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Morphometric Analysis of Burn-Induced Gastric Mucosal Injury and Effect of Melatonin

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Curling's ulcers are acute gastric mucosal injuries and are among the complications that affect the gastrointestinal system after severe thermal trauma. Melatonin, a multifunctional molecule, exerts various gastroprotective effects. We examined and presented a semiquantitative evaluation of the effect of melatonin on burn-induced acute gastric mucosal lesions. Melatonin (10 mg per kg body mass) was administered immediately and 12 hours after skin injury in a rat burn model. The therapeutic potential of melatonin was examined using macroscopic and microscopic morphometric analysis. The erosion index (EI) and percentage of protection (PP) were calculated. The depth of erosion, epithelial necrosis, hemorrhage, and inflammation were also evaluated. Melatonin successfully decreased the scores of all detected macroscopic and microscopic alterations and had therapeutic effects in 69,32% of cases with burn-induced gastric mucosal injury. In conclusion, the administration of melatonin can effectively improve gastric mucosal injury after a burn.

Key words: melatonin, burns, gastric mucosa, erosion

Introduction

Severe thermal injury continues to be a serious health problem [31]. Burns involving more than 30% of the total body surface area (TBSA) are accompanied by multiple complications affecting almost all organs and systems and cause burn shock [31, 28].

Systemic response manifestations and the development of complications further worsen the condition of patients with severe burns. Therefore, these critically ill patients require intensive care, a multidisciplinary approach, and prevention of possible complications that could affect different organs and systems [36, 29].

The gastrointestinal tract (GIT) is among the systems that are affected after burn trauma. The injures affect all of its parts – stomach, small and large intestines [8]. Gastrointestinal (GI) complications are often observed in patients with more than 20% TBSA burns [32]. Burns increase the risk of damage to the mucous membranes and the formation of ulcers, known as Curling's ulcers [10].

Curling's ulcers are acute gastric mucosal lesions and are one of the most common visceral complications early after severe burns. This lesion has been recognized as a potentially life-threatening event in critically ill patients [40]. In the past, the incidence of Curling's ulcers was higher [24], but in recent decades it has been drastically decreased, due to improved therapeutic approach and prophylaxis [10]. Nevertheless, a retrospective study updated the data and found mucosal postburn injuries and determined that the frequency of gastric ulcer is greater compared to the frequency of duodenal and both ulcer types [10].

Stress-induced damage to the gastric and duodenal mucosa can be observed after physical trauma, shock, hemorrhage, and sepsis [16]. For this reason, stress gastritis is defined as end-organ failure of the stomach in critical illness [16].

Despite successful prophylaxis of Curling's ulcers, the presence of severe burns or other severe clinical conditions poses an increased risk of developing gastric complications. In some cases, critically ill patients do not respond to medication treatment and this condition requires surgical intervention to control bleeding [16].

Melatonin (n-acetyl-5-methoxytryptamine), indoleamine derived from tryptophan, is a hormone secreted primarily by the pineal gland in the brain. Clinical and experimental research have shown a plethora of therapeutic effects of melatonin as an antioxidant, anti-inflammatory, and antiapoptotic agent [9], which determine its organoprotective properties. Additionally, melatonin is a promising adjunctive drug in critical situations such as hemorrhagic shock [37], sepsis [15], and surgery [13]. Melatonin has manifested strong protective effects on various organs following burn injuries [1, 2]. Experimental studies have shown that melatonin has gastroprotective activity against gastric mucosal injury induced by ethanol [6], non-steroidal anti-inflammatory drugs [27] and stress conditions [4, 11].

The aim of this study is to present a semiquantitative evaluation of the gastroprotective effect of melatonin in burn-induced gastric mucosal injury in an experimental rat model. For this purpose, a macroscopic and microscopic morphometric analysis of the changes in the gastric mucosa was carried out.

Material and Methods

Animals

The experimental procedures were approved by the Home Office for Care and Use of Laboratory Animals and were performed with careful consideration of the ethics of animal experimentation according to the International Guiding Principles for Animal Research approved in Bulgaria (No. 90000088/2008) and Directive 2010/63/EU on the protection of animals used for scientific purposes.

Experimental design

We used age-matched male Wistar rats weighing between 220 and 250 g. Animals were housed at 20 °C and offered standard rat chow and water *ad libitum.* They were kept in dark/light cycles (DL = 12:12 h) in individual wire-bottomed cages. The lights were turned off at 8:00 p.m. and turned on at 8:00 a.m. to achieve a physiological photoperiod. Rats fasted for 12 hours and were allowed free access to water before injury.

Thermal injury and melatonin treatment

Twenty-four animals were randomly divided into three groups (n = 8 in each group) as follows: the control, i.e., the non-burned (C), the vehicle-treated burned group (B), and the melatonin-treated burned group (B + M). After light ether inhalation, general anesthesia was performed using thiopental (30 mg/kg i.p.). In order to accomplish a third-degree burn over 30% of the total body surface area (TBSA), hot boiling water (90°C) was applied to the back of the animals for a period of 10 s. For those rats that were subjected to burn injury, 4 mL of physiological saline was applied intraperitoneally (i.p.) for immediate resuscitation following burn injury.

Melatonin (N-acetyl-5-methoxytriptamine, Merck, Darmstadt, Germany) at a dose of l0 mg/kg body weight (b.w.) dissolved in vehicle (2% ethyl alcohol diluted in physiological saline to constitute 5 mL/kg i.p.) was administered. Melatonin and vehicle were applied immediately i.p. after burns in the morning between 8:00 and 9:00 a.m. and 12 hours after thermal skin injury. All animals were given buprenorphine (0.3 mg/kg i.p. b.w.) twice daily for post-burn pain control. No animals died during the observed period.

The animals were re-anesthetized with thiopental and sacrificed 24 hours after the burn trauma. Stomachs were immediately removed and incision was made along the greater curvature and irrigated with saline solution (0.9% NaCl). Two blinded observers assessed macroscopic and microscopic changes.

Macroscopic assessment of gastric mucosal injury

The severity of erosions is scored with a hand lens using the following arbitrary scoring system modified by the method described by Srivastava et al. (1991) [35]: Shedding of epithelium = 10; Petechial and frank hemorrhages = 20; One or two erosions = 30; More than two erosions = 40; Perforation = 50.

The mean erosion score for each animal was calculated and expressed as the erosion index (EI) [39]. Evaluation and an erosion index in groups are calculated: $EI = EN + ES + EPx10^{-1}$. Where: EN = Average of number of erosions per animal; ES = Average of severity score; EP = Percentage of animals with erosions.

The percentage of erosion protection (PP) was determined as follows [39]: % Protection = ((C – T)/C) x 100. Where: C = Mean erosion index in the negative control group (the vehicle-treated burned group); T = Mean erosion index in the treated group (the melatonin-treated burned group).

Histopathological analysis

Stomach tissue specimens were fixed in 10% buffered formalin (pH 7.2), dehydrated in an ascending series of ethyl alcohol (70%-100%), and cleared in methyl benzoate. After that, they were embedded in paraffin wax. Tissue sections of 5 μm were stained with hematoxylin and eosin (H&E) and examined using a light microscope

(Olympus BH-2, Tokyo, Japan). The presence of hemorrhages, epithelial necrosis, and inflammatory cells infiltration were evaluated using the following scale: 0 – no; 1 – low; 2 – moderate; 3 – high. The presence and depth of erosions were assessed using the following scale: 0- none; 1- up to 1/3 of the total thickness of the mucosa; 2 – up to 2/3 of the total thickness of the mucosa; 3 - more than 2/3 of the total thickness of the mucosa is involved [34].

Statistical analysis

GraphPad Prism (version 9.0, GraphPad Software, San Diego, CA, USA) was used to conduct all statistical analyses. Numerical data were expressed as mean values and standard error of means. A one-way ANOVA test followed by Dunnett's post-hoc test was applied to evaluate the significant differences between the mean values of studied groups. The results were considered significant for a p-value less than 0.05.

Results

Macroscopic assessment

The gastric mucosa in the control group is pale pink and has preserved mucosal relief (**Fig. 1 A**). In the burned group (B), there is pronounced hyperemia of the gastric mucosa with erosions, located mainly on the surface of the mucosal folds. The bottom of the erosions is brownish in color (**Fig. 1 B1, B2**). In the melatonin-treated group, there are fewer erosions and only in separate areas, the mucosa is hyperemic (**Fig. 1 C**). Evaluation of the macroscopic changes of the gastric mucosa are given in **Fig. 1** and **Table 1**.

Microscopic assessment

The gastric mucosa of the animals from the control group had a preserved histological structure. Our results showed the presence of erosions in the gastric mucosa in all animals in the burn group. Morphometric analysis showed a prominent depth of the erosions, accompanied by epithelial necrosis. With a possible maximum score of 3.0, the average mean values were 2.75 ± 0.164 and 2.25 ± 0.25, respectively. Hemorrhagic changes were observed in three of the animals in the experimental group (37.5%), with a mean score of 0.375 ± 0.183. Thermal injury caused a prominent inflammatory reaction with a morphometric score of 1.625 ± 0.183.

Melatonin administration lowered all morphometric scores. The mean scores of the depth of erosions and epithelial necrosis were reduced by 54,55% ($p<0.0001$ compared to the burn group) and 66.67% ($p <0.0001$ compared to the burn group), respectively. Melatonin has a prominent effect on hemorrhagic changes, which were completely abolished in the treated group ($p = 0.0374$ compared to the burned group). Although not significantly, melatonin reduced the inflammatory infiltrate in the gastric mucosa compared to that observed in the burn group (1.25 ± 0.164). These results indicate the beneficial effect of the treatment on the maintenance of the integrity of the tissue structure. Microscopic alterations in gastric mucosa are summarily presented in **Fig. 2** and **Table 2**.

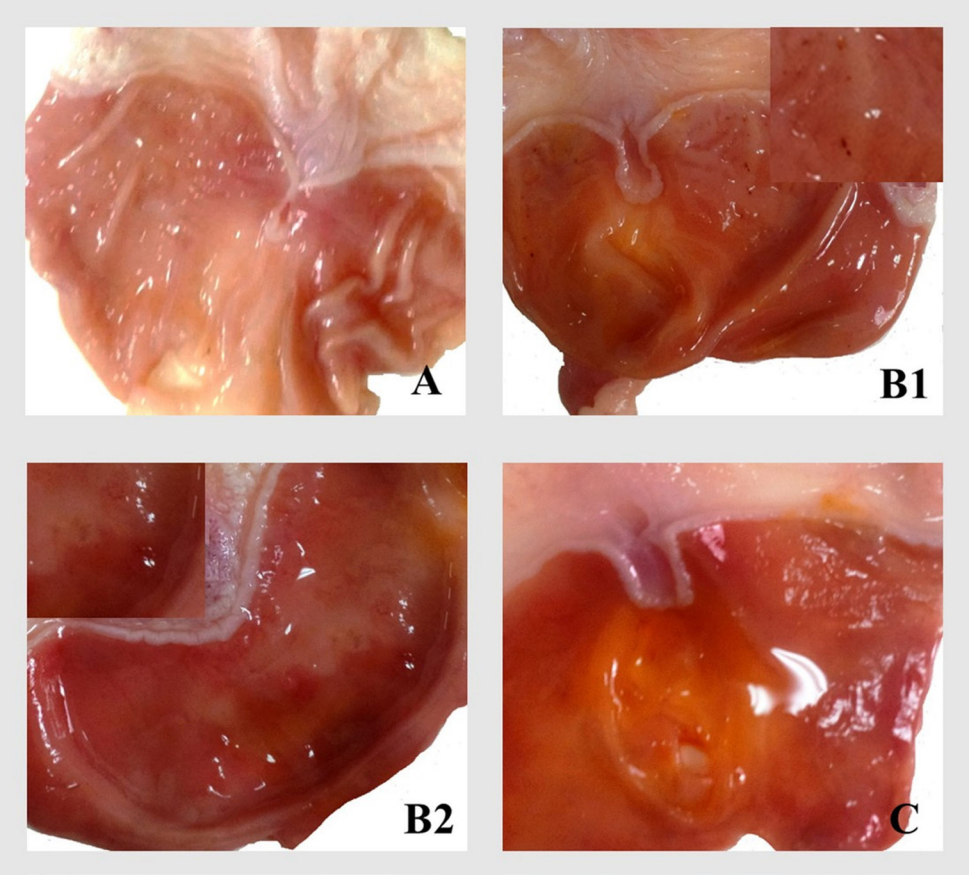

Fig. 1. Macroscopic changes in the gastric mucosa and the effect of melatonin in burn injury. Controls (A); burned rats (B1, B2); burned rats, treated with melatonin (C). Representative data.

Table 1. Melatonin effect on the macroscopic changes of the gastric mucosa after severe thermal trauma. Results are expressed as the mean ± SEM; **** $p < 0.0001$ vs. control group; * $p < 0.05$ vs. control group; ‴ $p < 0.005$ vs. burned, non-treated group. Controls (C); burned rats (B); burned melatonin-treated rats (B + M).

Group	ES	EN	EI	PP/ [%]
C	0.0	0.0	0.0	
B	45 ± 3,273****	5,125 ± 1,172****	50,13 ± 3,652****	
B + M	15 ± 7,319‴	0,375 ± 0,375‴	15,38 ± 7,651‴	69,32

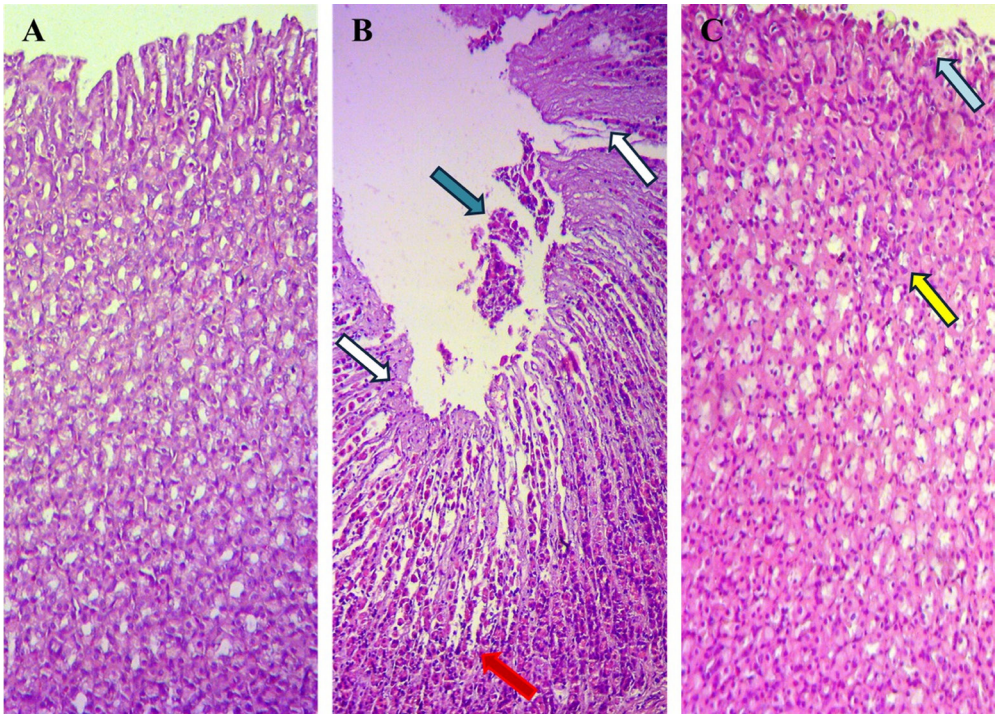

Fig. 2. Histopathological changes in the gastric mucosa and the effect of melatonin in burn injury. (A) Control group – the architecture of the gastric mucosa is preserved. (B) Burn group – erosion of the gastric mucosa with cellular debris (blue arrow), area of necrosis (white arrow), and underlying degenerative changes of the epithelial cells (red arrow). (C) Group with burn and melatonin treatment – normal preserved architecture of the gastric mucosa with desquamation of the covering epithelium (light blue arrow) and inflammatory infiltrate (yellow arrow) in the lamina propria of the mucosa. Representative images. Hematoxylin and eosin staining, magnification, 200x.

Table 2. Melatonin effect on scores of histopathological parameters in gastric mucosa after severe thermal trauma. Results are expressed as the mean ± SEM; **** $p < 0.0001$ vs. control group; * $p < 0.05$ vs. control group; †††† $p < 0.0001$ vs. control group; † $p < 0.05$ vs. control group; ▪▪▪▪ $p < 0.0001$ vs. burned, non-treated group; ▪ $p < 0.05$ vs. burned, non-treated group. Controls (C); burned rats (B); burned melatonin-treated rats (B + M).

Group	*Depth of erosion*	*Epithelial necrosis*	*Hemorrhage*	*Inflammatory infiltrate*
C	**0.0**	**0.0**	**0.0**	**0.0**
B	**2.75 ± 0.164******	**2.25 ± 0.25******	**0.375 ± 0.183***	**1.625 ± 0.183******
B + M	**1.25 ± 0.164$^{††††▪▪▪▪}$**	**0.75 ± 0.164$^{†▪▪▪▪}$**	**0.0$^{▪}$**	**1.25 ± 0.164††††**

Discussion

Acute postburn gastric mucosal injuries vary in degree of damage and can clinically manifest differently- from erosive gastritis, as asymptomatic lesions and occult GI bleeding to aggressive clinically significant GI bleeding, anemia, and death [29]. Every clinical manifestation is a sign of disrupted tissue homeostasis with limited survival strategies and a prevalence of processes that cause cell death [17].

This experimental rat model was used to monitor macroscopic and morphological changes in the gastric mucosa and the effect of intraperitoneally administered melatonin. Full-thickness skin injury results in damage to the stomach after 24 hours. Direct examination revealed changes in the mucosa in all animals exposed to burn, which proves the high risk of organ involvement in this type of trauma. The calculations based on the number of visible erosions, average values of the erosion severity, and the percentage of animals with erosions determined a high erosion index in the burn group. Light microscopic examination of the tissue samples showed that the depth of erosions, epithelial necrosis, hemorrhages, and inflammatory infiltrate were high in the burn group and corresponded to the results of the macroscopic examination.

Multiple factors are involved in the pathogenesis of stress ulcers, which contribute to the high mortality rate in critically ill burn patients [21]. Severe burn causes systemic stress and initiates a cascade of events, that compromises the blood perfusion to the gastrointestinal mucosa [29]. As a result, tissue hypoperfusion and hypoxia occur and lead to disrupted energy metabolism [40]. The production of gastroprotective factors (prostaglandins, bicarbonate, mucus) decreases, and this results in the development of multiple superficial erosions of the gastric mucosa due to loss of the integrity of the mucosal barrier [29]. Microcirculatory disorders are a trigger factor for cellular destructive processes such as oxidative stress and apoptosis [5, 18, 19, 21].

In our study, melatonin significantly reduced gastric mucosal injury. Macroscopic analysis showed a markedly reduced number and severity of erosions in the gastric mucosa, which leads to a reduction in the erosion index compared to the burn group. We also found a similar beneficial effect of melatonin on microscopic tissue changes. We observed a reduction of inflammatory cells infiltration and a significant limitation of the depth of erosions and the manifestations of epithelial necrosis and hemorrhages in animals treated with melatonin compared to those without treatment.

The change in macroscopic and microscopic scores can be explained by the properties of melatonin as a multifunctional molecule with a prominent pleiotropic biological action [12, 23]. Melatonin elevates the antioxidant capacity and increases the expression of antioxidant enzymes Cu/Zn superoxide dismutase [20] and heme oxygenase-1 [18] and suppresses lipid peroxidation in gastric mucosa postburn injury [5, 19]. Moreover, melatonin effectively ameliorates burn-induced gastric mucosal injury and modulate apoptosis through the expression of Bcl-2 family proteins [19].

The beneficial effects of melatonin on the gastric mucosa are not limited to the antioxidant and antiapoptotic action of the indole. Exogenously administered melatonin and its precursor L-tryptophan, are mucoprotecors that contribute to ulcer healing and enhance the microcirculation at the ulcer margins [7].

It is known that physical stress is the immediate response of the patient after a major thermal injury [30, 22]. Melatonin administration suppresses catecholamine synthesis [25] and significantly attenuates the adrenocortical secretory response to acute

and chronic stress [26]. In light of these facts, it may be considered that melatonin, by influencing acute stress, may have a beneficial effect on the organism, including the gastrointestinal system.

According to modern guidelines, proton pump inhibitors (PPIs) and histamine-2 receptor antagonists are used for Curling's ulcer prophylaxis [29]. The duration of stress ulcer prophylaxis is different [33]. PPIs are known to have adverse effects in short-term use, and especially in long-term use [38, 33]. In this regard, the low toxicity of melatonin [14] defines it as a molecule with promising therapeutic potential. In addition, co-treatment of rats with melatonin and ranitidine or omeprazole protects against stress ulceration in doses at which either of these alone could not protect the stomach [3].

Despite good management, Curling's ulcers remain a cause of considerable morbidity and mortality [29]. Therefore, response to treatment, prevention of ulcer recurrence, or ongoing gastrointestinal complications are essentially important for clinical practice [29].

Conclusions

The present study reveals a significant manifestation of the pathological changes in the gastric mucosa during burn. Melatonin successfully reduces both macroscopic and microscopic alterations. Melatonin provides a high level of protection and limits processes in the mucosal layer of the stomach wall. In this way, it can prevent the development of gastric ulcers and more serious postburn complications requiring surgical intervention.

These findings may inspire future research on the use of melatonin, alongside standard pharmacological agents, to prevent Curling's ulcers, acute gastric injuries from various causes, and other gastrointestinal diseases where oxidative stress, apoptosis, and inflammation play a key role in their pathogenesis.

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