Original Article

EFFECT OF MELATONIN ON BURN-INDUCED MORPHOLOGICAL CHANGES IN GASTRIC MUCOSA

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Summary

The pathophysiologic mechanisms leading to damage of gastric mucosa in burns are not completely understood. Melatonin, a compound with well-known antioxidant properties, reduces oxidative damage of the gastric mucosa and other organs. The aim of this study was to determine whether the protective effect of melatonin on burn-induced morphological changes in gastric mucosa in burns is mediated by peroxidative processs. Under anesthesia, the shaved dorsum of rats was exposed to a 90° bath for 10s to induce burn injury covering 30 % of total body surface area. Melatonin (10 mg/kg) was administrated intraperitoneally immediately, and on hour 12 after thermal skin injury. At the 24th hour after burn injury, gastric tissue samples were taken to determine biochemical and histological changes. Tissue samples were investigated by light microscopy. Mucosal malondialdehyde (MDA) and sulphydrils (SHs) as markers of oxidative status were measured in addition to histological analysis. Thermal skin trauma caused severe degeneration of the surface and glandular epithelium of gastric mucosa, degradation and loss of surface epithelial cells in gastric mucosa. MDA levels were increased significantly ($p < 0.01$), and SHs were decreased ($p < 0.05$) in the gastric mucosa. Treatment with melatonin significantly restricted oxidative damage and reduces degenerative changes in the gastric mucosa and preserved the integrity of mucosal epithelium after experimental thermal injury. In conclusion, we found that the protective effect of melatonin on burn-induced morphological changes in the gastric mucosa mediated by activated lipid peroxidative processes.

Key words: gastric mucosa, histopathology, melatonin, burns.

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Introduction

Extensive burn skin injury results in elevated risk of damage of organs distant from original burn wound, including gastric mucosa, which is a serious clinical problem [1, 2]. Pathophysiology of burn-induced gastric mucosa injury includes many mechanisms and has not been not entirely defined. Various cellular and molecular interactions such as neutrophil and macrophage activation, oxygen

radical and cytokine overproduction, depletion of glutathione and prostaglandin E_2 and mitochondrial dysfunction may be involved in the processes [3-5]. It has been reported that treatment with agents possessing antioxidant potential (alupurinol, rebamipide) or anti-inflammatory effect (cyclosporine A, oxytocin, monteukast) have a protective effect against burn-induced gastric oxidative injury [3, 6-9].

Melatonin is a hormone produced by the pineal gland, a tissue factor and an autocoid [10]. Recently, the gastrointestinal tract of vertebrates has been shown to be a rich source of extra pineal melatonin [11]. Melatonin is highly lipophilic, and for this reason all parts of the cell abound in melatonin. Being a scavenger of both reactive oxygen species (ROS) and reactive nitrogen species (RNS), melatonin protects the DNA, lipids and proteins against oxidative damage [12-13]. Melatonin stimulates the activity of a variety of antioxidant enzymes and reduces the production of cytokines [14-16]**.** Antioxidant and anti-inflammatory effects of melatonin have been shown in many studies [17-20]. Thermal trauma alters endogenous pineal melatonin secretion during the acute posttraumatic period. In a recent review, melatonin has been shown to reduce the toxicity of therapeutic agents used to treat thermal injury patients [21]. The protective effect of melatonin against various types of mucosal oxidative damage induced by ischemia reperfusion, as well as chemical agents such as indomethacin, aspirin and piroxicam is well documented [22-25].

Recently, we have shown that melatonin treatment restricts burn-induced oxidative damage of gastric mucosa by suppressing lipid peroxidation, oxidative stress and inflammatory response [26]. The literature data concerning the effect of melatonin on morphologic changes in gastric mucosa after burns are scarce.

The aim of this study was to determine whether the protective effect of melatonin on burn-induced morphological changes in gastric mucosa is mediated by peroxidation.

Material and Methods

Animals

The experimental procedures were approved by the Home Office for Care and Use of Laboratory Animals and performed with a strong consideration for ethics of animal experimentation according to the International Guiding Principles for Animal Research approved in Bulgaria. Same-age male rats weighing between 220 and 250 g were fasted for 12 h, and were allowed free access to water before injury. Animals were housed in a 20 °C room and offered rat chow and water ad libitum. They were kept in dark–light cycles (DL = 12:12 h) in individual wirebottomed cages and fed standard rat chow. The lights were turned off at 8:00 p.m. and turned on at 8:00 a.m. to achieve a satisfactory photoperiod.

Thermal injury and melatonin treatment

After mild ether inhalation, general anaesthesia was administered intraperitoneally (i.p.) using thiopental 30 mg/kg(-1) body weight. In order to accomplish a 30% third-degree burn, scalding hot water (90° C) was applied on the back of the animals for a 10 s. Following burn injury, 4 ml physiological saline was applied i.p. for immediate resuscitation. No animal died within the first 24 h post-burn period. Thirty male rats were randomly assigned to three groups: non-burned vehicle treated group (N-B, n = 7); burned +vehicle treated group (B, n = 7); and burned +melatonin treated group (B + M, n = 7).

Melatonin (N-acetyl-5-methoxytriptamine; Merck, Germany) in a dose of 10 ml/kg(-1) body weight, dissolved in a vehicle (2% ethyl alcohol diluted in physiological saline in a dose up to 5ml per kg body weight) was applied i.p. immediately and 12 hours after thermal skin injury. A solvent vehicle was administered to the burned and control rats (sham groups) i.p. immediately and 12 hours after thermal skin injury. All animals received buprenorphine in a dose of 0.3 mg/kg(-1) body weight i.p. twice daily for post-burn pain control. The animals were reanaesthetised with thiopental and sacrificed 24 h after the burns and stomachs were sampled.

Histological examinations

Tissue specimens were fixed in 10% buffered formalin (pH=7.2), dehydrated in ascending series of ethyl alcohol (70%–100%), cleared in methyl benzoate and embedded in paraffin wax. Tissue sections of 5 μm were stained with hematoxylin and eosin (H&E) and examined using light microscope (Olympus BH-2, Tokyo, Japan).The histopathological appearance of tissues in the different groups were compared.

Biochemical examinations

Tissue (stomach) samples were homogenized with ice-cold 150 mM KCl for the determination of malondialdehyde (MDA) and thiol levels. Membrane lipid peroxidation was assayed by MDA measured by its thiobarbituric acid (TBA) reactivity in gastric mucosa homogenates using the method of Porter et al [27]. Results were expressed as nmol MDA/g tissue were determined using the extinction coefficient of MDA-TBA complex at 532 nm =1, 56 x 10^{-5} cm^{-1} M^{-1} solution and results are expressed as nmol MDA/g tissue.

Gastric mucosal SH groups were determined by the method of Hu [28], based on the absorption of the color complex between SH groups and DTNB at 412 nm. Standard solutions of reduced glutathione were used to calculate the concentration of SH groups. Results are expressed in mmol SH/g tissue.

Statistical analysis

Data were analyzed statistically by one-way analysis of variance (ANOVA) and expressed as mean±SEM. A value of $p < 0.05$ was considered statistically significant. The statistical procedure was performed with GraphPadInStat software.

Results

Histopathological results

Severe skin burn caused degeneration of the surface and glandular epithelium of gastric mucosa and degradation and loss of surface epithelial cells (Fig. 1). Hyperemia (vascular congestion) and leukocyte infiltration detected in tissue sections suggested pronounced tissue injury. However, gastric mucosa of melatonin-treated rats demonstrated an almost intact gastric epithelium, with a slight damage in the surface epithelium being still prominent, while the congestion and leukocyte infiltration were minimal.

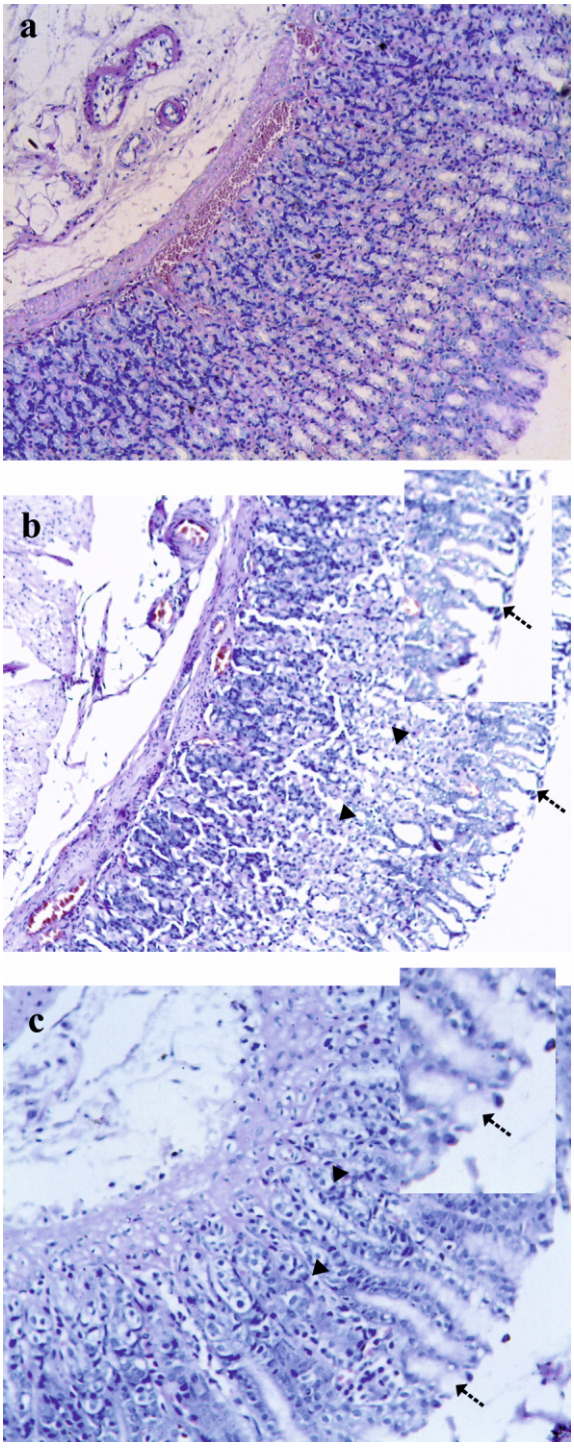

Fig. 1. Effect of melatonin on burn-induced histomorphological changes in gastric mucosa in rats: a) N-B - normal morphology of the surface and glandular epithelium of gastric mucosa; b) B - severe degeneration of the surface---►, glandular epithelium degradation and loss of surface epithelial cells ---►, hyperemia (vascular congestion) and leukocyte infiltration detected in tissue sections imply to a prominent tissue injury; c) B+Mel - normal surface and glandular epithelium in most areas of gastric fundus mucosa. H&E staining, original magnification, 200X, 400X

Biochemical results

MDA levels, measured as an index of tissue lipid peroxidation, were significantly higher in the gastric mucosa of the burn group as compared to control group ($p < 0.01$, Fig.2), while mucosal SH levels were significantly decreased following burn ($p < 0.05$, Fig.3). However, treatment with melatonin reversed the mucosal MDA and SH levels back to the control levels ($p < 0.05$) . In the intact animals treated with melatonin, the levels of MDA and SH levels were found to be similar to those of the control group.

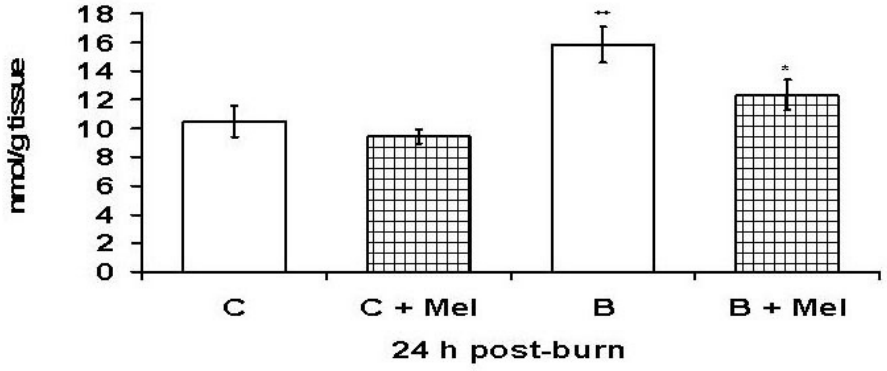

Fig 2. Effect of melatonin treatment on the mucosal malondialdehyde (MDA).
C-contol group; C+Mel-contol treated with melatonin; B-vehycle treated burn group; B+Mel-treated with melatonin burn group; ++ $p < 0.01$ compared with the control (C) group; *$p < 0.05$ compared with the vehicle treated burn group

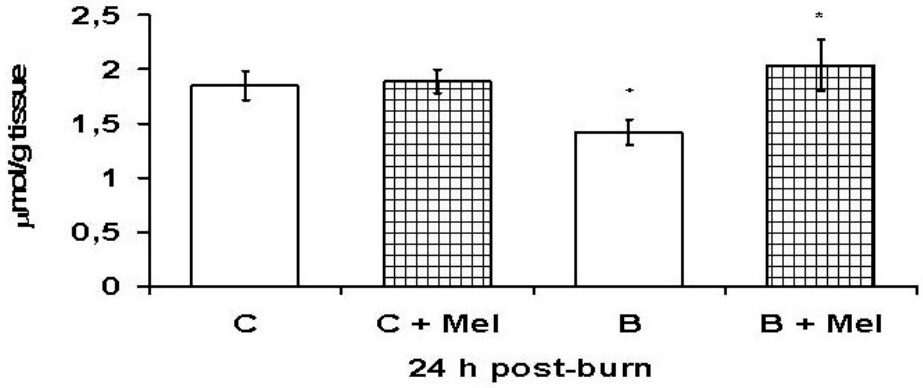

Fig 3. Effect of melatonin treatment on the mucosal thiols (SH).
C-control group; C+Mel-control treated with melatonin; B-vehycle treated burn group; B+Mel-treated with melatonin burn group; +$p < 0.05$ compared with the control (C) group; *$p < 0.05$ compared with the vehicle treated burn group.

Discussion

The results presented demonstrate that melatonin restricted burn-induced oxidative damage and degenerative changes in gastric-mucosa and preserved the integrity of mucosal epithelium as well.

One of the most important pathophysiological mechanisms of gastric mucosal damages is the excessive production of ROS and RNS production, and an unbalanced oxidant/ antioxidant process. [5, 27-31]. Our results show that thermal skin trauma induces oxidative stress in the gastric mucosa as evaluated by increased mucosal malondialdehyde (MDA) levels and sulphydril depletion. A significant increase of mucosal MDA has been reported in animal

models of burns along with evidence of pathological changes such as degeneration of the surface and glandular epithelium of gastric mucosa, as well degradation and loss of surface epithelial cells [3, 9].

ROS cause cellular injury via a mechanism including membrane lipid peroxidation, oxidative damage of proteins, nuclear and mitochondrial DNA, along with depletion of cellular antioxidants [32-33]. Oxidative stress is recognized as a strong mediator of apoptosis [34]. Free radical mediated mitochondrial dysfunction can lead to the release of cytochrome C (an initiator of apoptosis) and activation of caspases, playing essential role during apoptosis [35-38]. It has been reported that indomethacin, a non-steroidal anti-inflammatory drug (NSAID) induces gastric mucosal injury via epithelial cell apoptosis [39]. Augmentation of the latter, due to oxidative stress, is one of the main pathogenic events in the development of gastropathy [37]. NSAID-induced generation of mitochondrial ROS and depletion of GSH pool cause mitochondrial dysfunction and activation of the mitochondrial pathway of apoptosis in gastric mucosa [40]. It has been demonstrated that lipid peroxidation and oxidative stress play an important role in the process of apoptosis of gastric mucosa that results from various stressors such as ethanol, hydrogen peroxide [41-42], and several stress conditions such as ischemia reperfusion, water immersion and restrain stress [43-46].

Based on these data we could suggest that burn-induced peroxidative processes may activate apoptosis of gastric mucosal epithelium. These processes probably cause degenerative alterations, such as loss of surface epithelial cells and other damages in the gastric mucosa. Recently, apoptosis was found to be important in pathological changes occurring after burns. It has been shown that burns induce apoptosis of hepatocytes and gut mucosal cells as a result of "systemic apoptotic response" [47].

Recently it has been shown that melatonin prevents the development of mitochondrial oxidative stress and activation of mitochondrial pathway of indomethacin-induced apoptosis in the gastric mucosa [37]. On the other hand, histological studies document that melatonin provides an almost complete protection against indomethacin-induced gastropathy. In addition, melatonin reverses lipopolysaccharide-induced gastrointestinal motility disturbances through inhibition of oxidative stress [20].

Melatonin treatment restricts burn-induced oxidative damage of gastric mucosa by suppressing lipid peroxidation [26]. In addition, melatonin preserves GSH pool within the cell and mitochondria, which helps suppressing oxidative processes and restoring redox balance in these sites [30]. We hypothesized that melatonin as a scavenger of free radicals is capable of suppressing oxidative damage of gastric mucosal mitochondria and the mitochondrial apoptotic pathways as well. As an antioxidant, melatonin contributes to the reduction of degenerative changes in gastric mucosa and to preservation of the integrity of mucosal epithelium after experimental thermal injury.

Conclusion

In conclusion, the protective effect of melatonin on burn-induced morphological injury of gastric mucosa may relate to its antioxidant properties. It seems probable that melatonin could restore gastric mucosal damage following thermal skin injury.

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