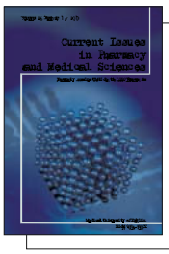

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Current Issues in Pharmacy and Medical Sciences

Formerly ANNALES UNIVERSITATIS MARIAE CURIE-SKLODOWSKA, SECTIO DDD, PHARMACIA
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Postprandial hyperglycemia changed fucosylated pattern of the oesophageal epithelial barrier activity through the nitrogen oxide mechanism

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ABSTRACT

The present study was designed to evaluate the role of postprandial hyperglycemia (PHG) on oesophageal epithelial barrier (OEB) integrity via evaluation expression of fucosylated glycans by PFA and LABA labeling and mechanism in formation PHG induced pre-ulcer lesions through the NO/NOS activity in OEB and therapeutic potential and mechamism of L-Tryptophan influence on OEB lesions. Fucosaylated glycans are contributed in OEB integrity. NO/NOS activity seem to play a critical role in OEB ulcerogenesis because blocking its activity aggravates experimental OEB lesions, most likely through the inflammation, vascular and perivascular changes.

Keywords: oesophagus, postprandial hyperglycemia, NO, histochemistry, fucosylated glycans

INTRODUCTION

Nowadays data demonstrates that correct functioning of the oesophageal epithelial barrier (OEB) is crucial to ensure health [3, 10]. Oesophageal diseases are more prevalent foregut pathology than "peptic ulcer" and characterized by hidden onset and cruel prognosis for malignancy transformation and, in addition, probably drug induced [4]. The OEB has to realize two major charges that can seem paradoxical: it must enable to resist to retrograde acidification and chemical (pepsin, trypsin, bile acids, etc) influence of gastric refluxant during postprandial state and spontaneous transient lower oesophageal sphincter (LOS) relaxation (TLOSR) while at the same time controlling the orthograde motor activity. This multitasking ability is permitted by the structural organization and compartmentalization of the oesophageal epithelium. The oesophageal enteroendocrinocyte's melatonin is a key issue in oesophageal cytoprotection [7, 13] but effects of its precursor L-Tryptophan (L-Try) on ulcer and pre-ulceric lesions in OEB still unknown. Also, experimental clinical and animal studies have clearly demonstrated that nitrogen oxide (NO) is important for the normal esophageal peristaltic and TLOSR [7, 13]. In addition, the daily wave-shaped postprandial hyperglycemia (PHG), typical dominated condition of standard modern diet, reflecting oxidative stress with the hyperproduction of reactive oxygen spices (ROS), stimulates pancreatic β-cell to hypersecrete insulin, keeping fasting plasma glucose concentrations are above the normal range for several years [10]. According to the recent data, glucose abnormal metabolism is the background of the one of the major worldwide common medical disease, as diabetes mellitus (DM) and over 90% of the total number of DM 366 million people currently have type 2 of DM (T2DM) [2]. However, whether the functioning of OEB is changed in patients with PHG remains elusive.

Animal models of OEB of oesophageal pathology are needed to investigate new biomarkers of early molecular and biochemical changes in the pathogenesis in oesophageal ulceration [11] and to test novel therapeutic approach [7]. We were first to use animal models of oesophageal non-erosive (e.g., induced by stress, streptozocin-induced hyperglycemia) lesions and our data expanded insight into the genesis of DM oesophageal lesions via

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DOI: 10.12923/j.2084-980X/26.1/ a.20

over-production of ROS by nitroxidative stress induced by disbalance in NO/NO-synthase (NOS) activity [14]. One of the very early events in experimental oesophageal ulceration is the rapid modification of glycoconjugate's pattern of OEB. Carbohydrate-containing compounds are the diverse members of glycome expressed at the cell surface which act as ligands for several signaling pathways and have important role as biomarkers in discovering many physiological and pathological processes, including degeneration, inflammation, atherosclerosis and cancer [4]. Modern data had shown that fucose (Fuc)-containing specific glycoconjuges, are implicated in recognition process for the selectin family of cell adhesion receptors expressed by platelets (P-selectin), endothelial cells (E- and P-selectin), and leukocytes (L-selectin) [1], in EGF domains of the Notch receptors [6], a family of transmembrane signaling proteins of signal transduction in neurogenesis, angiogenesis, and lymphoid development, as well as in tumor growth factor-beta family related Nodal [9]. Modification of fucosylated glycans recognized in gastric epithelial barrier induced autoimmune--mediated damage by *H. pylori* [6], however, their changes in OEB and its possible role as biomarkers of injury are still unknown.

The aims of the present study were: 1) to investigate the effect of PGH without and with pretreatment with L-Try on acute OEB lesions induced by non-topical ulcerogens (water immersion and restraint stress, WIS) and impact of Fuc-containing glycans in oesophageal mucosa of intact and exposed to induced damage rats by non-selective blocker of cyclooxygenase I and II (COX); 2) to determine the mechanism of L-Try oesophagoprotection related to activity of NO/NOS system changes and contribution of Fuc-binding glycans in OEB cytoprotection events.

MATERIAL AND METHODS

Male Wistar rats, weighing 180-220 g (n=70), with standard rat chow and free access to fluids were used in our studies. All rats were fasted 24 hours prior to the experimental procedure. All experiments were carried out according to the University Guidelines for the care and use of laboratory animals and the European Council Directive on 24 November 1986 for Care and Use of Laboratory Animals (86/609/EEC), and approved by the University Ethical Committee for Animal Research. PHG was induced by method Kozar V.V., 2009 in animals that had *ad libitum* accesses to 30% fructose during 28 days [8] versus to the control group with tap water access. The initial and final body weights of the various groups were recorded; the blood glucose concentration was measured from the tail vein by glycometr (Achtung TD-4207, Germany) every day. Acute oesophageal lesions were induced by the WIS by Takagi, 1964 [4]. For determination of influence NO/NOS mechanism on PHC-induced oesophageal lesions were used rats with modification COX activity by non-selective blocker COX indomethacin (COX) pretreatment in dose 10 mg/kg, intraperitoneally (i.p.) before 2 hrs of experiment. In studies on oesophagoprotection, induced by L-Try given intragastrically (i.g.), the following groups of rats were used: 1) control (Cont) – intact animals, 2) WIS with vehicle (1 ml saline i.p. and *per os*), 3) L-Try – 50 mg/kg, i.g. with vehicle, 4) L-Try- 50 mg/kg, i.g. with COX 10 mg/kg, i.p., 5) L-Try – 50 mg/kg, i.g. with WIS, 6) L-Try – 50 mg/kg, i.g. with COX 10 mg/kg, i.p. with WIS), After the end of experiment, rats were anaesthetized with phentobarbital (60 mg/kg i.p.), then the animals were sacrificed and the oesophagus was immediately removed for macroscopic analysis, opened and placed flat to count the number of oesophageal lesions by two investigators, unaware of the treatment given. The OEB lesions were defined as round or linear mucosal defects of at least 0.1 mm in diameter. For the microscopic examinations, the segments of the samples of the distal third esophagus were fixed in 10% formaldehyde for histological evaluation. In order to analyse the histological characteristics, the evaluation of the haematoxylin and eosin specimens were used due to the lesion index (LI) which evaluated as score sum according to classified system of the epithelial loss: 0 – none, 1 – preulcerative minimal changes and splitting, 2 – erosion, 3 – ulceration; combined with regenerative epithelial changes: 0–- none, 1– basal hyperplasia, 2– mitosis, ballon cells, akantosis, 3 – parakeratosis. For evaluation vascular index (VI) was used with score of vascular changes: 0 – none; 1 – edema, 2 – submucosal vascular dilation, 3 – perivascular hemorrhage; combined with leukocyte intraepithelial infiltration: 0 – none, 1 – mild, 2 – moderate, 3 – severe. For fucosylated glycans in OEB analysis the lower third of oesophagus were excised and used for lectin histochemistry. The set included lectins, which can bind L-fucose-rich glycoconjugates: the ovary of perch agglutinin PFA (Persa fluviatalis L., specific to Fuc α1-2 Gal α1-4Glc α-L-Fuc), the bark agglutinin from the shrub golden rain Laburnum anagyroides LABA (specific to Fuc α1-2 Gal α1-4Glc α-L-Fuc), conjugated to peroxidase (purchased from "Lectinotest Lab", Ukraine). Lectin labeling was routinely visualized. 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).

The content of nitrogen oxide in homogenate was determined as nitrites by the method of Green L., David A., 1992 [5] and expressed as ĕmol/g. NO-synthases activity was measured by the method of Sumbajev V., 2000 [12]. All results were processed by the methos of variation statistics.

RESULTS

The results demonstrated that the animal body weight from experimental groups was increased about 5-8% by PHG in compare to control. The blood glucose baseline of normal and experimental animals was 5.8±0.5 mmol/L. In the rats from control group OEB did not show any macroscopical or microscopical alterations. Macroscopic evaluation of impact PHG on OEB revealed mostly mucosal edema with focal superficial erosions in the distal part of oesophagus only in the rats with WIS versus to animals from other groups. To establish the microscopic changes in rat OEB were used LI and VI and their changes are shown in Fig.1 and 2, respectively. The nonspecific markers of PHG-related injury in epithelial part of OEB were seemed in the rats include increased mitoses in the epithelium, spongiosis and balloon-cell change (swelling) of keratinocytes. Main characteristic of stromal injury in OEB were vascular congestion in papillae, dilated vascular channels at the tips of the papillae. Furthermore, WIS-related lesions of OEB during PGH were characterized by signs of irregular hyperemia, stasis, perivascular diapedesis with microthroms and subepithelial excessive edema. L-Try treated animals had significantly less OEB lesions than in those receiving COX.

Lectin histochemistry of esophageal mucosa of vehicle pretreatment rats (control) revealed more sensitive and accurate picture of the structure-functional organization of esophageal mucosa and marked heterogeneity of PFA and LABA binding which was considered as norm (Fig.3 A, B). During labeling in the lower part of oesophagus there was showed a clear visualization of three layers of the esophageal epithelium: stratum corneum (SC), stratum spinosum (SS) and basal cell layer (BC) which were used into interpretation of lectin space orientation and ligand expression. PHG induced higher expression of Fuc-specific glycans labeling by PFA in the pre-epithelial and epithelial parts of OEB, as well as in subepithelial structures were in the rats with stress-associated oesophagitis. Moreover, Fuc-containing PFA expressing, increased in some cells in SS, as well as in dendritic cells, components of ENS, BC, and basal membrane in fibroblastic cells of lamina propria of OEB. In addition, in the subepithelial part of OEB expression of PFA lectin receptors were in the collagen fibers. In the muscular layer fucosylated PFA and LABA binding were poor, therefore in the external layer of OEB was minor expression of Fuc-specific glycoconjugates in the fibrils of connective tissues. Additionally, PGH with WIS elicited excessive expression of Fuc in internal elastic membrane and external layer in vessels in oesophageal microcirculation in OEB (Fig.3 C, D). An L-Try-treated rat had enhanced expression PFA and LABA in SC and SS in OEB on the background of decreasing oesophageal lesions appearance. De-fucosylation of nerve endings in OEB marked by PFA were in COX-treated rats, therefore administration of L-Try reversed these changes contributed to the main mechanism of maintenance of oesophageal mucosal integrity.

To determine the role of NO/NOS activity in PHG-inducted oesophageal injury, rats were pretreated by vehicle, COX and L-Tryp and underwent to WIS-induced injury. The basal level of NOS was 7.38±0.64 nmol /minmg protein, it should be noted that activity of iNOS was – 4.70±0.63 nmol /minmg protein, while eNOS – 2.68±0.20 nmol /minmg protein. Fig. 1 and Fig. 2 depicts the activities of total NOS, content of NO and iNOS and eNOS, respectively, in oesophagus of normal, PHG and WIS-inducted and L-Try/COX treated rats during PHG and WIS. The activity of NOS was significantly (*P.05*) decreased in rats pretreated by L-Try (Fig.1). The administration of COX to rats with PHG significantly (*P.05*) keeps NOS activity on the same level but decreased content of NO to 22% and iNOS activity in twice. The results of cotreatment of L-Try and COX decreased activity of iNOS and significally stimulated of eNOS activity in comparison to the data obtained in rats with WIS and without pre-treatment (Fig. 2).

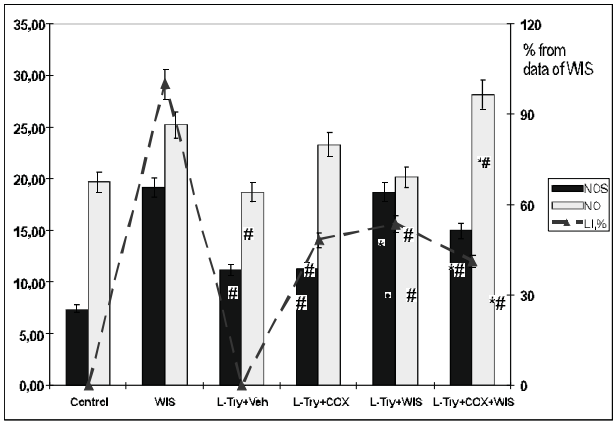

Fig. 1. The influence of postprandial hyperglycemia on oesophageal lesions and mucosal NOS expression, NO content (μmol/g) during WIS induction without/with COX and vehicle or L-Tryptophan (L-Try) pretreatment in rats (n=7-8), *p<0.05; #p<0.01versus control (Newman-Keuls's test).

DISCUSSION OF THE RESULTS

Recent epidemiological data showed that T2DM is one of wide distributed disease with high risk to tumorogenesis, including oesophageal adenocarcinoma [10]. Moreover, by 2030, the total number of people with T2DM will have risen twice, of whom over half will remain undiagnosed because studies suggest that the onset of T2DM can occur 5–10 years before clinical diagnosis [2]. Rayner CK, 1992 demonstrated that changes in blood glucose concentration have major effects on motor function in foregut [11]. Hy-

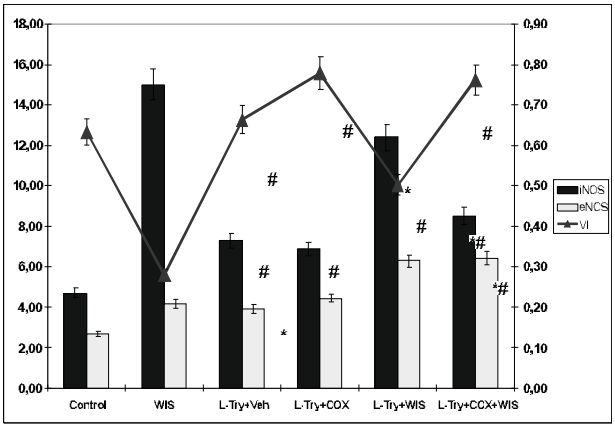

Fig. 2. The influence of postprandial hyperglycemia on Esophageal mucosal iNOS, eNOS expression and vascular index with vehicle and WIS induction without/with COX and L-Tryptophan pretreatment in rats (n=7-8), *p<0.05; #p<0.01 versus control (Newman-Keuls's test).

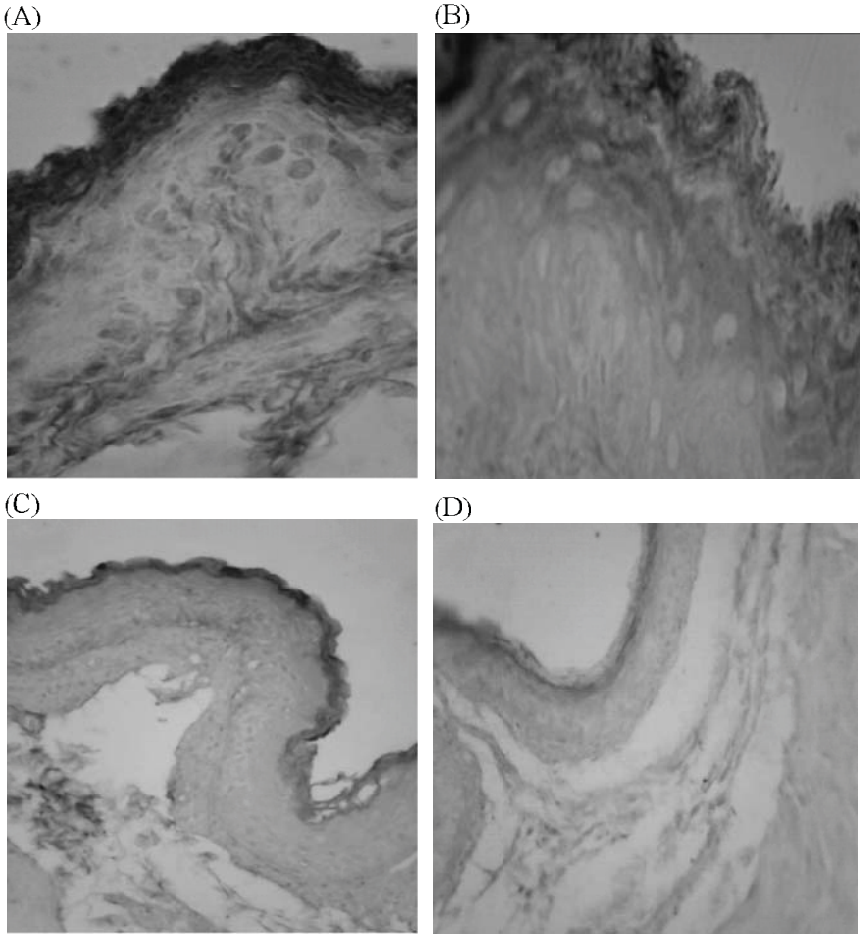

Fig. 3. Lectin histochemistry of oesophageal mucosa taken from the rats from control group (A, B) and subjected to porstprandial hyperglycemia and stress injury with indomethacin 10 mg/kg, applied (C and D). Dynamic changes in pattern of fucosylated glycans via expression of PFA (A, C), LABA (B and D) labeling in oesophageal epithelial barrier mucosa; magnification 300x (A, C, D) and 600x (B).

perglycemia may be an important factor contributing to the increased esophageal acid exposure in patients with diabetes mellitus also described by other authors [3]. Many animal and human studies reveled that TLOSR increased frequency and exposition OEB to harmful influence of the retrograde gastric content [4, 14] but mechanism underlying that effect remains underdetermined. Discovery of the mechanism of hyperglycemia-induced oesophageal pathophysiology will helps to find biomarkers of early pre-clinical changes in OEB. We previously demonstrated that control of the OEB integrity is highly modulated by components of its 'outer' epithelial microenvironment (chemicals, microflora, for example) and 'inner' sub-epithelial microenvironment (local blood flow, immune cells, the enteric nervous system [ENS]) [7, 15]. On the other hand, improved diagnostic process will enhanced therapeutic approach in upper gut ulcerogenesis. Little information is available regarding the contribution of glycoconjugates, which modern diagnostic technology represents, to the pathogenesis of OEB lesions. Lately, we have confirmed that sialoglycans and mannose glycans regulated recognition processes, immune defense and may be key biomarkers in ulcerogenic degenerative and regenerative processes in OEB [4]. In the present study, we demonstrated that PHG rats exhibited increased susceptibility to the oesophgeal damage as compared to control. One of the factors that might contribute to this increase in injury is the subepithelial vascular and perivascular changes (vascular leukocyte-adherence to the vascular endothelium) in OEB that represented by its structural-functional reorganization during PHG revealed by fucosylated glycans expression modification [9]. These observations imply the existence of mechanisms that coordinate PFA and LABA appearance in the different parts of OEB due to cellular and subcellular mechanism of cytoprotection induced by NO/NOS activity, key mediator of mucosal defence in foregut, as well as prostaglandin synthesis by COX. The results have proven that labening by PFA is highly effective for epithelial-glial-endothelial activity while LABA is a sensitive tool for cell injury, leukocytes recruitment, necrosis and also signs for apoptosis in the pre-epithelial and epithelial layer in OEB during PHG. We also extended our previous findings by esophagoprotection and demonstrated that pre-treatment with L-Try significantly reduced esophageal epithelial injury during PHG and this

effect was accompanied by the gradual increase in activity of eNOS and decrease activity of iNOS. Both, the changes in fucosylated pattern in OEB and decrease in score LI and VI, drop of iNOS activity, were observed in rats with PHG and L-Try pre-treatment but by the cytoprotective ability of eNOS.

CONCLUSIONS

Animal model of postprandial hyperglycemia exhibits preulcerogenic stage of OEB lesions which could be developed in the subset of T2DM in humans. Fucosylated glycans are contributed in oesophageal integrity. During induction of non-erosive stress-associated injury overexpression of fucosylated glycans were present in OEB pre-epithelial and epithelial layers labeled by LABA and in the epithelial-glial-endothelial activity by PFA. Pretreatment by L-Try is highly effective for oesophageal lesions inducted by PGH via modulation NO/NOS activity.

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