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Melatonin attenuates oxidative stress and modulates inflammatory response after experimental burn trauma

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Research Article

Melatonin attenuates oxidative stress and modulates inflammatory response after experimental burn trauma

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Abstract

Introduction. Thermal injury activates an inflammatory response. Melatonin possesses anti-oxidant and anti-inflammatory properties. The objective of the present work was to study melatonin effects on the inflammatory response under conditions of oxidative stress during the early stage of thermal injury.

Materials and methods. We used 24 white male rats of Wistar breed, randomly divided into three experimental groups. Group one was the control, group two was inflicted with burn trauma, and group three was inflicted with burn trauma, with melatonin application following the thermal injury. Melatonin was applied twice in doses of 10 g/kg b.m. immediately after the burn trauma and again at 12 hours. Plasma levels of tumor necrosis-factor-α (TNF-α), a pro-inflammatory mediator, and of interleukin-10 (Il-10), an anti-inflammatory mediator, were examined and their ratio was calculated. The levels of malondialdehyde (MDA), an oxidative stress marker, were also estimated.

Results. Thermal trauma significantly increased plasma TNF-α levels (ð<0.01) and TNF-α /IL-10 ratio but did not change IL-10 ones. Plasma MDA concentrations were significantly elevated as well (ð<0.0001). Melatonin application significantly reduced TNF-α (ð<0.05), increased IL-10 (ð<0.05), down-regulated TNF-α/IL-10 ratio and changed MDA concentrations (ð<0.01).

In conclusion, our results show that local alteration induces oxidative stress and inflammatory response with TNF-α /IL-10 disbalance. Melatonin modulates this response and attenuates oxidative stress in experimental burn injury.

Keywords : melatonin, inflammatory response, TNF-α, IL-10, burn

Highlights

- ✓ Thermal trauma significantly increased plasma TNF-α levels and TNF-α /IL-10 ratio but did not change IL-10.
- ✓ Melatonin attenuates oxidative stress and changes the disbalance between the pro- and anti-inflammatory mediators in favor of the anti-inflammatory ones.

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Introduction

Severe thermal trauma can lead to the development of systemic inflammatory response syndrome (SIRS) and sepsis. Generalization of the pathophysiological manifestations causes increased morbidity, poly-organ insufficiency, and mortality among burned patients (1). Locally, in a burn-induced wound, numerous cells such as neutrophils and macrophages are activated (2, 3). Their count in systemic circulation also increases. (4). These are the source of cytokines with pro-inflammatory action, activate the inflammatory cascade and, as along with them, they synthesize and liberate cytokines with anti-inflammatory action. The cellular response also underlies the generation of free radicals (5) which induce lipid peroxidation, cell membrane damage, and apoptosis (6, 7). The induced postburn oxidative and nitrosative remote organ damage disturbs immune system balance (8), contributes to immunosuppression development, and enhances the risk for the development of systemic inflammatory response syndrome (SIRS) and sepsis (9, 10).

Tumor necrosis factor-α (TNF-α) is a cytokine presenting with a variety of biological effects (11) and acting as a central inflammation mediator in sepsis, trauma, and burn (12-14). TNF-α induces gene expression of a series of pro-inflammatory cytokines and is capable of self-induction (11, 15).

Interleukin-10 (IL-10) has initially been described as an inhibitory factor for the synthesis of cytokines (TNF-α, IL-1, IL-6), chemokine, and adhesion molecules in the monocytes/macrophages and neutrophils (16-18). TNF-α reduction is considered the most important suppressive role of IL-10 (19). Data regarding how the elevated concentration of the pro-inflammatory mediators of thermal trauma prevail are presented in the recent literature, but there are relatively few investigations of the anti-inflammatory mediators.

Melatonin (N-acetyl-5-methoxytryptamine) is mainly a secretory product of the pineal gland. Its functions in the organism relate to numerous physiological and pathological processes. Melatonin exerts direct antioxidant effects via free radical scavenging and indirectly stimulates the activities of antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GRd), and catalase (20). Melatonin may exert an anti-inflammatory effect as well, by restricting the action of the free radicals and inhibiting the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) and related target genes, which participate in immunity and inflammation (21). Melatonin application has been found to reduce the manifestation of the systemic inflammatory response in experimental and clinical investigations (22-24). However, there are few studies regarding the melatonin effect on IL-10 levels under the conditions of experimental thermal trauma (25, 26).

Herewith we hypothesize that melatonin modifies cytokine secretion and modulates the systemic inflammatory response after burn trauma. To test this hypothesis, we examined melatonin effects on plasma levels of TNF-α, a pro-inflammatory mediator, and of Il-10, an anti-inflammatory mediator, during the early stage of thermal trauma.

Materials and Methods

Experimental design

The experimental procedure was approved by the Home Office for Care and Use of Laboratory Animals, and experiments were performed in accordance with the European Communities Council Directives 86/609/EEC. Age-matched male rats weighing between 220 and 250g fasting for 12 h were allowed free access to water before injury.

Animals were housed in individual wire-bottomed cages at 20º C and offered rat chow and water ad libitum. They were kept in dark/light cycles (12:12 h; lights on at 8:00 am) to ensure a satisfactory photoperiod. After light ether inhalation, general anesthesia was intraperitoneally performed using thiopental (30 mg/kg). In order to accomplish 30% of a third-degree burn, boiling water (98º C) was applied on the back of the animals for 10 sec. For those rats subjected to burn injury, 4 mL of physiological saline was intraperitoneally applied for immediate resuscitation after the trauma. No animals died within the first 24 h of the post-burn period. Twenty-four male rats were randomly assigned to three groups of 8 animals each: control, non-burned and non-treated (C), vehicle-treated burned group (B), and melatonin treated burned group (B+M).

Melatonin treatment

Melatonin (N-acetyl-5-methoxytryptamine, Merck, Germany) in a dose of 10 mg/kg body weight (b.w.) dissolved in vehicle, or vehicle alone (2% ethyl alcohol diluted in physiological saline in a dose 5 ml/kg) was administered to the appropriate group. Melatonin and vehicle were applied i.p. twice, immediately after burns in the morning between 8:00 a.m. and 9:00 a.m. and 12 hours after burn injury. All animals were given buprenorphine (0.3 mg /kg i.p. b.w.) twice daily for pain control post burn.

Animals from the all groups were anesthetized with thiopental and euthanized 24 h after burns.

Biochemical analysis

Blood was taken from the jugular vein and heparinized. Plasma was separated by centrifugation at 800 x g rpm for 10 min and aliquots were stored at -80oC until analysis. Plasma lipid peroxidation was assayed by MDA levels detected by thiobarbituric acid (TBA) reactivity as described by Porter et al. (27). Results were expressed as nmol MDA/mL plasma, using the extinction coefficient of MDA-TBA complex at 532 nm = 1.56 x 10–5 cm–1 M–1 solution.

Determination of plasma cytokine levels

Plasma levels of TNFα and IL-10 were determined by enzyme-linked immunosorbent assay (ELISA) using Gen-Probe Diaclone SAS kits (Besancon Cedex, France). Results were reported as pg/mL.

Statistical analysis

Statistical analyses utilized Graphpad Prism version 6.0. The results are shown as mean ± SEM and box plots. Significance was determined by unpaired Student's t test or the nonparametric Mann-Whitney-U-test. A P-value less than 0.05 two-tailed was considered significant.

Results

Examination of MDA in thermal trauma and melatonin effect

Plasma MDA levels were significantly increased by 39% ($p<0.0001$) in the burned rats compared to the control group (Fig. 1). Melatonin treatment significantly inhibited the elevation in plasma MDA level ($p<0.01$) and restored control values.

Examination of TNF-α in thermal trauma and melatonin effect

TNF-α levels increased significantly by 115% ($p<0.01$) in plasma of burned rats compared with controls (Fig. 2). Plasma TNF-α concentration decreased following melatonin treatment by 41% ($p<0.05$) in the burned rats but was 74% higher ($p<0.01$) relative to control rats.

Examination of IL-10 in thermal trauma and melatonin effect

Plasma IL-10 level did not change significantly in burned rats when compared to controls (Figure 3). Melatonin significantly elevated this level by 50% ($p<0.05$) in burned rats and was higher than that of the control rats.

Examination of TNF-α/IL-10 ratio in thermal trauma and melatonin effect

This ratio was higher by 114% ($p<0.05$) in the experimental group than the control group (Fig. 4). Melatonin treatment reduced this ratio by 37% ($p<0.05$), tending to restore values comparable to those of the control group.

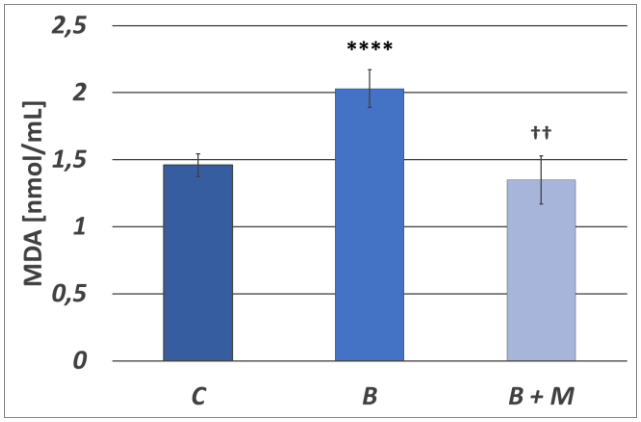

Figure 1. Effect of melatonin on MDA levels in plasma after burns. (C) control group; (B) burned, non - treated group; (B+M) burned, treated with melatonin group. Results are given as the mean ± SEM. $^{****}p<0.0001$ vs. control group; $^{††}p<0.01$ vs. burned, non - treated group.

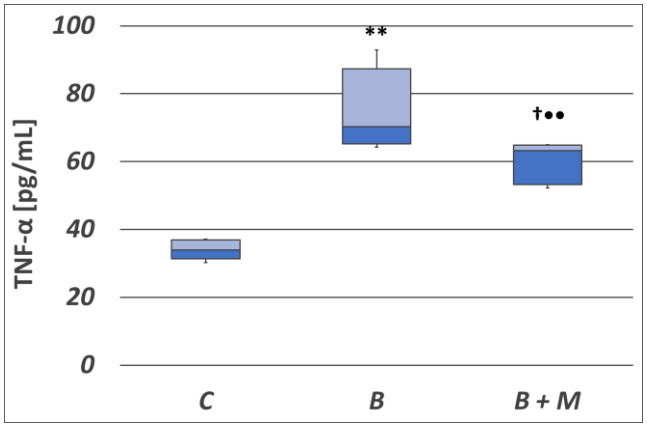

Figure 2. Effect of melatonin on TNF-α levels in plasma after burns. (C) control group; (B) burned, non - treated group; (B+M) burned, treated with melatonin group. Results are given as box plot, with median, 25th- and 75th-percentile values, min and max values. **p<0.01 vs. control group; †p<0.05 vs. burned, non - treated group; ••p <0.01 vs. control group.

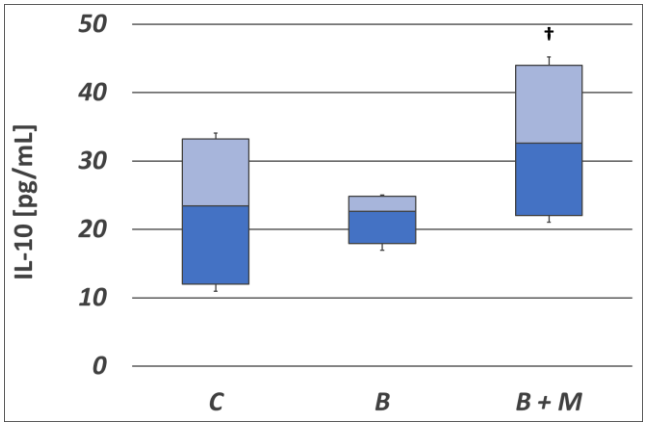

Figure 3. Effect of melatonin on IL-10 levels in plasma after burns. (C) control group; (B) burned, non - treated group; (B+M) burned, treated with melatonin group. Results are given as box plot, with median, 25th- and 75th-percentile values, min and max values. †p<0.05 vs. burned, non - treated group.

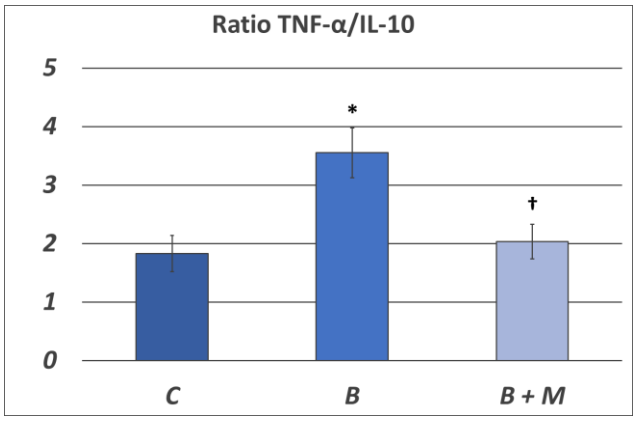

Figure 4. Effect of melatonin on TNF-α/IL-10 ratio in plasma after burns. (C) control group; (B) burned, non - treated group; (B+M) burned, treated with melatonin group. Results are given as the mean ± SEM. *p<0.05 vs. control group; †p<0.05 vs. burned, non - treated group.

Discussions

Thermal injury induces local tissue damage. The activated pro-inflammatory cells such as neutrophils and macrophages synthesize and liberate large amounts of chemokines, cytokines, adhesion molecules, and alarmines which when entering the circulation can cause a systemic inflammatory response (28). The enhanced production of pro-inflammatory cytokines, lipid peroxides, and acute-phase proteins, along with activation of the polymorphonuclear cells plays an important role in the systemic inflammatory response induced by thermal trauma (29, 30). Results from the present study demonstrate elevated plasma TNF-α concentrations after burn injury, providing evidence for the role of this mediator in the early local and systemic inflammatory response.

TNF-α represents not only an inflammation-inducing mediator but also an important factor in the course of the inflammation (31). It induces the expression of other inflammatory mediators such as IL-1α, IL-1β, IL-6 and IL-8, which form a cytokine network, adhesion molecules, acute phase proteins (32) and Il-10 as well (33). TNF-α augments ROS production by the pro-inflammatory cells. ROS (reactive oxygen species) activate lipid peroxidation and cause cell membrane damage in thermal trauma (6, 34). Both TNF-α and ROS enhance the expression of NF-kB, a transcription factor responsible for the production of other pro-inflammatory mediators with cytotoxic action. A correlation exists between MDA and TNF-α in liver during burns (35) as well as between plasma TNF-α concentration and the degree of thermal damage (36). Similar data have been obtained in clinical and experimental investigations of thermal injury (37, 38). But there are contradictory data about the changes of tissue and plasma TNF-α levels. Some investigators have failed to establish alterations in burned animals compared with not-burned ones, most probably due to the experimental design and the duration of the examination period (39).

Our results demonstrate that the level of the pro-inflammatory mediator significantly increases while that of the anti-inflammatory mediator shows no change. There are contradictory data about IL-10 concentrations in burn injury. While some authors report results similar to ours (40), others report elevated concentrations of two anti-inflammatory cytokines, IL-2 and IL-10, reaching their peak values during the initial hours after thermal trauma (41). The primary role of IL-10 is the suppression of the production of the pro-inflammatory mediators and the regulation of the inflammatory response (42). It seems possible that IL-10 accomplished its effects though the inhibition of the nuclear factor NF-kB (43) and through pathways that do not depend on NF-kB, such as activation of nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and heme oxygenase (HO-1) (44).

The examination of the balance between pro- and anti-inflammatory cytokines serves as a predictive marker in clinical practice (45). The mortality of the patients with sepsis is related to elevated TNF-α and IL-10 concentrations (46, 47). The IL-10: TNF-α ratio is low in surviving patients (47), so it has been assumed that TNF-α/IL-10 disbalance represents one of the triggering factors for the development of SIRS and polyorgan failure in the initial stage after burn injury (48). Further research is needed to clarify these alterations during the various stages of the severe thermal trauma. It is known that antioxidant application inhibits the systemic inflammatory changes in thermal injury (49) and other diseases in which pathogenesis of systemic inflammation is involved (50).

Our results demonstrate a disbalance between the pro- and anti-inflammatory cytokines under the conditions of oxidative stress induced by burn trauma. Melatonin application results in the establishment of a tendency towards restoration of balance more similar to baseline levels in control rats. The data also indicate that melatonin reduces plasma TNF-α level and enhances plasma IL-10, a finding consistent with other authors who have also established that anti-inflammatory melatonin action is related to reduced plasma TNF-α concentration and increased plasma IL-10 (51, 52). This effect is most probably due to melatonin's inhibitory effect on NF-kB (53), also evident from thermal trauma (26). As such, melatonin improves the pro-/anti-inflammatory balance and restricts the manifestations of the systemic inflammation.

In the present study, plasma MDA levels were significantly increased, thus demonstrating severe lipid peroxidation following considerable burn injury, a finding consistent with other studies (54, 55). The pathophysiological alterations are most likely a consequence of ischemia/reperfusion injury and polymorphonuclear cell

activation both locally and in the systemic circulation resulting in free-radical overgeneration (56, 4). On the other hand, the depletion of plasma glutathione and antioxidant enzymes has been shown as a cause for the manifestations of the systemic oxidative response and the aggravation of the pathological processes in thermal trauma (57). Melatonin administration significantly decreases plasma MDA levels, a consequence of the antioxidant and free-radical-scavenging capacities of melatonin and its metabolites (20).

Conclusions

Melatonin attenuates oxidative stress and changes the disbalance between the pro- and anti-inflammatory mediators in favor of the anti-inflammatory ones. Therefore, melatonin, by restricting the lipid peroxidation and by modulating the inflammatory response, can counteract the systemic inflammation and the subsequent development of sepsis and polyorganic insufficiency. These results confirm the broad therapeutic potential of melatonin and substantiate its possible application for the treatment of critical pathological conditions of the organism.

Acronyms and abbreviations

TNF-α: Tumor necrosis-factor-α
Il-10: Interleukin-10
MDA: Malondialdehyde
SIRS: Systemic inflammatory response syndrome
SOD: Superoxide dismutase
GPx: Glutathione peroxidase
GRd: Glutathione reductase
NF-kB: Nuclear factor kappa-light-chain-enhancer of activated B cells
Nrf2: Nuclear factor (erythroid-derived 2)-like 2
HO-1: Heme oxygenase

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