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Modification of glycoconjugates signaling pathway during esophageal barrier injury induced by experimental hypergastrinemia

Modyfikacja szlaku sygnałowego glikokonjugatów podczas uszkodzenia bariery przełykowej indukowanego doświadczalną hypergastrynemią

A prolonged gastroesophageal reflux is characterized by a change from normal squamous to columnar intestinal-type epithelium and is referred to as a sign of Barrett's esophagus and esophageal adenocarcinoma [1]. Therapies with acid suppressive agents, the proton pump inhibitors (PPIs) directly inhibit hydrogen ion exchange and acid secretion in response to all stimulatory agents and resulting hypergastrinemia. Clinical and rat model studies revealed the controversial role of plasma hypergastrinemia in gastrointestinal carcinogenesis [3]. Our previous studies showed that cell-surface-bound and secreted glycoconjugates from epithelial and other mucin-producing cells participate in the processes of epithelial permeability, leukocyte trafficking, angiogenesis, coagulation, as well as in regulation of apoptosis [6, 10]. Gastrin has been described as a growth factor for proliferation via both endocrine and autocrine/paracrine mechanisms [8].

The aim of the present study was to evaluate the effect of hypergastrinemia induced by omeprazole (OM), a PPIs, on esophageal mucosa by the investigation of the glycobiological characteristics of the rat esophagus as the indicator of morpho-functional integrity of the epithelial barrier.

MATERIAL AND METHODS

Male Wistar rats, weighing 180–220 g and fasted for 24 h with free access to water were used in our studies. All experimental procedures followed A Guide for Care and Use of Laboratory Animals. The rats were divided into 8 groups. For induction of hypergastrinemia, OM purchased by Doctor Reddis Laboratories Ltd, India was used in the dose of 14 mg/kg/day, intraperitoneally, with different duration of its administration: 7; 14; 21; 28 days. The other four groups served as a control for the corresponding groups. On finishing the experiment, blood was tested to define the concentration of plasma gastrin. Plasma gastrin was defined by radioimmunological method of the firm MP Biomedicals, LLC USA. For microscopic analysis, segments of the lower third of esophagus were used for the routine histological examination with hematoxilin-eosin staining and the lectin histochemistry. 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). Lectin labeling was visualized with diaminobenzidine (DAB) in PBS as described previously [10]. 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) and carried out by a semi-quantitative optical analysis, taking into account the intensity, indicated as absent (-), weak (+), moderate (++) or intense (+++). A statistical analysis was performed according to the semiquantitative scale and expressed as means ± SEM. For a comparison of the data we used a paired Newman-Keuls's test with a level of significance at $P< 0.05$.

RESULTS

It was determined that OM treatment in rats for 7 days caused the increase of plasma gastrin level from 59±35.5 pg/ml in comparison to the control of 181.18±59.68 pg/ml ($p<0.01$). While increasing the duration of OM injection to 14 days, plasma gastrin tended to rise; however, such changes were statistically insignificant. Continuation of duration of OM-treatment to 21 days caused an increase of plasma gastrin to 136.3±61.7 pg/ml ($p<0.01$). It should be noted that after 28 days of OM administration the concentration of plasma gastrin reached the average of 170.7±90.7 pg/ml ($p<0.05$) after 28 days. The conducted morphological investigation showed that the results of the continuous trophicus influence of gastrin (28 days) on the esophageal mucosa were exfoliation of the surface layer of epithelium, thinning of the epithelial layer, in special cases hydropic dystrophia of epitheliocytes. It is known from the data in medical literature that gastrin causes growth and proliferation of the cells of oesophageus mucous membrane, an increase in the number and hyperplasia of parietal cells, a change in the width of the mucous membrane [2], appearance of epitheliocytes with hypertrophicus nuclei and the symptoms of metaplasia (the transformation of columnal epitheliocytes into typical epitheliocytes with the formation of the main cellular layers peculiar to the epidermis of skin [3]. In the direction from basal to granular layer the vacuolization of cytoplasma and the pycnosis of nuclei were traced. Generally, we revealed an accelerated process of keratosis, intensification of keratinisation in the epithelial layer with accumulation of keratogialin in cellular cytoplasma. In the vasodilatation with signs of hemorrhage and mild leukocyte infiltration was observed. Intermittently, in the vascular lumens hyperaggregates of erythrocytes were found and prevalence of the signs of epithelial proliferation into lamina. Also, disorganization of fibrous structures was detected in the submucosa. The lower third of esophagus showed differential lectin-binding patterns in all parts of multilayered, stratified squamous epithelial barrier of esophageal mucosa in intact and studied groups of rats. Group 1 and group 8 (28 days' hypergastrinemia induced by IPP) revealed a marked heterogeneity of PNA, HPA, SNA and WGA binding profile of rat esophageal mucosa. The analysis of expression receptors of PNA, HPA, SNA and WGA labeling in esophageal epithelial barrier from rats of control group and 28-days' hypergastrinemia is presented in Table 1.

Table 1. Peculiarities of lectin cytotopography in the rat esophageal mucosa during 28-days' hypergastrinemia

Experimental Group	Control				Hypergastrinemia			
Esophageal mucosa / Lectins and their specification of carbohydrates	epithelial lamina			lamina propria	epithelial lamina			lamina propria
	basal layer	spinosum layer	superficial layer		basal layer	spinosum layer	superficial layer	
Peanut agglutinin, PNA, βDGal specific	-	+	+++	+	+	+	+++	++
Helix pomatia. agglutinin, HPA, DGalαNAc specific	-	-	+	-	+	+	+++	+ Intra-vascular RBC
Sambucus nigra, SNA, Neu5 Ac (2-6) Gal specific	+++	++	-	?	+	+	+++	+
Wheat germ agglutinin WGA, DGlcNAc specific	+++	+	+++	+++	+++	++	+++	+ Intra-vascular RBC & inner elastic membrane

Indication of (+++) – intensive; (++) – mild; (+) – weak; (-) – absent reactions

DISCUSSION

IPPs, potent antisecretory agents are intensively used to treat acid related disorders, especially gastroesophageal reflux. Prolonged inhibition of acid secretion by IPPs caused malfunction of foregut epithelial barrier by hypergastrinemia [8, 9]. Gastrin acts via the cholecystokinin type-2 receptor (CCK-2R), a member of the 7-transmembrane domain G-protein-coupled receptor superfamily. A novel splice variant named CCK-2Ri4sv has recently been described, which exhibits constitutive activation [7]. Gastrin exerts a trophic effect on the oxyntic mucosa, particularly on ECL cells, which are stimulated to replicate via gastrin/CCK-2 receptors expressed in ECL cells. An important downstream effect of CCK-2R activation is the phosphorylation/activation of the potent antiapoptotic factor, protein kinase B (PKB)/Akt [2]. Once phosphorylated PKB/Akt can itself inactivate a range of proapoptotic factors, including caspase-9, Bad, and the forkhead/winged-helix transcription factors important in the transcription of the cell death ligand Fas, as well as activating the antiapoptotic inhibitor κB kinase cascade [5]. In this study we established an esophageal epithelial barrier profile of glycoconjugates under hypergastrinemia as one of the most abundant defensive modifications. Changes of expression PNA, HPA, SNA and WGA lectin receptors observed in our study in esophageal barrier during hypergastrinemia is a result of involvement of glycosylation in a variety of important biological processes, such as morpho-functional signal transduction, cell growth, differentiation, adhesion, invasion, and immune surveillance. It is also known that changes in the expression of mucin and in their glycosylation state are closely associated with the development of cancer and cancer-related processes such as Barrett esophagus [4]. Of course, these investigations did not elucidate the mechanism of carcinogenesis. The demonstrated phenomenon regarding changes of the esophageal barrier induced by hypergastrinemia indicate accelerated synthesis of Neu5 Ac (2-6) Gal, DGlcNAc, βDGal specific glycoconjuges associated with signs of hyperkeratosis, hyperproliferation and local microcirculatory lesions. In fact, future studies with molecular tools are necessary for identification of the signaling pathways of aberrant glycosylation. Moreover, the glycoconjugates profile with malignant esophageal tissue shoud be investigated.

In summary, the results of the present study indicate that hypergastrinemia triggering induction of the defensive reaction of modification glycoconjugates signaling pathways is an essential component for esophageal barrier integrity.

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SUMMARY

The article presents lectinohistochemistry results of continuous 28 days' trophicus influence of gastrin on the esophageal mucosa, induced by daily administration of omeprasol, the potent proton pump inhibitor. Three-fold hypergastrinemia triggering induction of the defensive reaction of modification glycoconjugates signaling pathways is an essential component for esophageal barrier integrity. Evaluated changes of esophageal microcirculation and disorganisation in lamina were tracted as hyperproliferation.

STRESZCZENIE

W artykule przedstawiono wyniki badań lektynohistochemicznych śluzówki przełyku po 28-dniowej hypergastrynemii indukowanej podawaniem omeprazolu, silnego inhibitora pompy protonowej. Hypergastrynemia powodowała indukcję obronnej reakcji modyfikacji szlaku sygnałowego glikokonjugatów, będącej istotnym składnikiem integralności bariery przełykowej. Oceniane zmiany mikrokrążenia przełykowego i dezorganizacja blaszki zostały zakwalifikowane jako hyperproliferacja.