Melatonin Supplementation Under Hypobaric Hypoxia and Hypothermia Conditions

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The presence of oxidative and nitrosative stress under hypobaric hypoxia and hypothermia conditions led us to experimentally study the effect of the administration of melatonin as a non-nutritional antioxidant in rats. Morphological changes in the lungs and heart were monitored under hypobaric hypoxia and hypothermia conditions, with and without melatonin supplementation. Melatonin partially corrected the changes induced by hypothermia in the lungs and by hypoxia in the myocardium.

Keywords: melatonin, lung, heart, hypobaric hypoxia, hypothermia

Stress is a body state resulting from the interaction with various stressors – situations, events, objects or persons, which may cause a positive, beneficial, favorable stress response or a negative, harmful, unfavorable distress response of the body.

In living organisms, a biochemical disturbance of the oxidant/antioxidant balance occurs as a result of an overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS), products of cell metabolism, and as a result of a decrease in the antioxidant defense capacity of the body, with the development of oxidative stress (OS) and nitrosative stress (NS) [1-3].

The reactive oxygen species involved in OS are: superoxide anion, hydroxyl radical, peroxyl radical, hydroperoxyl radical, hydrogen peroxide, ozone, triplet and singlet oxygen. The reactive nitrogen species involved in NS are: nitric oxide radical, peroxynitrite, nitrogen dioxide radical and nitric oxide. About 100 diseases involving OS and NS have been described [2,4,5].

Antioxidants are substances that significantly reduce or inhibit the harmful effects of ROS and RNS. Melatonin (N-acetyl-5-methoxytryptamine) is an antioxidant synthesized in the pineal gland, where it is the major hormone, as well as extrapineally [6]. The determining factors of melatonin secretion are: non-ionizing light radiation, sympathetic overactivity, age, physical exercise, environmental stressors [7-12]. Melatonin secretion decreases under the action of light and increases in darkness.

It has a protective role in fighting intracellular OS, through: direct action on ROS as a scavenger and reduction of lipoperoxidation [13-18]; indirect action, by stimulation of antioxidant enzymes and suppression of pro-oxidant enzymes [19-22].

Environmental physical stressors such as temperature and pressure may cause changes in redox homeostasis, with the increase of OS and NS.

Altitude represents complex environmental stress through hypobaric hypoxia, low temperature, air humidity, ultraviolet radiation, and its interaction with the ozone layer, individual characteristics, physical exercise [4,24].

Exposure to cold may induce environmental hypothermic stress with the alteration of the body's homeostasis, the generation of ROS and RNS, and the reduction of AO defense capacity, oxidative stress varying in different tissues [23-31].

Literature data regarding biochemical OS and NS under hypobaric hypoxic and hypothermic stress conditions led us to study the effect of the administration of melatonin as a non-nutritional antioxidant.

Experimental part

Our experimental study monitored the morphological changes induced in the heart and lungs by physical stressors (low temperature and hypobaric hypoxia) andmelatonin administration associate with physical stressors.

Material and methods

The study was approved by the Ethics Committee of theIuliu Hatieganu University of Medicine and Pharmacy Cluj-Napoca, according to the requirements of the Declaration of Helsinki, the Amsterdam Protocol and Directive 86/609 CEE.

The experiment was conducted at the Experimental Research Laboratory of the Department of Physiology of the Iuliu Hatieganu University of Medicine and Pharmacy Cluj-Napoca, in the period May-June 2017.

Animal groups

The study was carried out on male Wistar rats aged 4-5 months, with a mean weight of 220±10 g, from the Biobase of the Iuliu Hatieganu University of Medicine and Pharmacy Cluj-Napoca. The animals were assigned to 5 groups (n=10 animals/group):

-group C, control group, supplemented with placebo and exposed to normoxia;

-group I, exposed to hypobaric hypoxia;

-group II, supplemented with melatonin and exposed to hypobaric hypoxia;

-group III, exposed to hypothermia;

-group IV, supplemented with melatonin and exposed to hypothermia.

The control group received lactose as placebo. Lactose was used as a negative control, due to its inert effects, and was administered by oropharyngeal gavage in doses of 0.1 mg/150 g animal daily, for 14 days. The animals of the control group were kept under adequate *vivarium* conditions: temperature 18-20°C and atmospheric pressure corresponding to the altitude of 364 m, O_2 concentration =20.94% and pO_2 air =117 mmHg.

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Groups I and II were exposed to hypobaric stress-advanced hypobaric hypoxia simulated in the hypobaric chamber with the KB0016D vacuum pump, in the Experimental Laboratory. Exposure was intermittent, for 12 hours daily, during 14 days, at an altitude of 5000 m and an air pressure of 405 mmHg; pO_2 =82 mmHg, O_2 concentration =11.2%. Daily interruption of hypobaric hypoxia was required in order to feed the animals and clean their cages.

Groups III and IV were exposed to hypothermic stress for 3 hours daily, during 14 days, at a temperature of 5ºC in the refrigerating chamber of the laboratory.

Groups II and IV were supplemented with melatonin Mellow Tonin (Secom®) administered in doses of 3 mg/kg body weightby oropharyngeal gavage, daily, for 14 days.

Histological examination

Histological examination was performed at the Morphopathology Laboratory of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Biological material was collected at the end of the experiment, after the animals were euthanized. The samples were collected according to the recommendations of the European Society of Toxicologic Pathology. The biological material consisted of lung and heart tissue samples.

The collected tissue samples were immersed in 10% buffered formol solution for 48 hour fixation. After fixation, the samples were processed using the paraffin technique.

The left lobe of the lung was collected, which was sectioned longitudinally. Cross sections of the right caudal and right cranial lobes were also cut.

The collected hearts were cut longitudinally, with the section perpendicular to the plane of the interventricular septum. After paraffin embedding, 4 micrometer thick serial sections were cut using the Leica RM 2125 RT microtome. The sections were placed on histological slides and stained with hematoxylin-eosin.

The histological preparations performed were examined with an Olympus BX 51 microscope; images were taken with an Olympus DP 25digital camera and processed using the Olympus Cell B image acquisition and processing software.

Results and discussions

The lungs of the animals in the control group (C) showed a normal histological appearance (fig. 1).

In group I (animals exposed to hypoxia) (fig. 2), lesions characteristic of pulmonary hypertension where observed. Thus, ahypertrophy of the pulmonary arterial media and intimawas evidenced, with the presence of inflammatory infiltrate dominated by neutrophils and eosinophils around these vessels. Smooth muscle cells in the arterial media were hypertrophic, so that the lumen was reduced in volume. In the parenchyma, a thickening of alveolar septa was seen.

In the case of group II animals (hypoxia +melatonin) (fig. 3), lesions characteristic of pulmonary hypertension persisted, with a thickening of arterial walls (of the media and intima), with an increase in the diameter of smooth muscle cells in the arterial media and endothelial cell vacuolization. Alveolar septal thickening was maintained, with a reduction of the air-blood exchange surface in the alveolocapillary membrane.

In group III (induced hypothermia) (fig. 4), there were discrete pulmonary edema lesions, significant pulmonary alveolar thickening with capillary congestion in the alveolar septa and the presence of discrete inflammatory infiltrate dominated by mononuclear cells. In the vicinity of pulmonary densification areas, compensatory pulmonary emphysema foci were observed.

In group IV (animals exposed to hypothermia + melatonin) (fig. 5), there was a significant reduction of pulmonary edema lesions and a significant reduction of pulmonary alveolar septal thickness. No changes in pulmonary blood vessels were seen.

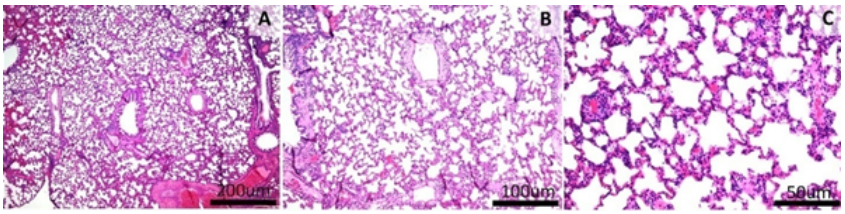

Fig. 1. Control group, lung sections; normal histological appearance. HE staining, scale bar =200 µm (A), scale bar =100 µm (B), scale bar =50 µm (C)

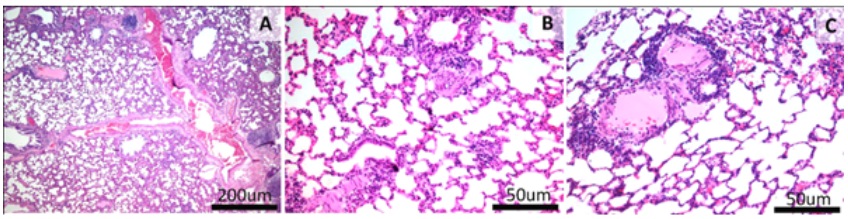

Fig. 2. Group I, lung sections; thickening of the arterial media with a reduction in the luminal diameter (B); inflammatory infiltrate with polymorphonuclear cells surrounding the pulmonary vessels (C). HE staining, scale bar =200 µm (A), scale bar =50 µm (B, C)

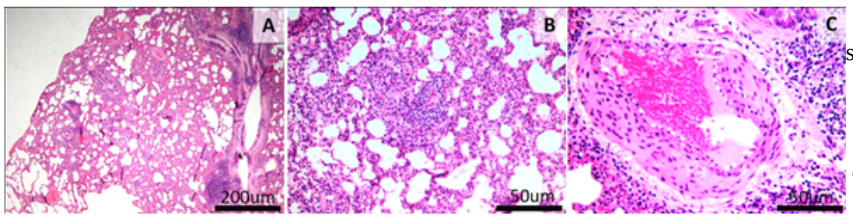

Fig. 3. Group II, lung sections; increase in the smooth muscle cell diameter in the vascular media with its thickening and a reduction in the luminal diameter (B, C); inflammatory infiltrate with polymorphonuclear cells around the pulmonary arteries (C); endothelial cell vacuolization (C). HE staining, scale bar =200 µm (A), scale bar =50 µm (B, C)

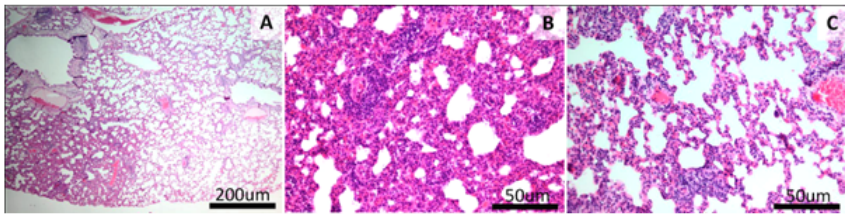

Fig. 4. Group III, lung sections; marked alveolar septal thickening (B, C), dilation of capillaries in the alveolar septa (A, B, C). HE staining, scale bar =200 µm (A), scale bar =50 µm (B, C).

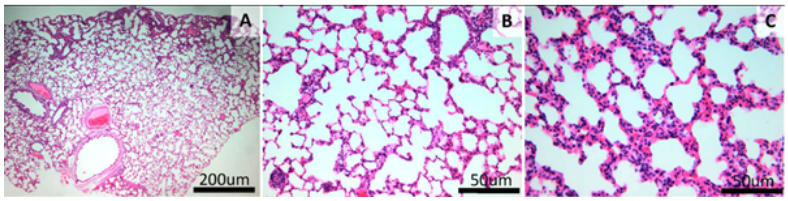

Fig. 5. Group IV, lung sections; normal alveolar septa (A, B, C), mild congestion of septal capillaries (C). HE staining, scale bar =200 µm (A), scale bar =50 µm (B, C).

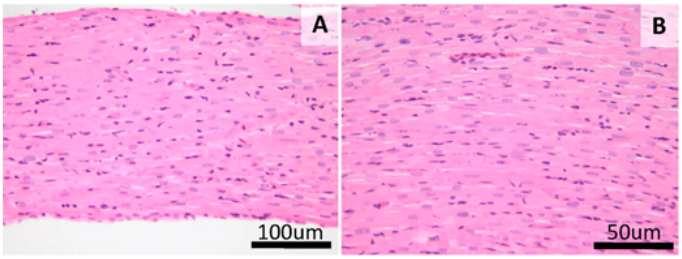

Fig. 6. Control group, heart sections; normal histological appearance. HE staining, scale bar =100 µm (A), scale bar =50 µm (B).

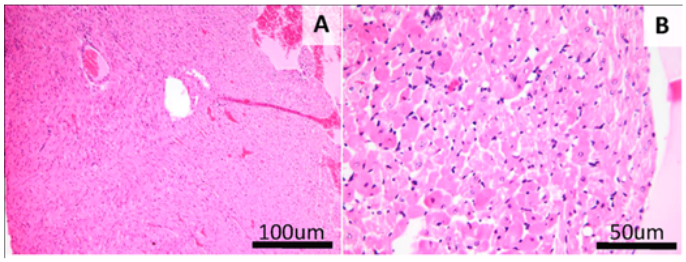

Fig. 7. Group I (hypoxia), heart sections; severe congestion of myocardial capillaries (A), many vacuolated or karyopyknotic cardiomyocytes (B). HE staining, scale bar =100 µm (A), scale bar =50 µm (B).

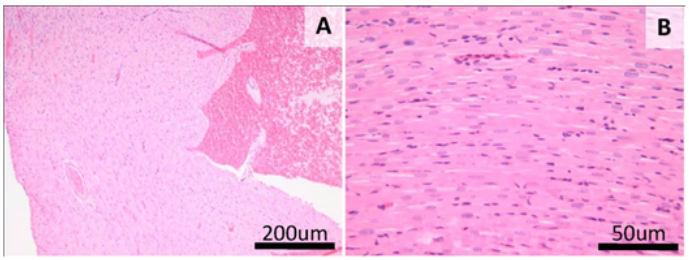

Fig. 8. Group II (hypoxia + melatonin), heart sections; mild congestion of myocardial capillaries (A), normal appearance of myocardial fibers (B). HE staining, scale bar =200 µm (A), scale bar =50 µm (B).

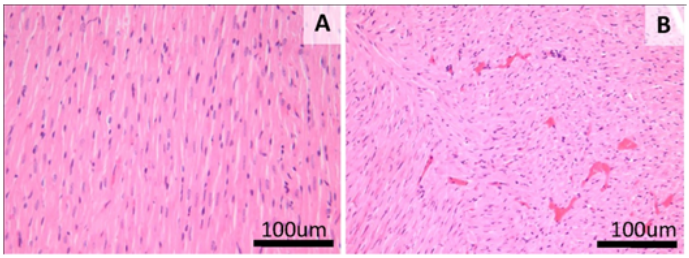

Fig. 9. Group III (hypothermia), heart sections; mild congestion of myocardial capillaries (B), normal appearance of myocardial cells (A, B). HE staining, scale bar =100 µm (A, B).

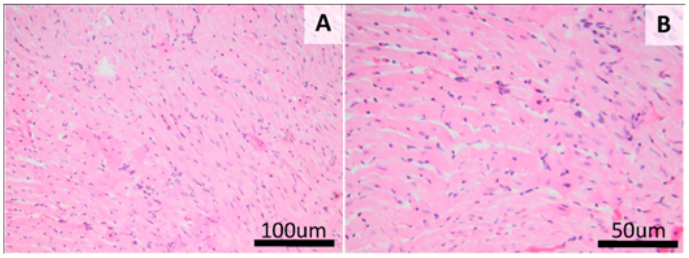

Fig. 10. Group IV (hypothermia + melatonin), heart sections; cardiac muscle cells with intensely acidophilic cytoplasm and pyknotic nuclei (A, B). HE staining, scale bar =100 µm (A), scale bar =50 µm (B)

In the heart sections of the control group (C) (fig. 6), no morphological changes in the myocardium, valvular and mural endocardium, or pericardium were observed.

In groupI animals (animals exposed to hypoxia) (fig. 7), there was severe congestion of myocardial capillaries and multiple myocardial degenerative and necrotic foci; cardiac muscle cells were vacuolated or with pyknotic nuclei and intensely eosinophilic cytoplasm.

In groupII animals (hypoxia +melatonin) (fig. 8), mild congestion of myocardial capillaries was observed, without the presence of myocardial necrotic or degenerative lesions.

In the sections performed in group III animals, exposed to hypothermia (fig. 9), severe congestion of myocardial capillaries was seen, without the presence of necrotic or dystrophic lesions.

In group IV animals, exposed to hypothermia and melatonin therapy (fig. 10), the presence of many cardiomyocytes with intensely eosinophilic cytoplasm and pyknotic nuclei (necrosis), and even myocardial microhemorrhage was observed.

Our research under diurnal conditions, in which endogenous melatonin secretion is reduced, demonstrates that pulmonary and myocardial histological changes are due to the administration of exogenous melatonin before exposure to hypobaric hypoxic stress and hypothermic stress.

Conclusions

The administered melatonin partially corrected the changes induced by hypothermia in the lungs and by hypoxia in the myocardium. Melatonin supplementation has a pulmonary protective effect under hypothermic stress conditions and a myocardial protective effect under intermittent hypobaric hypoxic stress conditions.

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Manuscript received: 14.02.2018