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Ulcerostatic Effect of *Bacillus mucilaginosus* Exopolysaccharide and its Possible Mechanisms

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The search for and detailed study of stimulators of repair processes in connective tissue are under special investigation at present. As has been shown, during gastric ulcer formation the level of polysaccharide components in the connective tissue of the stomach drops [2]. It may thus be expected that the administration of certain polysaccharides to the organism will produce an ulcerostatic effect. In this respect, investigation of exopolysaccharides from *Bacillus mucilaginosus*, which possess a biostimulating activity, seems rather promising [7,10]. Here, we report on a study of the ulcerostatic effect of *Bacillus mucilaginosus* polysaccharide.

MATERIALS AND METHODS

The experiments were performed on nonpedigree albino rats weighing 150-180 g. Gastric ulcer was induced by the acetate me- thod [9]. Polysaccharide from extracellular mucus of *Bacillus mucilaginosus* was isolated by precipitation, separation from ballast material and freeze-drying. Starting from the first day of the experiment, the animals received intraperitoneal injections of an aqueous solution of the polysaccharide (PS) in a dose of 5 mg/kg/day. The animals were divided into groups of 10 rats and were decapitated on the 7th, 14th, and 28th day. The stomach was removed and the area of lesion was measured. The ulcerated regions were subsequently excised and immersed in liquid nitrogen prior to monitoring the development of the connective tissue components.

The quantitative method was employed to study the glycopro- tein exchange in each group of aminals: the lesion tissue was dehydrated in acetone and delipidated subsequently in an ethanol- chloroform mixture and ethanol. The samples were homogenized and hydrolyzed. The total concentration of hexoses and hexuronic acids was determined in the first neutralized hydrolysate (0.5 n HCl, 100°C, 15 min). One of the homogenized samples was used for fractionation of glucosaminoglycans (GAG). Another sample was used for determination of sialic acids [5, 6, 8]. The effect of PS was compared to that of methyluracil (MU), which stimulates cell regeneration and lesion healing [1].

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An analogous experiment performed on untreated rats served as the control. The results obtained for intact animals were taken as the standard. The data were processed statistically using Student's t test.

RESULTS

The experiments showed that treatment with PS and MU accelerates the repair of experimental acetate-induced ulcer. The polysaccharide compared favorably with methyluracil in efficacy (Fig. 1).

The biochemical data on dynamic variations in the concen- tration of collagen (assessed from the oxyproline level) and noncollagen proteins (according to the tyrosine level) in the ulcerated tissue are presented in Table 1. We see that in the control collagen was rapidly accumulated by the 14th day after the operation and by the end of the observation its content exceeded the standard values approximately 1.5 times. On the other hand, the concentration of noncollagen proteins decreased, resulting in a lower tyrosine/oxyproline ratio of 0.92 compared to 1.91 in the intact wall of the stomach. In other words, in the ulcerated regions of the stomach fibrous tissue formed with a disturbed ratio of connective tissue proteins. At the same time, the hexose content did not change. This testified indirectly that the secretory activity of the gastric tissue remained at the same level.

Against the background of MU treatment the accumulation of collagen proceeded as in the control. However, MU influenced the level of noncollagen proteins and ultimately it led to an equalization of the tyrosine and oxyproline content. This is one of the pharmacological phenomena of

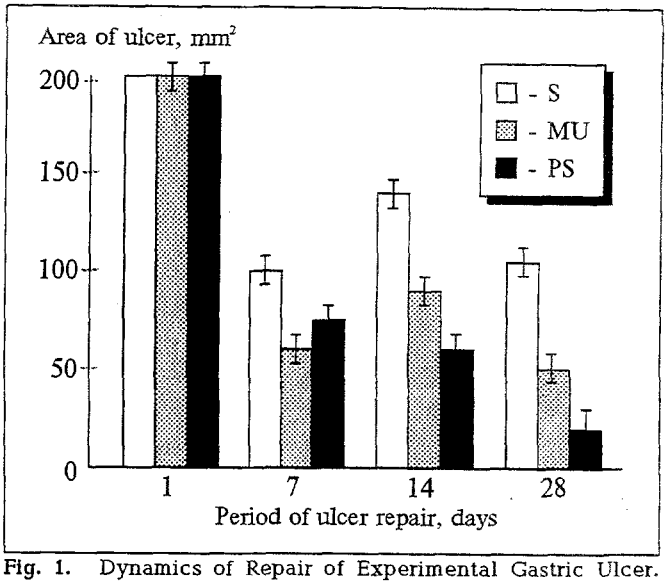

Fig. 1. Dynamics of Repair of Experimental Gastric Ulcer. S: standard (untreated animals); MU: control (animals treated with methyluracil); PS: experiment (animals treated with polysaccharide).

MU in the treatment of ulcers (Table 2). The effects of MU on the biochemical dynamics in gastric tissue during the development of an experimental acetate- induced ulcer and their interpretation were discussed in detail earlier [3, 4].

The dynamics of ulcer formation against the background of PS treatment reliably differed from that in the group of untreated animals and those treated with MU. The level of biochemical components in the stomach wall differed from the analogous indexes in the other groups (Table 3). In particular, the dynamics of the hexose level indicates that PS enhanced gastric secretion in the early stages of the process and produced a normal-

TABLE 1. Dynamics of Biochemical Indexes in Control Tissue of Acetate-Induced Gastric Ulcer (g/100 g Dried Delipidated Tissue, $M \pm m$)

Index	Period of ulcer formation, days		
	7	14	28
DNA (4.25±0.11)	4.35±0.07	4.39±0.06	4.01±0.01
RNA (0.98±0.05)	1.11±0.02*	1.07±0.07*	1.50±0.05*
Hydroxyproline (1.51±0.03)	1.44±0.04	1.93±0.02*	2.65±0.02*
Hydroxylysine (0.32±0.06)	0.32±0.01	0.32±0.01	0.25±0.01
Tyrosine (2.64±0.05)	2.41±0.03*	2.55±0.02*	2.48±0.02*
Arginine (3.88±0.2)	3.55±0.02	4.45±1.14*	4.25±0.24*
Hexosamines (1.28±0.03)	1.37±0.01*	1.42±0.03*	1.61±0.05*
Hexoses (1.58±0.04)	1.79±0.05*	1.73±0.09*	2.21±0.05*
Hexuronic acids (0.73±0.02)	0.75±0.01	0.66±0.02*	0.73±0.01
GAG fractions			
0.4 M NaCl (0.49)	0.42	0.32	0.41
1.2 M NaCl (0.19)	0.24	0.28	0.24
2.1 M NaCl (0.05)	0.09	0.06	0.08
Sialic acids (0.39±0.01)	0.70±0.04*	0.59±0.01*	0.52±0.01*

Note. Standard indexes for the intact wall of the stomach are indicated in parentheses; here and in Tables 2 and 3: * – significance of differences in comparison with the standard, $p<0.05$.

TABLE 2. Dynamics of Biochemical Indexes in Tissue of Acetate–Induced Gastric Ulcer under the Influence of Methyluracil

Index	Period of ulcer formation, days		
	7	14	28
DNA	4.31±0.09	4.39±0.09	4.54±0.11*
RNA	1.05±0.05*	1.14±0.05*	1.11±0.06*
Hydroxyproline	0.70±0.09	0.42±0.12	1.65±0.14
Hydroxylysine	0.41±0.05*	0.44±0.07*	0.33±0.04
Tyrosine	1.63±0.17	1.81±0.16	1.66±0.21
Arginine	3.95±0.15	4.29±0.18	4.51±0.21*
Hexosamines	0.77±0.13	0.76±0.16	0.88±0.12
Hexoses	1.78±0.04	1.91±0.03	2.09±0.15
Hexuronic acids	0.81±0.01	0.92±0.02*	0.90±0.01*
GAG fractions			
0.4 M NaCl	0.39±0.01	0.41±0.02	0.33±0.01
1.2 M NaCl	0.27±0.01	0.35±0.02	0.32±0.02
2.1 M NaCl	0.15±0.01	0.16±0.01	0.25±0.01
Sialic acids	0.68±0.08	0.61±0.05	0.47±0.05

izing effect later on. In addition, PS reduced inflammatory discharge (this being most evident in the middle of the observation period from the level of sialic acids), prevented the initial drop of the collagen concentration in the ulcerated and adjacent regions toward the 7th day, and accelerated scar formation by the end of the observation, when the collagen concentration in the control again decreased.

Thus, the revealed dynamics in the level of nucleic acids, collagen, noncollagen proteins, hexoses, and glucosaminoglycans points to their important role in the pathogenesis of experimental acetate-induced gastric ulcer, opens the door to the application of *Bacillus mucilaginosus* exopolysaccharide as an ulcerostatic remedy, and sheds light on various aspects of the mechanism of ulcerostatic action of different drugs.

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TABLE 3. Dynamics of Biochemical Indexes in Tissue of Acetate–Induced Gastric Ulcer under the Influence of Polysaccharide (g/100 g Dried Delipidated Tissue, $M\pm m$)

Index	Period of ulcer formation, days		
	7	14	28
DNA	4.28±0.005	4.47±0.13	3.88±0.26
RNA	1.08±0.033	1.06±0.02	0.86±0.04*
Hydroxyproline	1.65±0.028*	1.68±0.02*	1.85±0.03*
Hydroxylysine	0.37±0.013*	0.32±0.01	0.33±0.001*
Tyrosine	2.54±0.041*	2.52±0.01	2.50±0.04
Arginine	3.88±0.25	3.88±0.13*	4.65±0.15
Hexosamines	1.34±0.24	1.48±0.049	1.36±0.033*
Hexoses	2.30±0.156*	1.92±0.055	1.75±0.126*
Hexuronic acids	0.86±0.036*	0.69±0.020	0.63±0.011*
GAG fractions			
0.4 M NaCl	0.48	0.43	0.40
1.2 M NaCl	0.26	0.19	0.18
2.1 M NaCl	0.12	0.07	0.05
Sialic acids	0.62±0.018	0.52±0.025*	0.50±0.009

Note. * – significance of differences in comparison with the control, $p<0.01$.