



Physiology 2016

Abstracts

Joint Meeting of the American Physiological Society and
The Physiological Society

29 – 31 July 2016 · Convention Centre Dublin, Ireland

#physiology2016



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Ethical requirements

Experiments on animals or animal tissue

For work conducted in the UK all procedures must conform with current UK legislation. For work conducted elsewhere all procedures must accord with current national guidelines or, in their absence, with current local guidelines.

Experiments on humans or human tissue

All procedures must accord with the ethical standards of the relevant national, institutional or other body responsible for human research and experimentation, and with the principles of the World Medical Association's Declaration of Helsinki.

PCB180

Melatonin's role in skeletal muscle of rats with LPS induced endotoxemiaE. Ozkok³, H. Yorulmaz², G. Ates¹, O. Sahin⁴ and S. Tamer¹

¹Department of Physiology, Istanbul University, Istanbul Medical Faculty, Istanbul, Turkey, ²Department of Physiology, Halic University, Faculty of Medicine, Istanbul, Turkey, ³Department of Neuroscience, Istanbul University, Institute of Experimental Medicine, Istanbul, Turkey and ⁴Department of Neurology, Istanbul University, Istanbul Medical Faculty, Istanbul, Turkey

Motivation/Problem Statement Melatonin is known an effective anti-inflammatory agent in various animal models of inflammation and sepsis and functions stimulation of various antioxidant enzymes, contributing to enhance the antioxidant defense and to protective effects on mitochondrial function. In this study we aimed to investigate the effects of multidose melatonin treatment, which was injected before and/or after endotoxemia on biochemical and histologic changes in skeletal muscle and blood glutathione (GSH) level high dose of LPS induced endotoxemia.

Methods/Procedure/Approach: We divided rats into 4 groups, control, lipopolysaccharide (LPS) (20 mg/kg, i.p, single dose), melatonin (10 mg/kg, i.p. three times), and melatonin + LPS. Melatonin was injected i.p. 30 min before and after the 2nd and 4th hours of LPS injection. At the end of the experimental period, blood samples were taken from heart to determine serum concentrations of creatinine kinase (CK), aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose, and blood leucocyte. Blood GSH levels were measured spectrophotometrically using DTNB (5,5'-dithio-bis-(2-nitrobenzoic acid) reagent. For hematoxylin and eosin (H&E), the muscle tissue samples were fixed in 10% buffered formalin and embedded in paraffin wax. The slides were evaluated under light microscopy. Groups of data were compared with an analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Values of $P < 0.05$ were regarded as significant. **Results:** Serum CK, ALT, AST, and blood leucocyte values were found to be increased in LPS groups as compared with those of other groups ($P < 0.05$). In the LPS group, glucose levels were observed to be decreased compared to control group ($P < 0.01$). In the melatonin + LPS group; glucose levels were higher than the LPS group ($P < 0.01$). Antioxidant status was determined by GSH measurement in the blood. Section of skeletal muscle the sections were then stained with hematoxylin and eosin. The stained sections were visualized and photographed. In the Melatonin + LPS group, blood GSH levels were increased compared with the LPS group ($P < 0.01$). With H&E staining, we observed weak fibre boundaries and irregular - shaped nuclei in the LPS group. The appearance of muscle fibers in the melatonin + LPS groups was the same as those of the controls.

Conclusion/Implications: Our findings showed melatonin treatment prevented muscle damage by increasing glucose and blood GSH levels in rats with LPS induced endotoxemia. **Keywords:** glutathione, lipopolysaccharide, melatonin, skeletal muscle

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PCB181

Dynamics of cytokines concentrations in blood serum of larynx squamous carcinoma patientsI. Karmazina¹, A. Cherniakova², I. Isaeva¹ and N. Hloba¹

¹physiology, Kharkov National Medical University, Kharkov, Ukraine and ²Kharkov National Medical University, Kharkov, Ukraine

The pluripotent role of "cytokines network" in inflammation and tumor dissemination is still the issue of the day. Cytokine families are investigated to find out the reliable markers for the prognosis and monitoring of the course of cancer on the basis of the correlation between their concentrations in the blood serum and stages of cancer disease. The aim of our research was to investigate concentrations of TNF- α , IL-1 β , IL-6 (proinflammatory), IL-4, IL-1RA (antiinflammatory) in blood serum of patients with inflammation and different stages of squamous epithelium carcinoma of the larynx and to validate their role as predictors of tumor progression.

Informed consent was given by all patients. Serum samples were collected by centrifuging whole blood at 3000 rpm (1000g) for 10 minutes at 15°C: I set of samples–inflammation (n=22); II set of samples–squamous epithelium carcinoma transformation T1N0M0 (n=14), 2 group–tumor progression T2N0M0, T3N0M0 (n=26), 3 group–tumor dissemination T4N0M0, T4N1(2)Mx, T1NxMx (n=21); III set of samples–healthy persons (n=18). Concentrations of interleukins were investigated by commercially available validated kits (ELISA, Germany) by immunoenzyme method. Statistical analysis: STATISTICA 10.0, ANOVA.

Inflammation resulted in increase of both pro- and antiinflammatory interleukins: TNF- α -11.35 \pm 1.73 pg/ml vs.3.11 \pm 1.23 pg/ml control ($p < 0.01$); IL-1 β -12.41 \pm 2.88 pg/ml vs.4.37 pg/ml ($p < 0.01$); IL-6-9.99 \pm 1.76 pg/ml vs.3.89 pg/ml ($p < 0.01$); IL-4-17.45 \pm 2.45 pg/ml vs.7.04 \pm 2.66 pg/ml ($p < 0.01$); IL-1RA-2306 \pm 115.3 vs.521.8 \pm 36.22 pg/ml ($p < 0.01$). The stage-dependent significant changes of "cytokines network" were revealed in sera of patients with cancer. In 1 group of II set: TNF- α -15.91 \pm 1.19 pg/ml ($p < 0.01$); IL-1 β -6.34 \pm 2.21 pg/ml ($p < 0.05$); IL-6-7.85 \pm 1.98 pg/ml ($p < 0.01$); IL-4-11.26 \pm 2.02 pg/ml ($p < 0.01$); IL-1RA-804 \pm 64.5 ($p < 0.05$). In 2 group of II set: TNF- α -18.38 \pm 1.32 pg/ml ($p < 0.01$); IL-1 β -13.16 \pm 2.66 pg/ml ($p < 0.01$); IL-6-14.42 \pm 2.72 pg/ml ($p < 0.01$); IL-4-15.48 \pm 1.22 pg/ml ($p < 0.01$); IL-1RA-2016 \pm 198.6 ($p < 0.01$). In 3 group of II set: TNF- α -29.10 \pm 4.73 pg/ml ($p < 0.01$); IL-1 β -17.70 \pm 2.12 pg/ml ($p < 0.01$); IL-6-18.73 \pm 1.75 pg/ml ($p < 0.01$); IL-4-7.88 \pm 2.68 pg/ml ($p < 0.01$ 1 and 2 groups); IL-1RA-721.1 \pm 101.5 ($p < 0.05$ I set, $p < 0.01$ 2 group).

Therefore, at the initial stages of cancerogenesis TNF- α , IL-1 β and IL-6 may be generated by malignant cells as well as the "host's" immune system against the tumor antigens, and contribute in the tumor proliferation. At the stage of cancer dissemination proinflammatory cytokines reach their maximum with the significant drop of the antiinflammatory IL-4 and IL-1RA that confirm the malfunction of organism immune surveillance. The cooperative examination of pro- and anti-inflammatory cytokines levels in the blood serum may be useful biochemical factors for prognosis of carcinoma metastasis.

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