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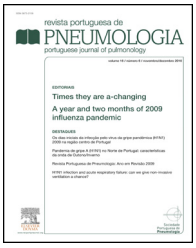

ORIGINAL ARTICLE

Melatonin attenuates lung injury in a hind limb ischemia–reperfusion rat model

Hamed Takhtfooladi[a], **Mohammad Takhtfooladi**[b,*], **Fariborz Moayer**[c], **Sayed Mobarakeh**[d]

[a] *Faculty of Veterinary Science, Karaj Branch, Islamic Azad University, Karaj, Iran*
[b] *Department of Surgery, Faculty of Specialized Veterinary Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran*
[c] *Department of Pathobiology, Faculty of Veterinary Medicine, Karaj Branch, Islamic Azad University, Karaj, Iran*
[d] *Faculty of Medical Sciences, Shahid Sadughi University, Yazd, Iran*

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Abstract
Objective: This study evaluated the protective antioxidant effect of melatonin on lung injury as a remote organ after skeletal muscle ischemia–reperfusion in rats.
Methods: Thirty male Wistar rats were allocated randomly into three experimental groups: operated with no ischemia (Sham) group, ischemia–reperfusion group and ischemia–reperfusion + melatonin group. Hind limb ischemia was induced by clamping the femoral artery. After 2 h ischemia, the clamp was removed and the animal underwent 24 h reperfusion. Rats in the ischemia–reperfusion + melatonin group received melatonin (10 mg/kg i.v.), immediately before the clamp was removed. At the end of the trial, animals were euthanized and the lungs were removed for water content determination, histopathological and biochemical studies.
Results: In the ischemia–reperfusion + melatonin group, tissues showed less intense histological abnormalities such as neutrophilic infiltration, intra-alveolar hemorrhage and edema compared with the ischemia–reperfusion group. Histopathologically, there was a significant difference ($P < 0.05$) between the two groups. The lung water content in the ischemia–reperfusion + melatonin group was significantly lower than the ischemia–reperfusion group ($P < 0.05$). Lung tissue myeloperoxidase (MPO) activity and nitric oxide (NO) level were significantly ($P < 0.05$) increased by ischemia–reperfusion. The increase in these parameters was reduced by melatonin.

Comparing the ischemia–reperfusion + melatonin group with the sham group, no significant increase in all analyzed aspects of the research was observed.

* Corresponding author.
E-mail address: dr_ashrafzadeh@yahoo.com (M. Takhtfooladi).

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Conclusions: These findings suggest that melatonin has preventive effects in lung tissue injury after transient femoral artery occlusion.

Introduction

The pathophysiology of ischemia–reperfusion injury has been previously described. Polymorphonuclear leukocytes and free radical species have been shown to have pivotal roles in the etiology.[1] Skeletal muscle ischemia–reperfusion resulting from trauma, limb revascularization, orthopedic surgery and free-flap reconstruction or any other condition not only leads to local damage, but also causes severe injury involving destruction of remote organs.[2–4]Many studies have shown that several agents such as caffeic acid phenethyl ester or erdosteine,[5,6] N-acetylcysteine,[4] zinc aspartate,[7] trapidil[8] and tramadol[9] are useful against lung injury induced by oxidative stress damage in ischemia–reperfusion.

Melatonin, the chief hormone of the pineal gland, is a well-known potent antioxidant and free radical scavenger[10,11] that can counteract the damaging effects of free radicals.[12,13] Melatonin also shows a protective effect on lung injuries induced by ischemia–reperfusion.[14] There have been no studies to test the effect of melatonin on lung histological changes induced by skeletal muscle ischemia–reperfusion. The aim of this study was to examine the effects of melatonin on acute lung injury in a rat model of skeletal muscle ischemia–reperfusion by transient femoral artery occlusion.

Materials and methods

All experimental procedures were performed according to the guidelines for the ethical treatment of experimental animals and approved by Islamic Azad University School of Veterinary Science, Animal Care and Use Local Ethics Committee.

Experimental groups

Thirty male Wistar rats weighing 300 ± 35 g were used in this study. They were maintained under constant room temperature of $25 \pm 2\,^{\circ}C$, relative humidity of $75 \pm 5\%$, 12 h/12 h light/dark cycle, with *ad libitum* access to water and commercial food and were placed in individual cages. Animals were randomly allocated into three experimental groups of ten rats each:

Group I – Sham group with no ischemia–reperfusion. The animals were subjected to all operative procedures, except arterial occlusion and reperfusion. After isolation and exposure of the femoral artery for 2 h, the animals received 2 ml of 0.9% saline via the external jugular vein.

Group II (ischemia–reperfusion group) – The animals were subjected to 2 h of ischemia followed by 24 h of reperfusion. Two milliliters of 0.9% saline was administered intravenously prior to reperfusion.

Group III (ischemia–reperfusion + melatonin group) – All animals underwent 2 hours of ischemia by occlusion of the femoral artery followed by 24 h of reperfusion. A solution of 10 mg/kg melatonin was administered immediately via the external jugular vein, with a total volume of 2 ml.

Surgery

Anesthesia was induced using ketamine plus xylazine (10 mg/kg and 50 mg/kg i.m., respectively). After induction of anesthesia, the left hind limb was prepared for sterile surgery. A skin incision was made on the medial surface of the left hind limb and the femoral artery was isolated and clamped with a vascular forceps for 2 h. Following the ischemic period, the vascular forceps was removed and then the surgical site was routinely closed. Rats were returned to their cages with a commercial balanced diet and water *ad libitum*.

Specimen collection

At the end of the trial, the rats were euthanized with an overdose of intraperitoneal pentobarbital injection (300 mg/kg) and the lungs were removed *en bloc*. The right lungs were used for water content determination and histopathological analysis under light microscope and the left lungs were stored at $-20\,^{\circ}C$ till the biochemical analysis. The lung tissue homogenate and supernatant samples were prepared as described by Yildirim et al.[15]

Biochemical assay

Myeloperoxidase (MPO) activity and nitric oxide (NO) levels were studied in lung tissues. The activity of MPO was analyzed spectrophotometrically as described by Wei et al.[16] NO level in lung tissue was measured by Griess reaction.[17]

Lung wet/dry weight ratio

Lung wet/dry weight ratio was used as an indicator of pulmonary edema. The lower lobe of the right lung from each animal was weighed and placed in a vacuum oven (at $54\,^{\circ}C$) until a stable,[18] dry weight was achieved. The ratio of lung wet weight to dry weight was then calculated.