Gastroprotective Effect of Natural Non-Starch Polysaccharides

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Experiments on mice and rats with modeled neurogenic and indomethacin-induced injuries to the gastric mucosa showed that preventive courses of calcium alginate, calcium pectate, and low-esterified pectin improved the resistance of the gastroduodenal mucosa to the destructive effect of the above factors.

Key Words: *experimental ulcerogenesis; non-starch polysaccharides*

Non-starch polysaccharides, belonging to low-esterified pectins, are perspective sources for the creation of new drugs, including those for the treatment of gastrointestinal diseases. According to published data, combined protocols for peptic ulcer treatment include successive use of antacid-alginate complex and H_2-histamine receptor blockers. Sodium alginate solution and colloid bismuth pectin preparation are effective in the treatment of *Helicobacter pylori*-positive duodenal ulcer [11]. Compositions of alginic acid or alginates with antacids are used in practical gastroenterology for the prevention of gastroesophageal reflux and arresting heartburn [12]. It was shown that pectin increased the height of intestinal villi and depth of small intestinal cryptae, increased plasma level of enteroglucagon, and stimulated the production of monocarbonic fatty acids in animals with experimental colitis [9].

We studied the effects of calcium alginate, calcium pectate, and low-esterified pectin on the development of neurogenic and indomethacin-induced lesions in rat and mouse gastric mucosa.

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MATERIALS AND METHODS

Experiments were carried out on 69 outbred female mice (29-30 g) and 81 outbred rats of both sexes (250-300 g) from Laboratory of Experimental Biosimulation, Institute of Pharmacology. The animals were handled in accordance with the regulations adopted by the European Convention for Protection of Vertebrates Used for Experimental and Other Research Purposes (Strasbourg, 1986).

Calcium alginate (77.3% alginic acids and 72.5 mg/g sample calcium, mol. weight 403 kDa), low-esterified pectin (69% anhydrogalacturonic acid, 1.2% esterification, mol. weight 39.3 kDa, ion-exchange capacity 3.92 mg/eqv/g), and calcium pectate (67.3% anhydrogalacturonic acid, 38 mg/g sample calcium, esterification below 1.2%, mol. weight 39.3 kDa) were used. For prophylactic purposes the preparations were administered intragastrically in doses 50 and 100 mg/kg to mice (4 days) and in doses of 25 and 50 mg/kg to rats (8 days). The choice of effective doses was based on published data and preliminary studies [4,5]. Taking into account the role of stress as the "triggering mechanism" in the development of gastroduodenal ulcers [6], the neurogenic injury to the gastric mucosa was induced by partial immobilization of mice (22-h fixation by the cervical fold with forceps [1]).

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Injury to the gastric mucosa was induced by a single intragastric dose of indomethacin (60 mg/kg in 1 ml saline) after a course of preventive treatment (on day 8) [7]. The mice received the last dose of polysaccharides 1 h before immobilization and the rats were treated 1 h before indomethacin. The animals were sacrificed by ether overdosage.

The number and length of lesions in isolated gastric mucosa were macroscopically evaluated; the lesions were differentiated into punctate, large, and strip-like. The mean number of ulcers per animal in the group and the percentage of animals with ulcers were estimated. Pauls index (PI) was determined as the integral indicator of the number of lesions by the formula: mean number of ulcers×% of animals with ulcers/100%. Antiulcer activity (AA) of the preparations was evaluated as follows: the control group PI was divided by the experimental group PI [2]. The test preparation was considered active if PA was at least two units. The degree of gastric mucosal lesions (DL) and severity of lesions were characterized by calculating the summary length of all lesions in the gastric mucosa (in mm) in each rat. The severity of lesions was evaluated as follows: control group DL-experimental group DL/control group DL×100% [7].

The results were statistically processed using Wilcoxon—Mann—Whitney nonparametric tests and Fisher's angular transformation. The differences were considered significant at $p<0.05$.

RESULTS

Polysaccharide treatment appreciably prevented the formation of lesions in mouse gastric mucosa under conditions of immobilization stress. Calcium alginate in doses of 50 and 100 mg/kg reduced the number of animals with ulcers (40% *vs.* 60% in control group) and the mean number of ulcers per animal (Table 1). Similar shifts were observed after treatment with calcium pectate in doses of 50 and 100 mg/kg, which was seen from AA values. The gastroprotective effect of pectin in a dose of 50 mg/kg was realized at the expense of decreased incidence of ulcer formation and decreased (6.7 times) number of lesions in comparison with the controls. Pectin in a dose of 100 mg/kg exhibited significant antiulcer activity (7.5 units), which manifested in decreased number of animals with ulcers and number of lesions per mouse in comparison with untreated animals (Table 1).

A special aspect in the treatment of gastroduodenal ulcers is the ulcerogenic effect of nonsteroid antiinflammatory drugs [3,6]. Treatment with calcium alginate, low-esterified pectin, and calcium pectate reduced the number and severity of gastroduodenal lesions in rats (Tables 2, 3). Treatment with calcium alginate in a dose of 25 mg/kg decreased the number of large ulcers ($p<0.01$) and the mean number of ulcers ($p<0.05$). The number of animals with strip-like ulcers and the number of these ulcers decreased (by 1.5 times; $p<0.05$) in comparison with untreated animals (Table 2). Summary length of all lesions per rat decreased by 1.8 times ($p<0.01$), due to which the index of severity of mucosa lesions decreased by 43.54%. Calcium alginate in a dose of 50 mg/kg reduced the degree and severity of lesions of the gastric mucosa (by 27.81%; Table 3). Pectin in a dose of 25 mg/kg significantly reduced the number of large and strip-like ulcers (5.3 and 2.1 times, respectively) and the mean number of lesions in comparison with untreated animals (Table 2). Pectin in a dose of 50 mg/kg also significantly reduced the incidence of lesions of all types (Table 2). These data indicate high efficiency of the preparation, manifesting in a signi-

TABLE 1. Effects of Preventive Courses of Calcium Alginate, Calcium Pectate, and Low-Esterified Pectin on Neurogenic Ulcer Formation in Outbred Female Mice

Group, drug dose		Percentage of mice with ulcers	Number of ulcers, $X\pm m$			Number of ulcers per mouse, $X\pm m$	PI	AA
			large	strip-like	punctate			
Stress control (n=10)		60	0	0.40±0.40	1.60±0.70	2.00±0.71	1.20	—
Alginate	50 mg/kg (n=10)	40	0	0	0.40±0.16	0.40±0.16	0.16	7.5
	100 mg/kg (n=10)	40	0	0	0.60±0.31	0.60±0.31	0.24	5.0
Pectate	50 mg/kg (n=9)	22.2*	0	0	0.22±0.15	0.22±0.15	0.05	24.0
	100 mg/kg (n=10)	40	0	0	0.90±0.41	0.90±0.41	0.36	3.3
Pectin	50 mg/kg (n=10)	30	0	0	0.30±0.15	0.30±0.15	0.09	13.3
	100 mg/kg (n=10)	40	0.10±0.10	0	0.30±0.15	0.40±0.16	0.16	7.5

Note. Here and in tables 2, 3: number of animals is shown in parentheses. *$p<0.05$, **$p<0.01$ compared to the control (stress control).

TABLE 2. Effects of Preventive Courses of Calcium Alginate, Low-Esterified Pectin, and Calcium Pectate on the Development of Indomethacin Lesions in the Gastric Mucosa of Outbred Rats

Group, drug dose		Number of ulcers, $X \pm m$			Number of ulcers per mouse, $X \pm m$	PI	AA
		large	strip-like	punctate			
Series I (male rats)							
control (n=11)		11.36±1.77	7.09±1.28	16.00±2.35	34.45±3.21	34.45	—
alginate	25 mg/kg (n=10)	5.20±1.47**	4.60±1.19*	12.50±2.17	22.30±3.65*	22.30	1.54
	50 mg/kg (n=11)	8.55±1.43	6.18±1.51	14.45±1.89	29.18±3.30	29.18	1.18
Series II (female rats)							
control (n=9)		10.00±1.79	12.33±1.52	25.89±1.65	48.22±2.11	48.22	—
pectin	25 mg/kg (n=10)	1.90±0.89**	5.80±1.44**	15.40±1.93**	23.10±2.75**	23.10	2.09
	50 mg/kg (n=10)	1.50±0.45**	8.70±1.22*	16.80±2.99*	27.00±3.45**	27.00	1.79
pectate	25 mg/kg (n=10)	2.20±0.85**	9.10±1.39	15.10±2.03**	26.40±2.90**	26.40	1.83
	50 mg/kg (n=10)	6.27±0.60**	9.10±1.24	13.60±2.16**	23.30±2.62**	23.30	2.07

ficant reduction of the severity of lesions in the gastric mucosa of treated rats in comparison with the control group (Table 3).

Calcium pectate in a dose of 25 mg/kg significantly reduced the number of large ulcers (by 4.5 times) and the number of animals with this type of destruction (by 40%) in comparison with the control group (Table 2). Similar changes were observed with regard to strip-like and punctate lesions: their number decreased by 1.4 (p<0.01) and 1.7 (p<0.01) times, respectively. The use of this polysaccharide in a dose of 50 mg/kg reduced the number of animals with strip-like ulcers (by 60%), with large (by 16.7 times, p<0.01) and punctate lesions (by 1.9 times, p<0.01). The degree and severity of lesions decreased significantly (by 39.4 and 39.2%, respectively) (Table 3).

The cytoprotective effects of polysaccharides, which are due to the formation of gel on the surface of gastric mucosa, can play the key role in the realization of their antiulcerogenic effect [4,5]. It was shown that non-starch polysaccharides, for example, calcium alginate and pectin, inhibit the formation of LPO products, thus reducing the degree of the prooxidant/antioxidant imbalance during exposure to ulcerogenic factors [10]. Presumably, the gastroprotective effect of non-starch polysaccharides is realized at the expense of the biological activity of products of their enteric enzyme cleavage — monocarbonic acids (acetic, propionic, and butyric), providing up to 10% of basal energy requirement of the gastroduodenal epithelial cells, this significantly reducing the level of energy metabolism disorganization in the pathogenesis of ulcer for-

TABLE 3. Effects of Preventive Courses of Calcium Alginate, Low-Esterified Pectin, and Calcium Pectate on the Degree and Severity of Gastric Mucosal Lesions in Rats on the Model of Indomethacin Ulcerogenesis

Group, drug dose		Mean length of lesions ($X \pm m$), mm			DL of gastric mucosa ($X \pm m$), mm	Severity of gastric mucosal lesions, %
		strip-like	large	punctate		
Series I (male rats)						
control (n=11)		29.14±6.40	22.73±3.55	8.00±1.18	59.87±7.71	100
alginate	25 mg/kg (n=10)	17.15±6.25*	10.40±2.93**	6.25±1.09	33.80±3.42**	56.46**
	50 mg/kg (n=11)	18.91±5.95	17.09±2.86	7.23±0.95	43.22±5.20*	72.19**
Series II (female rats)						
control (n=9)		20.00±5.50	44.67±6.57	12.94±0.82	77.61±4.71	100
pectin	25 mg/kg (n=10)	3.80±1.78**	20.55±4.52**	7.70±1.00**	32.05±4.80**	41.3**
	50 mg/kg (n=10)	3.00±0.91**	34.70±6.08**	8.40±1.50*	46.10±6.00**	59.4**
pectat	25 mg/kg (n=10)	4.40±1.71**	35.05±4.75	7.55±1.02**	47.00±5.91**	60.6**
	50 mg/kg (n=10)	1.20±0.53**	39.15±6.57	6.80±1.08**	47.15±6.40**	60.8**

mation [8]. Short-chain fatty acids improve microcirculation in the gastric mucosa and intestinal motility. Due to stimulation of epithelial cell proliferation, monocarbonic acids promote healing of the damaged surface [9]. The protective effect of alimentary polysaccharides, preventing rectal cancer, is attributed to the formation of monocarbonic acids [4,5]. The detected gastroprotective activity on the model of neurogenic injury to the mucosa can play an important role in the prevention and therapy of gastroduodenal ulcer, which is now referred to dysadaptation diseases.

Hence, preventive course of low-esterified pectin, calcium pectate and alginate appreciably improved the resistance of the gastric mucosa in mice and rats to the ulcerogenic exposure on the models of neurogenic and indomethacin injuries, this suggesting further studies of the gastroprotective activity of these preparations on other models.

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