# ANNALES

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# *Effect of pro-inflammatory cytokine-mediated mechanism on quality of gastrointestinal restitutio ad integrum*

Wpływ mechanizmu prozapalnego mediowanego cytokinami na jakość restytucji żołądkowo-jelitowej *ad integrum*

## INTRODUCTION

The existing treatment of acid-related diseases is mostly based on potent acid suppression. Recent studies have revealed that the widespread use of prolonged acid suppression is associated with dismotility in the upper gastrointestinal (GI) tract, hypergastrinemia, dysbiosis [1]. Chronic gastroesophageal reflux disease (GERD) bears the risk of Barrett's esophagus that is a premalignant stage of esophageal adenocarcinoma. WHO warns that the risk of Barrett's esophagus increases by 30 to 50% per decade of life in patients with GERD. Inflammatory reaction is central to the majority of manifestations of GERD and a starting point for abnormal *restitutio ad integrum*. *Restitutio ad integrum* is a total recovery performed by natural healing systems of an organism and the key characteristic of the GI epithelial barrier [6, 11]. Melatonin (MT) may take part in protecting the esophagus from tissue damage [2, 7]. It is well known that melatonin possesses GI resistance to luminal damaging agents due to the free radical scavenging cascade, vasodilatatory and cytoprotective effects [4, 5, 9]. The main role in the *restitutio ad integrum* is played by intercellular and apical glycoconjugates. They represent a signaling system of cell functioning. The aim of our study was to examine if inflammatory signaling contributed to the esophageal phenotype of cytoprotection and healing activity during MT pretreatment.

## MATERIAL AND METHODS

Male Wistar rats which were used in our studies were divided into five groups. One was the intact control group. The other rats were inducted erosive esophageal acidic lesions by perfusion solution of 0.25 N HCl with pepsin and non-erosive esophageal mucosa (EM) lesions by water-immersion restraint *stress* (WRS) by Takagi, 1964 without and with melatonin (20 mg/kg/ip) treatment. The

healing process of EM was monitored after 24h of injury. To estimate the damage, inflammation and hyperplasia of the EM and lectin histochemistry examination, segments of the lower third of esophagus were extracted from the animals body. A score system was used for estimation of the degree of epithelial changes. The experiment included revealing of both the macroscopic morphological changes and histological changes. To access the glycoconjugates of EM lectin sets were used. The lectin set included using peanut agglutinin (PNA, specific to BDGal→DGalNAcDGal), Sambucus nigra agglutinin (SNA, specific to Neu5 Ac(2-6)Gal), Helix pomatia agglutinin (HPA, specific to DGalaNAc), wheat germ agglutinin (WGA, specific to DGlcNeuNAc) conjugated to peroxidase (purchased from Lectinotest Lab, Ukraine). Images of histological slices were investigated using a digital video camera connected to a microscope (MBI-15-2, LOMO, Russia) and were processed using the AVerMedia FZC Capture image analysis program (AVerMedia Technologies, Inc., USA). To study the functioning of the proinflammatory signaling mechanism in the serum; IL-1beta, TNFalpha cytokines were examined by enzyme-linked immuno sorbent assay (ELISA) by kits "Diaclone" (France). For a comparison of data, we used a paired Newman-Keuls's test with the level of significance at $P< 0.05$.

## RESULTS

In the groups of rats with the MT pre-treatment less damage to EM, hyperplasia of basal membrane and akantosis was revealed. There was a variety of expressions and epithelial patterns of lectins (PNA, SNA, WGA) in different groups of rats. Generally, the lectin expression decreases after acidic perfusion in different epithelial layers, but it increases in rats with the MT pretreatment by modification of glycoprotein pattern in EM. These changes are accompanied by increased activity of mucus-producing cells. Lectin expression in NEE inducted by stress is different compared to the expression caused by acidic injury. PNA expression is weaker, but SNA expression is stronger in different epithelial layers. With melatonin pretreatment the most intense PNA expression was found in stratum spinosum and basal cells. The strongest SNA expression was found in the basal layer. WGA expression shows no visible differences. Moreover, changes in carbohydrate moieties of glycoconjugates revealed MT-induced activation of cytoprotection mechanism that caused marked thinning of esophageal epithelium and a decrease of papillary due to faster luminal cell migration and more rapid epithelial regeneration after injury. In the control group, IL-1beta content was 24.16±0.30 pg/ml, TNFalpha – 1.05±0.11 pg/ml. Induction of acid-peptic esophageal lesions led to increased IL-1beta to 55.35 ± 7.88 pg/ml, TNFalpha – 5.56 ± 0.55 pg/ml ($p <0.05$). Melatonin treatment reduced IL-1beta to 46.4% and TNFalpha – 16.2% in comparison to the previous group ($p<0.05$). In WRS-induced EM lesions IL-1beta increased to 38.7%, TNFalpha – 65.6% relatively to control ($p <0.05$). Melatonin caused a decrease of IL-1beta to 21.8%, TNFalpha – 35.1% compared to the control. Acidic perfusion induced topical destructive EM lesions and HSI was 2.5-folds time more than with melatonin treatment; WRS-induced non-erosive esophagitis was with constantly increased HSI in 150% in comparison to melatonin treated rats showing the same effect as in the model of acidic esophagitis. These changes show an obvious anti-inflammatory effect of melatonin.

## DISCUSSION

MT, potent antioxidant, vasodilatatory agent is intensively studied in recent investigations, especially in experimental and clinical gastroenterology. Prolonged inflammation is the key factor in abnormal *restitutio ad integrum* which caused metaplasia in foregut epithelial barrier [3, 8]. The presented *in vivo* studies demonstrated in two experimental models of esophageal injury that acidic-pepsin erosive esophagitis and stress-induced non-erosive esophagitis that MT strongly exhibits anti-inflammatory activity via decreased synthesis of the IL-1beta TNFalpha. The exposed phenomenon regarding changes of the esophageal barrier induced during MT treatment healing indicate modification of the synthesis of DGlcNeuNAc, Neu5 Ac(2-6)Gal, DGalaNAc specific glycoconjuges associated with signs of hyperkeratosis, hyperproliferation and local microcirculatory lesions. MT exerts an anti-inflammatory effect on the EM, particularly on inhibition leukocytes infiltration, which stimulate the quality of *restitutio ad integrum.* Also, important changes in the EM expression of mucin and in their glycosylation state were shown in this stress-induced studies of non-erosive esophagitis because they are closely associated with the development silent signs of precancer-related processes such as Barrett esophagus. In this study we established the phenotype of esophageal epithelial barrier profile of glycoconjugates during the healing process is a result of involvement of glycosylation in the essential biological process of inflammation [10] and modification of glycoconjugates are novel valid biomarkers of it. Of course, these investigations did not elucidate the full mechanism of *restitutio ad integrum*. In fact, future studies with molecular tools are necessary for identification of the signaling pathways of aberrant glycosylation. Moreover, the glycoconjugates profile with pre-malignant esophageal tissue (during Barrett esophagus) shoud be investigated.

In summary, the results of the present study indicate that the quality of *restitutio ad integrum* of erosive and non-erosive esophagitis is related to pro-inflammatory cytokine-mediated mechanism, which is the basis for the formation phenotype of EM protective responses. MT, perspective and potent anti-inflammatory agent, would be beneficial to antiulceric and healing action.

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## REFERENCES

1. Baatar D., et al.: Esophageal ulceration triggers expression of hypoxia-inducible factor 1α and activates vascular endothelial growth factor gene: implications for angiogenesis and ulcer healing. Am. J. Pathol., 161, 1449, 2002.
2. Bubenik G.A.: Therapeutic perspectives of gastrointestinal melatonin. Melatonin: from molecules to therapy. Eds: R.S. Pandi-Perumal, D.P. Cardinali, Nova Publishers 2006.
3. Carrillo-Vico A., Guerrero J.M., Lardone P.J., Reiter R.J.: A review of the multiple actions of melatonin on the immune system. Endocrinology, 27, 189, 2005.
4. De Filippis D., et al.: Melatonin reverses lipopolysaccharide-induced gastro-intestinal motility disturbances through the inhibition of oxidative stress. J. Pineal. Res., 44, 45, 2008.
5. Erren T.C., Reiter R.J.: Light Hygiene: Time to make preventive use of insights--old and new--into the

nexus of the drug light, melatonin, clocks, chronodisruption and public health. Med. Hypoth., 73, 537, 2009.
6. Konturek S.J., Zayachkivska O.S., Brzozowski T. et al.: Protective influence of melatonin against acute esophageal lesions involves prostaglandins, nitric oxide and sensory nerves. J. Physiol. Pharmacol., 58, 371, 2007.
7. Konturek S.J., Konturek P.C., Brzozowska I., Pawlik M.: Localization and biological activities of melatonin in intact and diseased gastrointestinal tract (GIT). J. Physiol. Pharmacol., 58, 381, 2007.
8. Mayo J.C., Sainz R.M., Tan D.X., et al.: Anti-inflammatory actions of melatonin and its metabolites, N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK) and N1-acetyl-5-methoxykynuramine (AMK) in macrophages. J. Neuroimmunol., 165, 39, 2005.
9. Motilva V., Cabeza J., Alarcon de la Lastra C.: New issues about melatonin and its effect on the digestive system. Curr. Pharmaceut. Design., 7, 909, 2001.
10. Perianayagam M.C., Oxenkrug G.F., Jaber B.L.: Immune-modulating effects of melatonin, N-acetylserotonin, and N-acetyldopamine. Ann. N.Y. Acad. Sci., 1053, 386, 2005.
11. Zayachkivska O., Gzhegotsky M., Yaschenko A. et al.: Effects of nitrosative stress and reactive oxygen-scavenging systems (ROSS) in esophageal physiopathy under streptozotocin-induced experimental hyperglycemia. J. Physiol. Pharmacol., 59, 77, 2008.

## SUMMARY

We studied the morphofunctional changes in esophageal mucosa after the experimental injury by acid-pepsin perfusion and stress inducted injury with and without melatonin treatment. Melatonin-treatment enhanced epithelial proliferation and accelerated the healing activity of the esophageus tissue. Melatonin, a perspective and potent anti-inflammatory agent, can be used with antiulceric and healing scope.

*Keywords:* esophageal mucosa, melatonin, ulcer

## STRESZCZENIE

Oceniono zmiany morfotyczno-funkcjonalne w śluzówce przełyku po jej eksperymentalnym uszkodzeniu pod wpływem perfuzji kwasowo-pepsynowej i uszkodzeniu indukowanym stresem bez leczenia i po leczeniu melatoniną. Podawanie melatoniny zwiększało proliferację nabłonka i przyspieszało proces zdrowienia tkanki przełyku. Melatonina, potencjalny środek przeciwzapalny, może być stosowana w celu zapobiegania rozwojowi wrzodów jak też w celu ich leczenia.

*Słowa kluczowe:* śluzówka przełyku, melatonina, wrzód