free radicals appear to be produced by the accumulation of hypoxanthine under anaerobic conditions and activated neutrophils in ischemic tissue (9).

During the mitochondrial electron transfer chain, free radicals are produced that cause the protein to be destroyed. Metabolic processes may cause oxidative protein damage (10). Protein damage comes in two forms; Non-specific damage (global) and site-specific damage (localized). Non-specific damage is caused by reactive oxygen species, leading to protein fragmentation and deformation of almost all amino acids. Site-specific damage occurs when reactive oxygen species (ROSs), such as hydroxyl, are present at the junctions of metal ions in proteins (11). Catalytic metal ions, such as iron and copper, bind to

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proteins, and the hydroxyl formed by O_2^- and H_2O_2 reacts with the target molecules at the junctions of copper or iron (9, 12). The damaging effects of hydroxyl are observed at the specific site where the catalytic ions are attached. The defensive function of free radical scavengers to remove hydroxyl from specific sites reduces damage. Arginine-lysine, cysteine, and proline are sensitive to this type of oxidation. Ischemia-reperfusion-induced damage is prevented or treated by antioxidants, free radical scavengers, and other agents (5).

Melatonin is a product secreted by the pineal gland, which affects the circadian rhythm by acting on the suprachiasmatic nuclei (13, 14). The chemical composition of melatonin is N-acetyl 5-methoxytryptamine. This compound is a derivative of serotonin, which is produced from tryptophan (15). Serotonin is first acetylated by N-acetyltransferase and then methylated by hydroxy indole orthomethyltransferase to form melatonin. Exposure to light inhibits N-acetyltransferase and stops melatonin production. Exposure to darkness causes neuronal messages to be transmitted by neurons to the suprachiasmatic nuclei. From there, the nerve message reaches the paraventricular nuclei and travels through the upper thoracic spinal nerve to the upper cervical ganglion (16). Following this, sympathetic nerve fibers go to the pineal gland and secrete norepinephrine. Norepinephrine is received in pinealocytes by alpha and beta-adrenergic receptors, increasing the production of cyclic AMP and converting serotonin to melatonin in the melatonin synthesis pathway. Melatonin enters the blood and ventricles of the brain and enters the cerebrospinal fluid from the ventricles (17).

Melatonin has several functions, including the treatment of insomnia and delaying the aging process. Melatonin affects many physiological activities such as neuroendocrine function and puberty (17, 18). In addition, melatonin has potent antioxidant activity that protects cells, tissues, and organs from damage by reactive oxygen species (ROS) (19). Melatonin also accumulates free radicals and prevents fat peroxidation. Different melatonin levels stimulate or increase the expression of genes in antioxidant enzymes such as SOD, catalase, and glutathione. One study found that mRNA levels increased SOD isoforms such as Mn-SOD, and Cu, Zn-SOD by 35-51% after melatonin administration (18).

Free radicals cause oxidative damage that reduces cell capacity. They also reduce the antioxidant capacity, which the use of antioxidant drugs can increase (20). Melatonin acts to detoxify free radicals such as hydroxyl by donating electrons directly. This substance protects cells, tissues, and organs from oxidative damage induced by various free radical production processes and agents (21). It is effective in safeguarding nuclear DNA, membrane fats, and cytoplasmic proteins and improves the antioxidant defense capacity of the whole organism. The function of melatonin as a free radical scavenger and antioxidant is probably due to its easy passage through body barriers such as the blood-brain barrier and its entry into cells and cell organs (22). Thus, in the present study, the effect of melatonin as an antioxidant and free radical scavenger was investigated on ischemia-reperfusion.

Materials and methods

Chemicals

The study was performed on 36 male Wistar-Albino rats weighing 120-180 g. The rats were placed in separate cages at a temperature of 20-25 ° C and a humidity of 70-80% and exposed to light for 12 hours. They were also fed standard rat food. They were not fed for 12 hours before the test but consumed water. Upon completion of the work, food and water were provided to the rats without any restrictions. All methods of studying animals were in accordance with NIH rules.

We categorized 36 rats into six groups of 6 in the following order:

Group 1: Control

Group 2: Sham underwent surgery to dissect the upper mesenteric artery and injected the same volume of solvent.

Group 3: Ischemia-reperfusion (IR)

Group 4: IR with melatonin at a dose of 10 mg/kg

Group 5: IR with melatonin at a dose of 20 mg/kg

Group 6: IR with melatonin at a dose of 30 mg/kg

Rats were weighed, and 10% ketamine and 2% xylazine (Sigma-Aldrich, USA) were used for anesthesia according to their weight. Ketamine at a dose of 40-50 mg/kg and xylazine at 4-5 mg/kg were injected intraperitoneally (IP) into rats. After opening

the rat abdomen, using two sterile forceps, the intestines were gently pushed to the left and the aortic artery beat was observed. The superior mesenteric artery should be found in the upper left abdomen, where it separates from the aorta and enters the intestinal mesentery. The length of the artery is short and about 1cm, which is located below the posterior peritoneum of the abdomen. The artery was removed from the back of the peritoneum using a pair of forceps and released. Small clamps for neurosurgery and aneurysms were used to close the artery. Behind the clamp and at the beginning of the artery, the pulse of blood flow was observed. The cessation of blood flow was also seen in the branches isolated from the superior mesenteric, in the mesentery of the intestine. The superior mesenteric artery remained clamped for 30 minutes.

During this time, to prevent the intestines from drying out in the air, a sterile gauze moistened with 0.9% sterile normal saline was placed on the rat's open abdomen to keep the area moist. During the operation, the rat's heart rate and respiration were monitored regularly, and a temperature lamp was used to prevent the rat's body temperature from decreasing. At the end of 30 minutes, the clamp was gently removed from the artery using an applicator, while the return of blood flow to the intestine was fully observed. Prepared melatonin for rats was injected intramuscularly before and after clamp removal. In the next step, the intestines were gently placed, and the peritoneum and skin were repaired together using 4-0 silk thread. The suture site was disinfected with betadine, and the rats were transferred to their cage.

For the treatment of rats, 48 mg of melatonin (Sigma-Aldrich, USA) was dissolved in 1 ml of 98% ethanol. Ethanol was diluted with normal saline 0.9% in a ratio of 1:10 to reduce the effect and toxicity of ethanol.

Malondialdehyde (MDA) factor was used to evaluate the effect of melatonin on the elimination of free radicals and reduction of fat peroxidation. Using the thiobarbituric acid method can measure the aldehydes resulting from tissue peroxidation. In this method, Aldehyde from peptide peroxidation displayed by TBARs (mostly MDA) reacts with thiobarbituric acid at acidic pH and forms a color complex capable of absorbing light at 532 nm. Protein concentration was measured using the Bradford Protein Assay kit. The test in this kit is based on the reaction of pyrogallol red with protein in the presence of molybdenum and acidic conditions. The color complex created at a wavelength of 600 nm has the highest absorption.

To evaluate the results of the experiments performed in this study for the variables of Malondialdehyde and tissue protein, one-way analysis of variance and t-test were used. Significant differences between the means of different groups were assessed by the Tukey test.

Results and discussion

Data obtained from MDA (as an indicator of lipid peroxidation) and the effect of melatonin on this index showed that the amount of tissue MDA in the ischemia-reperfusion group (group 3) compared with the control group (group 1) was significantly increased (P<0.01). Tissue MDA levels were significantly lower in the melatonin-treated groups at different doses (group 4, 5, and 6) than in the IR group (group 3) (p<0.05) (Table 1). In this regard, Group 1was the control group, Group 2 was the Sham group (underwent surgery to dissect the upper mesenteric artery and injected the same volume of solvent), Group 3 was Ischemia-reperfusion (IR), Group 4 was IR with melatonin at a dose of 10 mg/kg, Group 5 was IR with melatonin at a dose of 20 mg/kg, and Group 6 was IR with melatonin at a dose of 30 mg/kg.

Table 1. Changes in intestinal malondialdehyde (MDA) levels in the study groups; control (C) and group (G)

Variable	C1	G 2	G 3	G 4	G 5	G 6
Mean Tissue MDA (µg/µl)	0.8135	1.361	2.579ǂǂ	1.319*	1.398*	1.672*
Standard Error	0.2816	0.3556	0.3216	0.2641	0.0669	0.1521

*: Significant difference with group3 (P<0.05); ǂǂ: Significant difference with group1 (P<0.01)

The data obtained from the study of tissue protein in the intestine showed that the amount of tissue protein in group 3 was significantly reduced compared to group 1 (P <0.001). There was no significant difference between groups 1 and 2 (P = 0.12). The amount of tissue protein in group 4 was significantly increased compared to group 3 (P <0.001). There was

no significant difference between the amount of tissue protein in groups 5 and 6 compared to group 3 (P = 0.191) (Table 2).

Table 2. Changes in intestinal tissue protein levels in the study groups; control (C) and group (G)

Variable	C 1	G 2	G 3	G 4	G 5	G 6
Mean Tissue Protein (mg/dl)	16.121	13.138	4.328ǂǂ	16.099***	6.786	5.342
Standard Error	1.543	2.898	0.7473	2.654	1.102	1.184

***: Significant difference with group3 (P<0.05); ǂǂ: Significant difference with group1 (P<0.01)

Several studies have shown that free radicals are involved in the pathogenesis of induced damage in IR (23, 24). Xanthine oxidase system and activated neutrophils are also engaged in producing toxic free radicals and damage during IR. In the ischemic phase of the xanthine oxidase system, cessation of blood flow and lack of oxygen leads to anaerobic metabolism and lack of energy and a decrease in ATP and accumulation of hypoxanthine in ischemic cells (25). Lack of energy affects the ATPase ion pump in the cell membrane, leading to calcium, sodium, and water accumulation in the cell and its swelling (26).

Xanthine dehydrogenase, a natural enzyme in the body, is converted to xanthine oxidase (by calcium-dependent protease)(27). In the reperfusion phase, xanthine oxidase converts hypoxanthine to xanthine and also produces uric acid, superoxide radicals and hydrogen peroxide. This condition leads to fat peroxidation and oxidation of cell membrane protein (28). Neutrophils, on the other hand, are activated during reperfusion and migrate to connective tissue. Oxidation in neutrophils during reperfusion leads to the production of reactive oxygen species. These toxic extracellular secretions of neutrophils cause fat peroxidation and oxidation of cell membrane proteins (29). Some studies have shown that the use of xanthine oxidase inhibitors, such as allopurinol or oxypurinol, reduces IR damage (30).

Researches have also shown that removing leukocytes from the circulatory system greatly reduces IR damage (31, 32). The damage caused by the reperfusion phase is much more severe than the ischemic phase due to a large amount of free radicals. But the need for perfusion to maintain ischemic tissue is undeniable. This is because it provides cellular energy and removes toxic metabolites (33).

Melatonin is a product secreted by the pineal gland that has various functions, including antioxidant properties. Melatonin either collects free radicals directly or indirectly by collecting the power of antioxidant enzymes in the body to collect free radicals (17). Melatonin effectively protects the DNA of the nucleus and membrane lipids, and cytoplasmic proteins and improves the antioxidant capacity of the whole organism. The function of melatonin as a collector of free radicals and antioxidants is probably related to the easy passage of melatonin through body barriers such as the blood-brain barrier and its entry into cells and cell organs (34).

Lipid peroxidation is one of the damages caused by free radicals. The malondialdehyde (MDA) index was used to measure fat peroxidation. MDA is the end product of fat peroxidation (33). In the present study, it was found that melatonin at doses of 10, 20, and 30 mg/kg significantly reduced tissue MDA and reduced ischemia-reperfusion injury. This finding was consistent with previous studies (35-37). Protein damage is one of the damages caused by free radicals during ischemia-reperfusion. This damage occurs in both global and localized forms in the protein (37). The present study results also showed that the free radical caused by ischemia-reperfusion causes protein oxidation. Its amount in the ischemia-reperfusion group is significantly lower than in the control group.

Our study showed that melatonin administration at a dose of 10 mg/kg could significantly reduce protein oxidation compared to the ischemia-reperfusion group. But melatonin at doses of 20 and 30 mg/kg did not show a significant change in protein oxidation compared to the ischemia-reperfusion group.

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