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*Defence factors of mucosa in duodenum ulcer model
and influence fenugreek extract in rats*

Czynniki ochronne śluzówki w modelu wrzodów dwunastnicy u szczurów oraz wpływ
ekstraktu z kozieradki pospolitej

INTRODUCTION

Investigation of properties of biologically active substances provides perspective of their use in pathologies of different genesis. Search for new biologically active substances (BAS), which could increase total resistance of the organism and stimulate development of protective reactions in response to the action of different disturbing factors, became an urgent problem. In this aspect, BAS of plant origin are of special importance. In particular, fenugreek extract is a potential source of BAS complex, which contains galactomanans, amino acids, lipids, unsaturated fatty acids, polysaccharides, steroid sapogenins, alkaloids, flavonoids, vitamins, microelements.

The purpose of this work was to research the effect of fenugreek extract on the state of duodenum mucosa cells in conditions of cysteamine model ulcer in rats. Experiments were carried out on plasma membranes (PM) of duodenum mucosa cells; also, the content of glycoproteins (GP) in parietal mucous of duodenum was evaluated. Lipid composition and the level of activity of membrane bounded enzymes were studied to estimate the structural and functional state of plasmatic membranes (PM).

MATERIAL AND METHODS

Investigations were carried out in acute experiments on male rats. Animals were divided into the groups: first – control; second – animals with cysteamine model ulcer, whose development was controlled by histological examinations; third – the group of animals that were given fenugreek extract (FE) in dose 50 mg/kg during 7 days. A complex of biochemical methods were used,

including preparative centrifugation, enzyme analysis, thin layer chromatography, electrophoresis, spectroscopy.

RESULTS

In the development of pathological processes, there is more attention paid to the destruction of adaptation of the organism as living conditions are changed. One of the conceptions of pathogenesis of ulcerous disease in stomach and duodenum is seen from the point of view of the theory of adaptation [1, 6]. Under the influence of etiological factors some mechanisms that control functional renewal and self-regulation are disrupted.

When duodenum ulcer develops, there are changes in the biochemical structure of the mucus walls. Under the effect of various ulcerous factors there is a change in glycoproteins in mucous membranes of the stomach which plays an important role in protective mechanisms of cells from pathogenic influence of aggressive factors. Because in different pathological conditions there is a destruction of hydrocarbon components of glycoproteins which form protector features, their content has an important value.

Our data show that in cysteamine model duodenum of rats has an increase of galactose 2.7 and fucose 1.8 times, *n-acetyl neuraminic acid* 2.4 times, but a significant decrease in the amount of hexozamines 3 times.

Synthesis and excretion of glycolipids of mucous membranes, their biochemical and structural features can not be seen apart from membrane complex of the cells and their microenvironment.

As our results show (Table 1), in conditions of cysteamine model in duodenum, plasma membranes of the cells has a decrease of total phospholipids (PL) 2.1 time, as well as some fractions of PL: phosphatidyl ethanolamine (PE) 2.6, phosphatidylcholine (PC) and sphingomyeline (SM) 2.7, phosphatidyl inositol (PI) in 1.5 times, but quantity of lyso phosphatidylcholine (LPS) increased 1.5 times. Therefore, the rate of PC/ LPS in comparison to control in animals with cysteamine treatment decreased 3 times.

Table 1. Lipid content (mg/mg protein) in plasma membranes of rat duodenum mucosa cells in experimental cysteamine ulcer model ($M \pm m$; $n = 12$)

Animal groups	Ch	PC	PI	SM	PE	LPC
Control (first)	42.9 \pm 3.2	30.9 \pm 0.5	10.5 \pm 0.3	13.3 \pm 1.2	22.7 \pm 0.7	5.0 \pm 1.1
Cyst. model (second)	85.8 \pm 2.9*	11.4 \pm 0.7*	6.9 \pm 0.9*	4.9 \pm 1.4*	8.7 \pm 0.8*	7.5 \pm 1.3
Cyst.model + FE (third)	47.3 \pm 3.1*	27.9 \pm 2.3**	9.9 \pm 1.1**	12.8 \pm 1.4*	19.8 \pm 1.2**	5.4 \pm 0.7

*Difference between the second group and control is statistically significant ($p < 0.05$);

**Difference between the second group and the third is statistically significant ($p < 0.05$)

When cysteamine model was used the increase of relative quantity of cholesterol (Ch) is noted by 2 times because of decrease of relative content of other lipid components in PM, such as PE, PC, SM, PI. The decrease of PL may be due to lipid peroxidation (POL) or also due to disruption of lipid synthesis and metabolism in cells in experimental models.

Decrease of PC causes changes of phase changes of the membrane that influence membrane transport, transmembrane signal transfer and activity of membrane bound enzymes [2]. PC is one of the main sources of fatty acids that the cell uses for further synthesis of biologically active components like prostaglandins that can also be protective mediators from ulcer.

Today, the importance of apoptosis in pathogenesis of ulcer disease is observed. It was found out that factors that stimulate apoptosis also inhibit synthesis of PC in the cells, which also explains a significant decrease of its content in conditions of experimental ulcerogenesis. Decrease in the content of PE in cell PM may be explained by its high metabolic activity and active introduction of this fraction of PL in synthesis of PC. Also, conditioned rats had a decreased content of PI by 1.5 times compared with the control group of animals that could be related to the activation of phospholipid hydrolysis. PI is a unique bridge between hydrophilic and hydrophobic signal molecules and also it regulates localization of proteins in the membrane.

There was an increase of fraction LPC and a decrease of the levels of PC in experimental models. LPC is a product of hydrolytic breakage of PC that may indicate activation of phospholipase A2.

LPC shows membrane destructive action and its accumulation may be a result of POL of lipids that play an important role in ulcer development. Increase of POL processes results in destruction of lipid bilayer, disruption of membrane penetration, activation of most important enzymes and change in the functional state of membrane receptor complex. Our research showed decrease of the content of SM by 2.7 times compared to the control that relates to the changes of its metabolism in cells of duodenum in experimental model.

Since one of the ways of ceramids formation is dissociation from SM of phospho choline fragment, it can be assumed that a decrease in the content of SM in PM is due to hydrolysis under the action of enzymes sphingomelinases. Signal processes under the action of ceramids play an important role in death of cells via apoptosis in conditions of experimental ulcer formation.

Decrease in the content of SM influences the physicochemical properties of PM, in particular via redistribution of CH and fluidity of microdomains. Changes in sphingolipid content of PM results in modification of intermolecular interactions which influence the function of membranebounded proteins that take part in signal transduction [5].

Therefore, in experimental ulcer formation in rats there is a change in the content of all PL that relates to the injury of PM cells in duodenum.

DISCUSSION

Body has a unique system of biochemical adaptation that forms a certain level of tolerance to stress factors. One of the effective mechanisms that form adaptation of the animals to the aggressive factors of the environment is via the restructuring of the membranes and subcellular components of the cell [3].

In most cases in research that are dedicated to the understanding of the ulcerous disease, the main

attention was to the level of acidity without taking into account the state of mucous-electrolitical protective secretion that not always permits to assess correctly the level of destruction in balance between aggressive factors and protection, and choose the adequate healing method [4].

Cholesterol together with phospholipids are the main lipid components of PM of the cell. Today there is no doubt that properties of the membranes depend not only on their PL content but also on the presence of the Ch. Ch plays a role in the stabilization of cell membranes. Its main function is the formation of a certain level of rigidity necessary for its functioning. Changes in cholesterol content result in significant disturbance of physical properties of the membranes.

Difference in relative rates in various fractions of PM indicate changes in structural and functional organization of mucosa cells in duodenum that may be understood as adaptive or even desadaptive reactions. Accumulation of degraded, metabolically inactive PL complexes such as LPC is characteristic of destabilized membrane.

According to the publications, intensive accumulation of lysed forms of PL in cell membranes relates to the tendencies of transport of these degraded forms of PL in serum with the following elimination from the body or introducing them in processes of resynthesis some cellular PL.

Disrupted lipid exchange lies at the basis of development of destructive processes in membrane structures. Lipid components of membranes are important regulatory factors of membrane enzymes: ionic transporters and cell receptors and, therefore, our next step was to evaluate the activity of main membrane bounded enzymes.

Analysis of the activity of membrane bounded enzymes (5'-nucleotidase, Ca^{2+} , Mg^{2+} -ATPases) in PM of mucosa cells in duodenum indicates that their activity was decreasing in relation to control in conditions of ulcer formation. In cysteamine model of ulcer, the activity of H^+ , K^+ -ATPase in plasma membranes of cells in duodenum was decreasing while the activity of Na^+ , K^+ -ATPase, in turn, was increasing in comparison to the control.

Analysis of our data and data from other scientific sources on the subject of structural and functional status of PM of duodenum in rats in conditions of ulcer formation allows to make a conclusion that effects of lipid content, content of glycolipids, activity of membrane bounded enzymes are parts of the dynamic process in cells of mucosa lining that can coordinate, synchronize and integrate cellular status and regulation of various biochemical processes.

Active protection and prevention from the destructive agents such as exogenic and endogenic formation as well as recovery of cellular homeostasis resulting from regulatory disbalance are made so via adaptive and protective structures and mechanisms.

CONCLUSIONS

Fenugreek extract introduced into animals with cysteamine ulcer resulted in recovery of PL content, decrease of cholesterol, normalization of the activity of the studied membrane bounded enzymes nearly to the control level. Our results show that fenugreek extract may accelerate recovery processes and lead to normalization of structure-functional state of cells in damaged mucosa in the condition of duodenum with ulcer. It is of future benefit to study compounds that would influence various levels of pathogenesis ulcerous disease of stomach and duodenum.

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SUMMARY

The state of duodenum mucosa cells in cysteamine model ulcer in rats and the effect of the fenugreek extract in that conditions were studied.

Keywords: mucosa, duodenum, glycoproteins, plasma membranes, phospholipids, membrane bounded enzymes, biologically active substances

STRESZCZENIE

Zbadano stan komórek śluzówki dwunastnicy w modelu cysteaminowym wrzodów u szczurów oraz wpływ ekstraktu z kozieradki pospolitej.

Słowa kluczowe: śluzówka, dwunastnica, glikoproteiny, błony plazmatyczne, fosolipidy, enzymy związane z błonami, substancje aktywne biologiczne