TRANSLATIONAL MEDICINE

UDC 611.33-018.25:[611-018:547.96]:577.112:577.152]-08 doi: https://doi.org/10.15407/ubj92.02.033

LECTINOCYTOCHEMICAL STUDY OF RAT STOMACH MUCOSA UNDER THE CONDITIONS OF CYCLOOXYGENASE-1/-2 BLOCKAGE AND PRETREATMENT WITH H-GLU-ASP-GLY-OH

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Received: 22 December 2019; **Accepted:** 27 March 2020

Assessment of glycoconjugate expression on cell membranes using the lectin histochemistry technique may be a feasible approach for evaluating the functional state of the cell. The aim of this study was to evaluate carbohydrate determinants of rat stomach mucosa cell membranes under the conditions of COX-1/-2 blockage with indomethacin and pretreatment with the tripeptide H-Glu-Asp-Gly-OH. Male Wistar rats were divided into 3 groups (n = 6 per group): 1st group (control) received vehicle; 2nd – indomethacin (35 mg/kg); 3rd – H-Glu-Asp-Gly-OH (10 μg) 30 min before indomethacin. Rats were sacrificed 24 hours later. Gastric mucosa (GM) carbohydrate determinants were studied by lectin-peroxidase technique. The lectins panel included α-fucose- (LABA), syalo- (WGA, SNA), mannose- (Con A, LCA) and galactose-specific (HPA, PNA, SBA) lectins. Intensity of lectin-receptor reaction was scored: 0 – no reaction; 1 – weak; 2 – mild; and 3 – strong reaction. COX-1/2 blockage caused GM lesions, attenuated by H-Glu-Asp-Gly-OH. WGA and SNA showed the highest affinity to GM. Indomethacin decreased SNA-labeling of epitheliocytes and mucocytes and LABA-labeling of chief cells. H-Glu-Asp-Gly-OH reversed the glycosylation changes, caused by COX-1/COX-2 blockage only in regards to labeling of chief cells with LABA, epitheliocytes and mucocytes with LCA, mucocytes with SNA. Predominantly H-Glu-Asp-Gly-OH under COX-1/COX-2 blockage had an effect opposite to indomethacin alone but glycosylation changes under these conditions differed significantly also from the control. COX-1/COX-2 blockage causes alteration of glycosylation processes in rat GM, mainly reduction of NeuNAc(α2-6)DGal and α-Fuc content. H-Glu-Asp-Gly-OH under the conditions of COX-1/COX-2 blockage leads to more profound changes in GM lectin-binding pattern compared to the independent effect of indomethacin and to control.

K e y w o r d s: lectin histochemistry, glycosylation, oligopeptides, H-Glu-Asp-Gly-OH, cyclooxygenase (COX), gastric lesions, indomethacin.

Introduction

Nonsteroidal antiinflammatory drugs (NSAIDs), whose therapeutic effect is based on the inhibition of cyclooxygenase (COX) activity, are the most commonly prescribed medications worldwide for the management of pain and inflammation, however adverse effects of NSAIDs, in particular stomach mucosa toxicity, present one of the major health care challenges [1, 2, 4-6]. Research data from the last decade provide scientific evidence that various short chain peptides may be worth attention as promising gastroprotective agents, especially for chronic NSAIDs users [2-6]. Short derivatives of regulatory peptides are known to be involved in the

regulation of acid/base secretion in the gastrointestinal tract, motility, enzymatic activity, production of prostaglandins and nitric oxide and interaction with the neuroendocrine system [2, 4-6]. Important characteristics of oligopeptides, in contrast to conventionally used proton pump inhibitors or blockers of histamine-2 receptors, are lack of side effects and extremely low effective doses [2, 4, 5]. Oligopeptides were also reported to be resistant to the influence of gastric and enteric proteinases, which allows their oral administration to the patient [4].

Our previous studies demonstrated the gastroprotective effects of hexapeptide Arg-α-Asp-Lys-Val-Tyr-Arg and tripeptides H-Glu-Asp-Gly-OH and H-Lys-Glu-Asp-OH, that are mediated by the decrease of nitrosooxidative stress in the stomach, although the molecular mechanisms of biological effects of oligopeptides need to be elucidated [2, 3].

Glycans decorate the surface of most living cells and organisms, creating a complex landscape of recognition sites and barriers that generally represent the first point of contact at the interface of a cell's biotic and abiotic environments [16]. Disease-associated glycosylation changes can derive from a huge number of molecular causes as glycan biosynthesis is, unlike the synthesis of DNA, RNA and proteins, a non-template-driven process involving numerous different participants [14]. Glycosylation as a common post-translational modification, occurring in most human proteins, plays crucial role in molecular recognition, cell-cell adhesion, molecular trafficking, receptor activation and signal transduction [23] Changes in glycosylation pattern was reported in many inflammatory and malignant gastrointestinal tract disorders [13, 15, 20, 23]. In experimental studies, loss of intestinal mucin-type O-glycans was reported to promote the development of spontaneous duodenal tumors and increase susceptibility to colitis [13, 15].

In recent years, the study of glycosylation processes is progressing remarkably by the development of glycan analysis systems, including histochemical methods, based on the use of lectins microarrays [7, 12, 24]. Lectins are carbohydrate binding proteins, other than enzymes or antibodies, which may be extracted from plants, animals and microorganisms but compared to antibodies against glycans, lectins are less expensive and have useful specificities for complex glycans [12]. Systematic analysis of the change in expression pattern of glycogens and lectins can bring about a comprehensive understanding of the genetic basis of glycobiological changes occurring in pathological condition [15].

Thus, the aim of this study was to evaluate carbohydrate determinants of rat stomach mucosa cell membranes under the conditions of COX-1/-2 blockage with indomethacin and pretreatment with the tripeptide H-Glu-Asp-Gly-OH.

Material and Methods

Animals and experimental design. The studies were conducted on 18 male Wistar rats, aged 8 weeks, weighting 180–220 g, obtained from the Experimental Medical Laboratory of Danylo Halytsky Lviv National Medical University (Lviv, Ukraine). The studies were approved by the Institutional Ethic Committee of Danylo Halytsky Lviv National Medical University (Protocol No 2 of February 16th, 2015). The experimental procedures were carried out in accordance with the international guidelines for the use and care of laboratory animals. The rats were kept in self-ventilating cages (6 animals per cage) at room temperature with light exposure following a 12/12 hour light/dark cycle. The animals were fed with concentrate feed K 120/2 for mice, rats and hamsters ("Dutch Concentrate Feed", Ukraine). All rats underwent a 24 hour fast prior to drug exposure, with water ad libitum.

To assess changes of the gastric mucosa glycoprofile under the conditions of COX-1/-2 blockage with indomethacin and pretreatment with tripeptide H-Glu-Asp-Gly-OH, the rats were randomly divided into 3 groups (n = 6 in each group). The control group received the vehicle, infused intragastrically using a probe (isotonic solution of sodium chloride). The second group was intragastrically administered indomethacin at a dose of 35 mg/kg (Sopharma, Bulgaria), dissolved in slightly alkaline distilled water [21]. The third group was pretreated with H-Glu-Asp-Gly-OH (10 μg), dissolved in saline 30 min before indomethacin introduction.

Determination of gross mucosal damage. Twenty-four hours after the experimental procedures the animals, anesthetized by urethane at a dose of 1.1 mg/kg, were sacrificed by decapitation. The stomachs were rapidly excised, cut along the lesser curvature, washed in isotonic sodium chloride solution and pinned open for macroscopic examination. After gross inspection, hemorrhagic and ulcerative lesions of the gastric mucosa were counted and their lengths (mm^2) were measured with the method of planiometry by a researcher blind to the experimen-

tal grouping. The lesions were scored according to macroscopic degree of injury: hyperemia (2); pellicle and small hemorrhages (4); pellicle and superficial erosion (6); several superficial erosions or one deep erosion (8); ulcer (10); and ulcer, erosions, hemorrhages (12) [2].

Histologic evaluation of gastric mucosa and lectin histochemistry analysis. The pieces of stomach wall were excised, fixed in 4% neutral formalin, and embedded in paraffin wax according to the standard protocol. For general morphology studies, 5- to 7-μm-thick sections were stained with haematoxylin and eosin.

Lectin histochemistry investigations were conducted using the peroxidase-diaminobenzidine visualization protocol with diaminobenzidine tetrahydrochloride (Sigma, USA) [10]. The lectin panel is presented in Table 1. All lectins used and their peroxidase conjugates were prepared by Dr. V. Antonyuk ("Lectinotest", Ukraine). Microscopy was conducted and pictures taken using Granum R6053 photomicroscope (China), equipped with Echo-Imager 502000 (China), and the ToupView 3.7 computer program (USA). Peroxidase activity and relevantly the sites of lectin binding with glycoconjugates were determined according to the brown staining of oxidative polymerization of 3.3-diaminobenzidine product. Semi-quantitative evaluation of the staining intensity in stomach mucosa cells was performed by three independent observers, as described in the literature [22]. Staining intensity was classified into 4 categories: no reaction (0); weak (1); mild (2) and strong reaction (3).

Statistical analysis. Statistical analysis was performed using Origin Pro, GraphPad Prism and Microsoft Excel software. Since the data distribution in our study was normal, the mean values, standard errors and standard deviations, and upper and lower 95% confidential intervals for the means were calculated. Student's *t*-test was used, with differences of $P < 0.05$ considered to be significant. Results are expressed as means (M) ± standard error (m).

Results

Gross and histomorphologic assessment of the gastric mucosa under the conditions of COX-1/-2 inhibition and pretreatment with H-Glu-Asp-Gly-OH. In our studies the mucous membrane of control group rats was relatively intact (Fig. 1, *A*), whereas COX-1/-2 inhibition with indomethacin caused the development of hyperemia, edema and abundant erosive and ulcerative lesions of the gastric mucosa with the mean area = 11.20 ± 0.65 mm^2, and score of damage = 9.60 ± 0.61 (Fig. 1, *B*, Fig. 2, *A, B*). Pretreatment with H-Glu-Asp-Gly-OH did not reverse the ulcerative effect of COX-1/COX-2 inhibition, but resulted in the decrease of the gastric lesion area (Fig. 1, *C*, Fig. 2, *A, B*) and a minor trend of decreased damage score ($P > 0.05$).

Histologic studies did not reveal pathological changes in the stomachs of the control group of rats, whereas indomethacin caused the development of

Table 1. Lectins and their respective carbohydrate specificities

No	Lectin designation, abbreviation	Specific monosaccharide	Complementary oligosaccharide/ polysaccharide
1.	Canavalia ensiformis agglutinin, Con A	αDMan>αDGlc	Man(α1-2)Man(α1-2)Man in N-glycans, nonfucosylated
2.	Lens culinaris agglutinin, LCA	αDMan/αDGlc	GlcNAc-Oligomannose core of N-glycans
3.	Laburnum anagyroides bark agglutinin, LABA	LFuc	Gal(β1-4)Fuc(β1-3)Glc
4.	Helix pomatia agglutinin, HPA	αDGalNAc	GalNAc(α1-3)GalNAc
5.	Peanut agglutinin, PNA	DGal	DGal(β1-3)GalNAc
6.	Sambucus nigra agglutinin, SNA	NeuNAc(α2-6)DGal	NeuNAc(α2-6)Gal(β1-4) GlcNAc(β1-2)
7.	Soybean agglutinin, SBA	αDGalNAc>βD GalNAc	GalNAc(α1-3)Gal(β1-3)GalNAc
8.	Wheat germ agglutinin, WGA	DGlcNAc>NeuNAc	NeuNAc(α2-6)Gal(β1-4) GlcNAc, Man(β1-4) GlcNAc

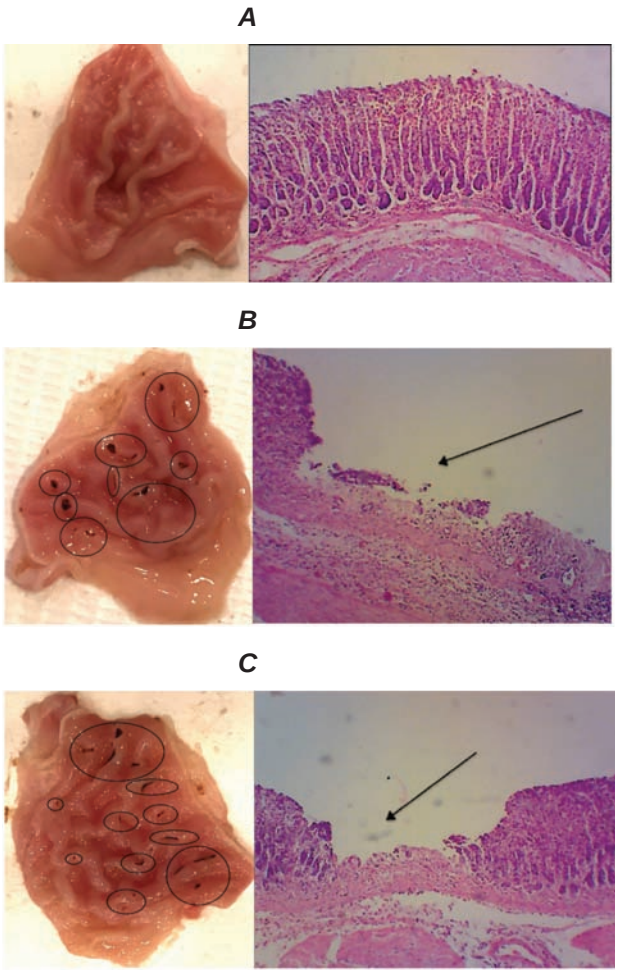

*Fig. 1. Morphologic parameters of rat stomach mucosa under conditions of COX-1/COX-2 blockage and pretreatment with H-Glu-Asp-Gly-OH. (**A**) Control group, (**B**) Effect of indomethacin, (**C**) H-Glu-Asp-Gly-OH pretreatment on the background of indomethacin. Arrows indicate injury to the stomach mucosa. Hematoxylin and eosin staining (magnification 150x)*

edema, loss of the surface epithelial cells, leukocyte infiltration in the submucosal layer, and decrease of the periulcerative mucosa thickness (Fig. 1, *B*). In the H-Glu-Asp-Gly-OH-pretreated group, better preservation of mucosal structural architecture (crypts and gastric glands) was noted as well as increased content of mucocytes (Fig. 1, *C*).

Assessment of α-fucose-specific carbohydrate determinants in rat stomach mucosa. Staining with *Laburnum anagyroides* bark agglutinin (LABA) revealed the presence of fucose-specific receptors in almost all cell components of the mucous-epithelial layer of the gastric mucosa, besides endocrinocytes (Fig. 3). The reactivity of gastric mucosa cells to

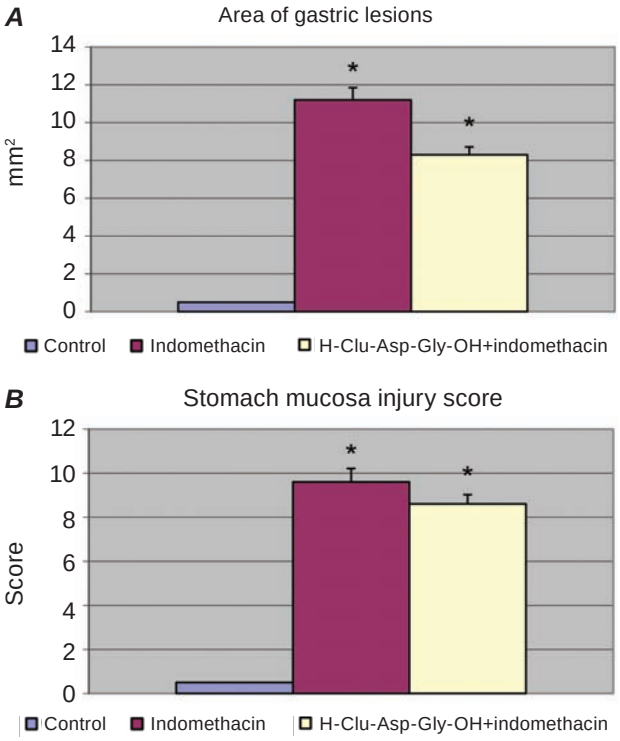

*Fig. 2. Macroscopic evaluation of gastric mucosa injury. (**A**) Area of gastric lesions. (**B**) Stomach mucosa injury score. * $P < 0.05$, compared to control*

LABA in control rats was as follows, mucocytes > chief cells > epitheliocytes > parietal cells (Table 2). Cyclooxygenase blockage with indomethacin caused a tendency to increase the α-Fuc content in mucocyes and epitheliocytes ($P > 0.05$), whereas the number of fucose receptors decreased by 48% ($P < 0.05$) in chief cells and more than 2 times in parietal cells compared to control animals. Pretreatment with H-Glu-Asp-Gly-OH had a tendency to reverse the changes of α-Fuc content in chief cells ($P > 0.05$) caused by indomethacin and showed a tendency to increase LABA-receptors in parietal cells ($P > 0.05$), compared to the effect of cyclooxygenase (Fig. 3). But intriguingly H-Glu-Asp-Gly-OH introduction enhanced the decrease of LABA-reactivity of epitheliocytes (50%, $P < 0.05$) and mucocytes (50%, $P < 0.05$) caused by indomethacin, and in the tripeptide-pretreated group α-Fuc content in these cells was also lower compared to the control group ($P < 0.05$).

Assessment of syalo-specific carbohydrate determinants in rat stomach mucosa. Staining with both syalo-specific lectins wheat germ agglutinin (WGA) and *Sambucus nigra* agglutinin (SNA) was shown to be feasible for identifying the epithelio-

Table 2. Intensity of lectin-receptor reactions in gastric mucosa cells under the conditions of cyclooxygenase-1/2 blockage and pretreatment with H-Glu-Asp-Gly-OH in Wistar rats

Lectins and their carbohydrate specificity	Experimental groups											
	Control				Indomethacin-induced gastric lesions				H-Glu-Asp-Gly-OH + Indomethacin			
	EP	CC	PC	M	EP	CC	PC	M	EP	CC	PC	M
Laburnum anagyroides bark agglutinin (LABA)	2.17±0.18	2.50±0.37	0.50±0.24	2.83±0.18	2.67±0.23	1.33±0.23$^{\#}$	0.17±0.18	2.67±0.23	1.33±0.23$^{\#}$*	2.50±0.24*	0.30±0.23	1.30±0.23$^{\#}$*
Wheat germ agglutinin (WGA)	2.66±0.36	2.83±0.18	2.83±0.18	3±0	2.83±0.18	2.67±0.23	2.50±0.24	2.66±0.23	3±0	2.83±0.18	1.66±0.36$^{\#}$	2.83±0.18
Sambucus nigra[l] agglutinin (SNA)	3±0	1.00±0.28	2.17±0.18	2.83±0.18	2.33±0.23$^{\#}$	1.67±0.23	2.17±0.18	2.17±0.18$^{\#}$	2.83±0.18	1.17±0.18	1.17±0.18$^{\#}$*	2.83±0.18*
Canavalia ensiformis agglutinin (Con A)	2.60±0.23	0	0	0	2.00±0.28	0	0	0	0.50±0.24$^{\#}$*	0	0	0
Lens culinaris agglutinin (LCA)	2.66±0.23	1.00±0.28	0.66±0.36	1.83±0.33	3±0	0.50±0.24	0.50±0.24	0.83±0.33	2.17±0.18*	0.17±0.10$^{\#}$	0.17±0.1	2.33±0.36*
Helix pomatia agglutinin (HPA)	0.50±0.24	0.16±0.05	1.50±0.24	1.8±0.3	0.33±0.23	0.17±0.01	2.33±0.36	2.33±0.23	0.33±0.23	0.83±0.18$^{\#}$	1.67±0.23	3±0$^{\#}$*
Peanut agglutinin (PNA)	0.167±0.01	0.50±0.24	1.00±0.28	1.00±0.28	0.167±0.01	0.167±0.01	1.83±0.33	0.33±0.23	0.667±0.23	0.50±0.24	0.33±0.23*	2.67±0.23$^{\#}$*
Soybean agglutinin (SBA)	2.66±0.23	1.00±0.28	1.00±0.28	2.66±0.23	2.50±0.37	0.83±0.33	2.00±0.28$^{\#}$	1.00±0.28$^{\#}$	2.83±0.18	1.17±0.18	1.33±0.23*	1.00±0.28*

Gastric mucosa structural components: EP – epitheliocytes; CC – chief cells; PC – parietal cells; M – mucocytes. [1]Values are expressed as means *(M)* ± standard error (*m*). Student's *t*-test was used, differences of $P < 0.05$ were considered statistically significant. #$P < 0.05$, compared to control; *$P < 0.05$, compared to indomethacin effect

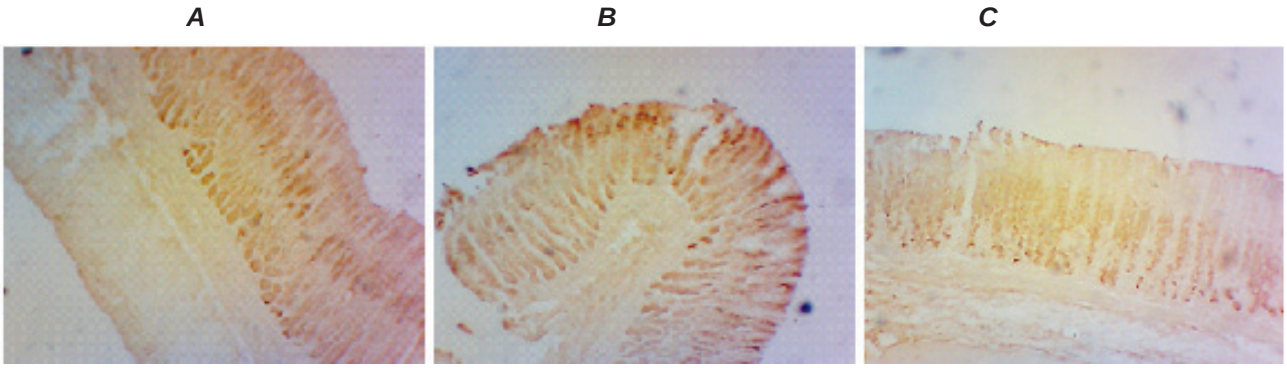

*Fig. 3. Laburnum anagyroides bark agglutinin (LABA) staining of stomach mucosa. (**A**) Control group, (**B**) Effect of indomethacin, (**C**) H-Glu-Asp-Gly-OH pretreatment on the background of indomethacin. Magnification 150x*

cytes, chief and parietal cells as well as mucocytes of the gastric mucosa (Fig. 4, Fig. 5). Mucocytes of the control animals showed the highest reactivity to WGA among the visualized cell components of the gastric mucosa, however, other cells, apart from endocrinocytes, were also well stained (mucocytes > chief cells = parietal cells > epitheliocytes (Table 2). No statistically significant changes of WGA-receptor number were revealed under the conditions of indomethacin-induced gastric lesions compared to control rats, but there was a tendency for increased WGA-reactivity in epitheliocytes ($P > 0.05$) and decreased WGA-reactivity in chief, parietal cells and mucocytes ($P > 0.05$, Fig. 4). Pretreatment with H-Glu-Asp-Gly-OH resulted in increased numbers of WGA-receptors in mucocytes, chief cells and epitheliocytes, that were decreased by cyclooxygenase blockage. However, the effect of the tripeptide enhanced the decreased WGA-staining of parietal cells, caused by indomethacin, so that the reactivity of these cells in the H-Glu-Asp-Gly-OH-pretreated group was 33% lower ($P < 0.05$) compared to control rats according to semi-quantitative evaluation.

Concerning SNA-labeling of the mucous-epithelial barrier of the stomach, the reactivity of the cells was epitheliocytes > mucocytes > parietal cells > chief cells (Fig. 5). Under the conditions of cyclooxygenase blockage with indomethacin, NeuNAc(α2-6)DGal content decreased by 22.2% ($P < 0.05$) in epitheliocytes and by 23.5% ($P < 0.05$) in mucocytes compared to control. In chief cells, SNA-labeling slightly increased ($P > 0.05$) but did not change in parietal cells, in contrast to control animals. Pretreatment with H-Glu-Asp-Gly-OH reversed indomethacin-induced alterations of mucocyte carbohydrate determinants ($P < 0.05$) and showed a tendency to increase syalo-specific receptors (labeled by SNA) in epitheliocytes, compared to the effect of cyclooxygenase-1/2 blockage ($P > 0.05$, Fig. 5). Administration of H-Glu-Asp-Gly-OH did not affect the staining profile of chief cells compared to indomethacin, but decreased SNA-reactivity of parietal cells almost 2 times compared to indomethacin-treated and control rats ($P > 0.05$). As mentioned before, NeuNAc(α2-6)DGal content in parietal cells of control rats and animals with gastric lesions was considered to be the same, based on the evaluation of the lectin-receptor reaction.

Assessment of mannose-specific carbohydrate determinants in rat stomach mucosa. Among mannose-specific lectins in our studies, the gastric mucosa of experimental rats showed stronger reactivity to *Lens culinaris* agglutinin (LCA) than to *Canavalia ensiformis* agglutinin (Con A). Con A labeling in all groups of animals was restricted to only epitheliocytes, and a very slight reactivity to this lectin was marked in 2 animals under the conditions of ulceration, that anyway was supposed to be statistically insignificant for calculations (Table 2). Cyclooxygenase blockage with indomethacin caused 25% reduction of αDMan content in epitheliocytes compared to control and the loss of ConA-receptors was enhanced by the effect of H-Glu-Asp-Gly-OH on the background of indomethacin.

The reactivity of gastric mucosa cells to LCA was epitheliocytes > mucocytes > chief cells > parietal cells. Blockage of cyclooxygenase-1/2 with indomethacin tended to increase LCA-labeling in epitheliocytes ($P > 0.05$) and to decrease labeling in chief cells, parietal cells, and mucocytes ($P > 0.05$).

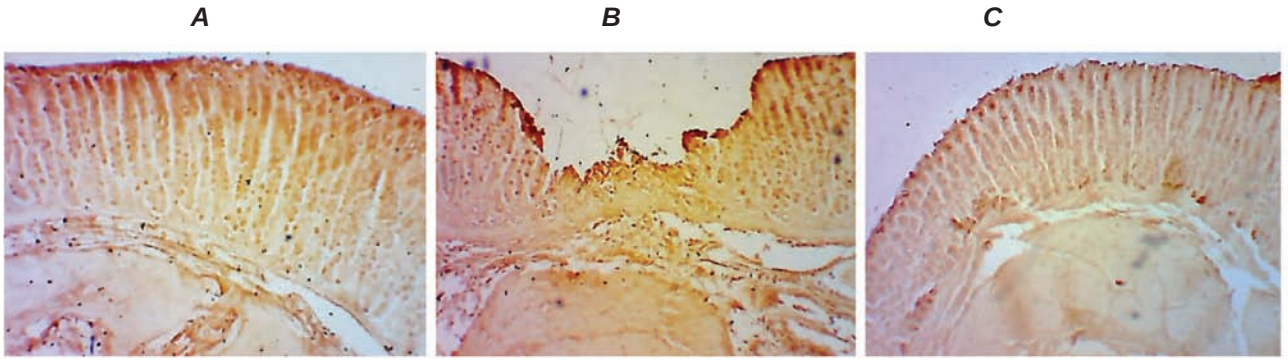

*Fig. 4. Wheat germ agglutinin (WGA) staining of stomach mucosa. (**A**) Control group, (**B**) Effect of indomethacin, (C) H-Glu-Asp-Gly-OH pretreatment on the background of indomethacin. Magnification 150x*

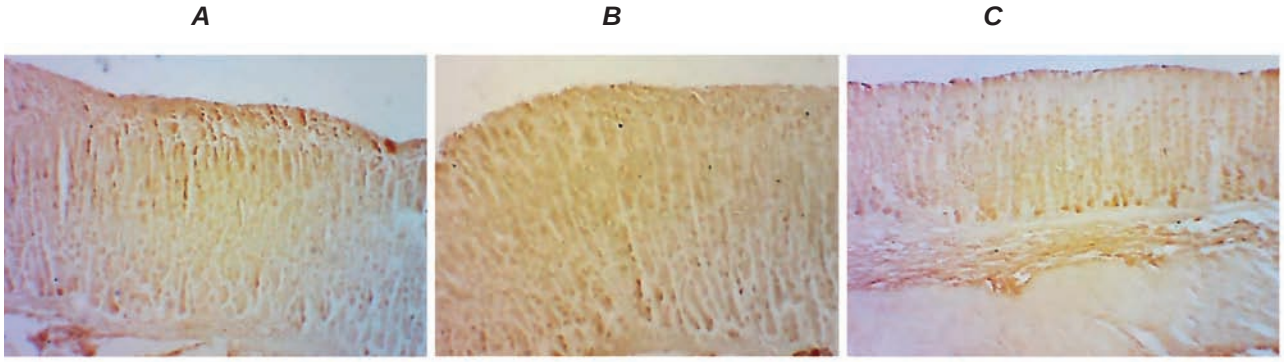

Fig. 5. Sambucus nigra agglutinin (SNA) staining of stomach mucosa. (A) Control group, (B) Effect of indomethacin, (C) H-Glu-Asp-Gly-OH pretreatment on the background of indomethacin. Magnification 150x

In the H-Glu-Asp-Gly-OH-pretreated group the number of LCA-receptors decreased by 28% ($P < 0.05$) in epitheliocytes and increased by 64.3% in mucocytes ($P < 0.05$) compared to indomethacin alone, approaching the glycome of control rats. However, the noted loss of parietal cell and chief cell reactivity to LCA under the conditions of cyclooxygenase-1/-2 blockage was enhanced by pretreatment with H-Glu-Asp-Gly-OH. LCA-labeling of chief cells under the conditions of H-Glu-Asp-Gly-OH administration on the background of indomethacin was 6 times lower compared to control rats ($P < 0.05$).

Assessment of galactose-specific carbohydrate determinants in rat stomach mucosa. Three galactose-specific lectins were used to assess the changes of the gastric mucosa glycopropfile under the conditions of cyclooxygenase inhibition and pretreatment with the tripeptide H-Glu-Asp-Gly-OH. The reactivity of gastric mucosa cell constituents to *Helix pomatia* agglutinin (HPA) in control rats was mucocytes > parietal cells > chief cells > epitheliocytes (Table 2). Development of indomethacin-induced gastric lesions was accompanied by decreased HPA-labeling of epitheliocytes, but increased reactivity of parietal cells and mucocytes to this lectin (Fig. 6). Pretreatment with H-Glu-Asp-Gly-OH caused changes in the lectin-binding pattern of the gastric mucosa compared to the effect of indomethacin, in particular the statistically significant increase of HPA-receptors in mucocytes ($P < 0.05$) was noted and this difference was also detected versus the control group of animals ($P < 0.05$). Introduction of H-Glu-Asp-Gly-OH simultaneously with cyclooxygenase inhibition with indomethacin also resulted in a 79% increase of HPA-labeling of chief cells compared to indomethacin alone and the control group (Fig. 6). Pretreatment with H-Glu-Asp-Gly-OH did not affect the lectin-receptor reactions in epitheliocytes compared to the effect of indomethacin.

Reactivity of gastric mucosa cells to peanut agglutinin (PNA) was parietal cells = mucocytes > chief cells > epitheliocytes. Blockage of cyclooxygenase with indomethacin did not affect the glycoprofile of epitheliocytes but led to a tendency for

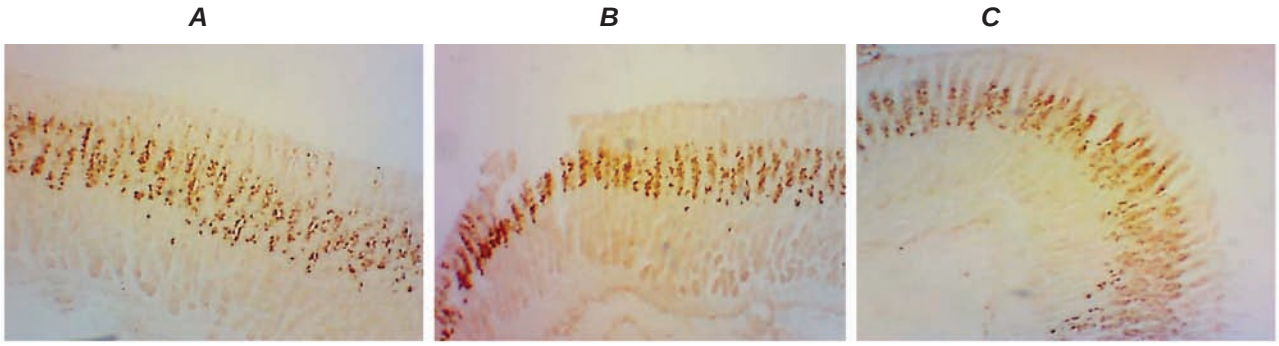

*Fig. 6. Helix pomatia agglutinin (HPA) staining of stomach mucosa. (**A**) Control group, (**B**) Effect of indomethacin, (**C**) H-Glu-Asp-Gly-OH pretreatment on the background of indomethacin. Magnification 150x*

decreased PNA-labeling of chief cells ($P > 0.05$) and mucocytes ($P > 0.05$) and a tendency toward increased parietal cell reactivity ($P > 0.05$). The effect of H-Glu-Asp-Gly-OH on PNA-labeling of all cells, apart from chief cells, caused significant changes in glycosylation processes compared to the effect of indomethacin alone but this dynamic was not consistent with the glycoprofile of control rats. Thus, pretreatment with H-Glu-Asp-Gly-OH led to an almost 3-times decrease in PNA-labeling of parietal cells versus control ($P < 0.05$), statistically significant enhancement ($P < 0.05$) of mucocyte staining compared to control, and an almost 4-times increase in epitheliocyte reactivity to this lectin.

The sensitivity of gastric mucosa cells to soybean agglutinin (SBA) was epitheliocytes = mucocytes > chief cells = parietal cells. Gastric ulceration, caused by cyclooxygenase activity blockage with indomethacin, was accompanied by 50% increased SBA-labeling of parietal cells ($P < 0.05$) and by 62.5% of mucocytes ($P < 0.05$). The tendency for a decrease in numbers of SBA-receptors in epitheliocytes and chief cells was noted in rats exposed to the ulcerative effect of indomethacin. Pretreatment with the tripeptide H-Glu-Asp-Gly-OH did not affect the glycoprofile of mucocytes compared to the effect of indomethacin alone, but decreased SBA-labeling of parietal cells, chief cells and epitheliocytes approached the glycome of control rats.

Discussion

This study provides the first description of the rat stomach mucosa glycoprofile in health and under the conditions of COX-1/COX-2 blockage with indomethacin according to the staining with a comprehensive set of lectins with 4 different carbohydrate specificities (α-fucose-, syalo-, mannose- and galactose). The changes of glycosylation processes in rat stomach mucosa under the conditions of tripeptide H-Glu-Asp-Gly-OH administration on the background of COX-1/COX-2 blockage are also reported.

COX-1 is a constitutive enzyme, catalyzing the reaction of prostaglandin production [2]. Prostaglandins are known to play a crucial role in the processes of gastric mucosa integrity maintenance, including contributing to the decrease of hydrogen chloride production in stomach in response to stimuli, the increase of bicarbonate secretion, production of the protective mucus in the gastrointestinal tract, phospholipid biosynthesis, and enhanced blood flow [1, 2]. Gastrointestinal complications are probably the most critical adverse effects of NSAIDs and the indomethacin model of experimental gastric lesion induction is well described in the literature [1, 2, 5]. In our studies nonselective cyclooxygenase-1/2 blockage with indomethacin also resulted in extensive gastric mucosal damage. Pretreatment with a tripeptide, containing lysine, glutamate and asparaginate provided a mild cytoprotective effect in idomethacin-induced gastric lesions in rats. According to data reported in the literature, the tripeptide H-Glu-Asp-Gly-OH stimulates cell proliferation and protein biosynthesis, and significantly inhibits apoptosis in fibrobasts, increasing resistance of their mitochondria to damaging agents, as revealed in cultured human stomach epitheliocytes [11]. Short peptides were reported to affect the processes of gene expression and H-Glu-Asp-Gly-OH was shown to decrease the synthesis of the proinflammatory enzyme COX-2 along with TNF-α [11, 17]. Taking into account that COX-2 is an inducible enzyme, upregulated in inflammation, that leads to overproduction of prostaglandin E2, a well established mediator of pain and inflammation [1, 2, 5], it may be presumed

that COX-2 inhibition with H-Glu-Asp-Gly-OH is one of the explanations for the mild gastroprotective effect of this tripeptide. Attenuation of experimental gastric mucosa damage in rats under the conditions of selective COX-2 inhibition was reported in our earlier studies and research of other authors [2].

In the model of cistamine-HCl-induced gastric ulceration on the background of *Helicobacter pylori* infection it was shown that subcutaneous administration of H-Glu-Asp-Gly-OH during 5 days led to the decrease of gastric lesions and inflammatory changes around the ulcerative defect as well as enhanced ulcer healing [15]. Both our previous studies and data in the literature showed the decrease of expression and activity of NO-synthase, lipid peroxidation processes, level of heat shock protein (HSP) 70 and p65 in experimental gastric lesions in animals under the effect of H-Glu-Asp-Gly-OH [3, 11]. Stem cells, capable of differentiation to other cells, and intracellular signal cascades have also been reported to be some of the targets for the effects of the short peptides [11, 17].

In our studies all 8 used lectins labeled the mucous-epithelial barrier of the gastric mucosa, although other scientists have reported the lack of LABA-, PNA- and SNA-labeling of healthy rat stomach mucosa [18]. However, in our research the cell reactivity to Con A (αDMan>αDGlc) was restricted to epitheliocyte labeling that is concordant with the data of other researchers [19]. Syalo-specific lectins WGA (DGlcNAc>NeuNAc) and SNA (NeuNAc(α2-6)DGal) showed the highest affinity to gastric mucosa in 3 experimental groups of rats, compared to other lectins (α-fucose-, mannose- and galactose-specific) and reactivity to WGA was higher in contrast to SNA. In a study by Falalyeyeva et al (2011) WGA, specific to NAcDGlc→NAcNeu, was also shown to exert the highest affinity to stomach mucosa in rats [18]. Development of indomethacin-induced ulceration of gastric mucosa caused the statistically significant decrease of SNA-labeling of epitheliocytes and mucocytes that is indicative of the decrease of NeuNAc(α2-6)DGal content in tissue and LABA-labeling of chief cells, mediated by the diminished α-Fuc level. Roy et al. (2014) reported the increased reactivity of human gastric mucosa to WGA in gastritis [20] but our studies in rats showed only increased WGA-reactivity in stomach mucosa epitheliocytes.

Interestingly, introduction of the tripeptide H-Glu-Asp-Gly-OH on the background of COX-1/-2 blockage had a more profound effect on glycosylation processes in gastric mucosa compared to indomethacin alone. Pretreatment with H-Glu-Asp-Gly-OH in only a few cases (LABA-labeling of chief cells, LCA-labeling of epitheliocytes and mucocytes, SNA-labeling of mucocytes) reversed the glycosylation changes, caused by COX blockage with indomethacin. In most cases this tripeptide on the background of indomethacin administration had an effect opposite to indomethacin alone but glycosylation changes under these conditions differed significantly also from the control group. These changes may also be explained by masking of relevant carbohydrate determinants of stomach mucosa by other glycans.

A limitation of this study is the lack of data on the independent effect of the tripeptide H-Glu-Asp-Gly-OH on glycosylation processes in gastric mucosa.

Conclusions

Nonselective COX-1/COX-2 blockage with indomethacin causes alteration of glycosylation processes in rat stomach mucosa, mainly reduction of NeuNAc(α2-6)DGal and α-Fuc content. Pretreatment with H-Glu-Asp-Gly-OH under the conditions of COX-1/COX-2 blockage leads to more profound changes in the lectin-binding pattern of the gastric mucosa compared to the independent effect of indomethacin and to control rats, which presumably shows that one of the molecular mechanisms of action of oligopeptides may be interaction with glycoconjugate procession. Lectins may serve as feasible and available molecular probes for the evaluation of glycosylation processes in gastric mucosa.

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

Acknowledgements. The authors are grateful to Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program, the Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association) for their support of our organization as a participating Cedars-Sinai Medical Center.

Data on financial support. The cost of reagents used in this research was covered by the authors.

ЛЕКТИНОЦИТОХІМІЧНЕ ДОСЛІДЖЕННЯ СЛИЗОВОЇ ОБОЛОНКИ ШЛУНКА ЩУРІВ ЗА УМОВ БЛОКУВАННЯ ЦИКЛООКСИГЕНАЗИ-1/2 ТА ВВЕДЕННЯ H-GLU-ASP-GLY-OH

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Дослідження експресії глікокон'югатів на мембранах клітин за допомогою техніки лектинної гістохімії може бути одним із підходів для оцінки функціонального стану клітини. Метою роботи було оцінити зміни вуглеводних детермінант клітинних мембран слизової оболонки шлунка щурів за умов блокування ЦОГ-1/2 індометацином та попередньою обробюкою трипептидом H-Glu-Asp-Gly-OH. Щури-самці лінії Wistar були поділені на 3 групи (n = 6 в групі): 1-а (контроль) отримувала плацебо; 2-а – індометацин (35 мг/кг); 3-я – H-Glu-Asp-Gly-OH (10 мкг) за 30 хв перед введенням індометацину. Через 24 год щурів декапітували. Вуглеводні детермінанти слизової оболонки шлунка (СОШ) визначали з використанням лектинопероксидазної техніки. Панель лектинів включала α-фукозо- (LABA), сіало- (WGA, SNA), манозо- (Con A, LCA) та галактозоспецифічні (HPA, PNA, SBA) лектини. Інтенсивність лектин-рецепторної реакції оцінювали: 0 – відсутня; 1 – слабка; 2 – помірна; 3 – сильна реакція. Блокування ЦОГ-1/2 призводило до розвитку виразкових уражень СОШ, які зменшувалися за дії H-Glu-Asp-Gly-OH. Найспецифічнішою до СОШ були WGA та SNA. Індометацин зменшував зв'язування SNA епітеліоцитами та мукоцитами, та зв'язування LABA головними клітинами. H-Glu-Asp-Gly-OH запобігав змінам процесів глікозилювання, зумовленим блокуванням ЦОГ-1/2, лише по відношенню до зв'язування LABA головними клітинами, LCA – епітеліоцитами та мукоцитами, SNA – мукоцитами. Загалом H-Glu-Asp-Gly-OH за умов блокування ЦОГ-1/2 чинив дію протилежну індометацину, але процеси глікозилювання відрізнялися також і від контрольної групи. Зроблено висновок, що блокування ЦОГ-1/2 змінює процеси глікозилювання в СОШ щурів, зокрема призводить до зниження вмісту NeuNAc(α2-6) DGal та α-Fuc. Дія H-Glu-Asp-Gly-OH за умов блокування ЦОГ-1/2 спричинює більш глибокі зміни зв'язування лектинів СОШ порівняно з самостійною дією індометацину та з контролем.

Ключові слова: лектинна гістохімія, глікозилювання, олігопептиди, H-Glu-Asp-Gly-OH, циклооксигеназа (ЦОГ), виразка шлунка, індометацин.

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