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Novel aspects of melatonin-induced defence on oral mucosa during experimental erosive esophagitis

Nowe aspekty indukowanej melatoniną obrony śluzówki jamy ustnej
w trakcie doświadczalnego nadżerkowego zapalenia przełyku

Mucosa of oral cavity and esophagus is subject to environmental factors and repetitive deformation of the passage of luminal nutrients and retrograde refluxate injury. Animal experiments and studies of human mucosa of foregut demonstrated that cytoprotection is a multiplayer process [1]. It involves various mucosal components: epithelial, endothelial, lymphoid, neuroendocrine (NE) and muscle cells, fibroblasts and extracellular matrix, and different players: cytokines, chemokins, defensins, PG/COX and NO/NOS signaling pathways, adhesion molecules, and the process of apoptosis. The upper part of digestive system is rich in NE cells. 50 years after the discovery melatonin (MT) by Dr. A. Lerner and coworkers as pineal gland hormone, it is known that this indolamine endogenously produced by NE cells exerts a wide range of biological effects on gastrointestinal neuromodulation, motility, mucosal defence, inflammation and secretory function [5]. MT may take part in protecting the oral cavity from tissue damage due to oxidative stress [3], and it may contribute to the regeneration of alveolar bone through the stimulation of type I collagen fiber production and the modulation of osteoblastic and osteoclastic activity [7, 11]. Precise molecular mechanisms of oral mucosal dysfunction, important implications for pathogenesis of dental disorders, especially in periodontal disease, are still unclear and the effect of MT on oral mucosa lesions (OML) induced during esophageal injury has not been studied. We examined the hypothesis that MT overcome the damaging effect of exogenous acid-pepsin injury on the upper part of the digestive system and implicated for OML healing and cell proliferation and the mechanisms involved in this action.

MATERIAL AND METHODS

This study was carried out with accordance to the statements of Helsinki Declaration and Bioethical Committee of European Union regarding handling of experimental animals. The animals were fasted for 24 h prior to the experiments but they were allowed free access to water. All experiments were performed at the same day time. EE were induced in male adult Wistar rats per seven days of acid-pepsin perfusion with a tube inserted through the oral cavity with the tip in the esophagus. The following experimental groups were included in the study: control, with or without inhibition PG/COX activity by intraperitoneally indomethacin (INDO) in the dose 2 mg/kg/day and MT–20 mg/kg/day pretreatment. All compounds were purchased from Sigma. The OML and esophageal injury was assessed by score systems of microscopic histological changes of the epithelial

loss, thickness of the basal cell layer (BCL), length of the papilla (PL), severity of intercellular space dilations (DIS) and presence of intraepithelial leucocytes infiltration (LI) and submucosal disorders via hematoxylin-eosin staining. The content of plasma final products of NO (NO_n) was determinated by generally accepted Grease's reagent. The statistical analysis used the values expressed as means ± SEM and variance followed by Newman-Keuls aposterioric test. P values of less than 0.05 were considered as statistically significant.

RESULTS

Plasma NOn levels in vehicle-saline treated control rats averaged 28.8±3.1 and esophageal acid-pepsin perfusion raised this level to about 39.4±4.0 (μmol/L). In rats pretreated with indomethacin the plasma levels of NOn drop significantly above those observed in control group. Plasma NOx levels were also significantly increased in rats treated with melatonin (41.1±1.8 μmol/L), but they showed a significant decrease after inhibition of COX/PG system with INDO (18.4±3.5 μmol/L). Seven days' esophageal perfusion induced clearly delineated deep esophageal mucosal defects (erosions and ulcers), but their mean area was twice smaller than those observed in rats with exogenous INDO injections. Assessing PL, DIS, ILI of oral mucosal samples obtained from rats with EE was judged as important, leading to additional information of foregut cytoprotection. It is of interest that microscopically epithelial OML induced by EE were also more pronounced in INDO-treated rats than those in animals without such pretreatment. Increased DIS in OML in INDO-treated rats indicated increased epithelial permeability. The quantative assessment of the signs of irregular hyperemia, stasis, restricted perivascular diapedesis with hemorrhage and focal edema in submucosa were significantly less severe in MT-treated animals than in those receiving cotreatment with INDO. Then normal size of PL was present in the mucosa covering the granulation tissue, but the thickness of the epithelial basal zone in MT-treated rats was decreased in comparison to the than control.

DISCUSSION

Disorders of the oral mucosal epithelium are multifactorial and may be attributable to both extrinsic factors (eg. dentures) or changes (eg. vitamin B_{12}/folate deficiency) and intrinsic factors such as endothelial dysfunction or oxidative stress or physiological drop of melatonin secretion, etc [3, 2]. Esophageal lesions are heterogeneous disorders and the extraesophageal manifestations are even more difficult to diagnose because they are typically associated with a negative endoscopic finding [4, 10]. Clinical studies evaluated the relationship between alteration of oral mucosa and the condition of morpho-functional integrity of esophageal mucosa [9]. In this study we demonstrated that experimental esophageal injury induced by acid-pepsin perfusion is associated with OML. We analyzed whether OML is mediated by acidic esophageal injury with implication PG/COX and NO/NOS signaling pathways and for the first time melatonin-induced healing is shown on oral mucosa during EE. Dysfunction of the normally protective endothelium is a key component in different gastroenterological diseases; however, in oral disorders it remains unclear. Endothelial dysfunction includes a pro-inflammatory, pro-coagulant and proliferative condition. NOS dysfunction affects NO production and increasing the concentration of oxidants such as superoxide and hydrogen peroxide, a phenomenon described as "NOS uncoupling" [5, 12]. It was recently revealed that high levels of NO were found to be generated in the esophageal lumen during reflux esophagitis as a result of reduction salivary nitrite content by acid [7]. Our previous studies showed that melatonin possessed

vasodilatatory, anti-inflammatory, antioxidant effects [14]. The presented studies demonstrated that melatonin strongly improved local blood flowit, prevened ischemia, decreased ILI and epithelial alteration via modulatory effect on NO/NOS and PG/COX signaling pathways. The identification of biological markers of endothelial dysfunction could help in an early diagnosis of OML and may represent a preferable novel therapeutic approach in the treatment of oral disorders.

Results of the present study indicate that esophageal ulceration triggers induction of modification activity of NO/NOS and PG/COX signaling pathways is an essential component for oral mucosa defence. Our demonstration that melatonin accelerates healing of experimental oral mucosal lesions due to a decrease of endothelial dysfunction may provide a rationale for future preclinical studies aimed at evaluating melatonin for the treatment oral disorders.

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SUMMARY

We studied the morphofunctional changes in oral mucosa lesions during the experimental erosive esophagitis induced by acid-pepsin perfusion and modification of PG/COX activity and melatonin treatment. Melatonin-treatment enhanced epithelial proliferation and accelerated healing in foregut mucosa. Melatonin is a likely mediator of oral mucosa cytoprotection and ulcer healing due to diminished endothelial dysfunctions.

STRESZCZENIE

Badano zmiany morfologiczno-czynnościowe śluzówki jamy ustnej w przebiegu doświadczalnego nadżerkowego zapalenia przełyku, indukowanego w efekcie perfuzji kwasowo-pepsynowej modyfikacji aktywności PG/COX i po leczeniu melatoniną. Leczenie melatoniną zwiększało proliferację nabłonka i przyspieszało zdrowienie śluzówki. Melatonina wydaje się mediatorem procesów cytoprotekcyjnych śluzówki i zdrowienia wrzodów w efekcie zmniejszenia dysfunkcji nabłonka.