Gastroprotective Effect of Nonstarch Polysaccharide Calcium Pectate under Experimental Conditions

S. G. Krylova, L. A. Efimova, E. P. Zueva, M. U. Khotimchenko*, E. N. Amosova, T. G. Razina, K. A. Lopatina, and U. S. Khotimchenko**

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Course prophylactic administration and single therapeutic treatment with calcium pectate improved resistance of rat gastroduodenal mucosa to ethanol-induced ulcers, prednisolone-induced ulcers, and H. Shay ulceration of the gastric mucosa.

Key Words: *experimental ulcerogenesis; calcium pectate*

Studying of the relationships between the structure of chemical compounds and their effect on living organisms is a fundamental problem of theoretical pharmacology. Pectin substances are a convenient model for evaluation of these relationships. These compounds serve as a promising source of new medicinal preparations and bioactive food additives. Moreover, they can be used in modern pharmaceutical technologies [6]. Structural characteristics of polysaccharide molecules contribute to a variety of physicochemical properties of pectins, which is manifested in biological and pharmacological activity. Pharmacotherapeutic activity of pectins includes the hypolipidemic, antiproliferative, bactericidal, immunomodulatory, antimutagenic, antitumor, hepatoprotective, disintoxication, and antiviral effects [5,9,13]. However, little is known about the relationship between the structure and pharmacological activity of pectins. Significant differences in the results and interpretation of studies are related to the absence of standardized pectin preparations.

Our previous experiments showed that nonstarch polysaccharide calcium pectate with certain physicochemical properties produces a strong gastroprotective effect in mice and rats during neurogenic and indomethacin-induced injury to the gastric mucosa [2].

Here we studied the preventive and therapeutic effects of nonstarch polysaccharide with a certain structure on the model of ulcerogenesis.

MATERIALS AND METHODS

Experiments were performed on 114 male and female outbred rats weighing 250-300 g and bred at the Laboratory of Experimental Biological Modeling (Institute of Pharmacology). The study was conducted according to the rules of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986).

Calcium pectate was obtained from commercial preparation of highly esterified citrus pectin (Copenhagen Pectin A/S, Lille Scensved). Pectate contains 67.3% anhydrogalacturonic acid and 38 mg/g calcium sample (esterification 1.2%, molecular weight 39.3 kDa).

The study of ethanol-induced ulcers and H. Shay ulceration included the pretreatment with calcium pectate in doses of 25 and 50 mg/kg. Famotidine and Maalox served as the reference drugs and were administered in daily doses of 5 and 317

Institute of Pharmacology, Tomsk Research Center, Siberian Division of the Russian Academy of Medical Sciences; *Institute of Sea Biology, Far Eastern Division of the Russian Academy of Sciences, Vladivostok; **Vladivostok State Medical University, Russia. ***Address for correspondence:*** zep0929@mail.ru. E. P. Zueva

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mg/kg, respectively, for 7 days (E. J. Freireich). The last dose was given 1 h before ulcerogenic exposure [1,3]. Reference drugs and effective doses were selected from published data and results of preliminary studies [1,2]. In experiments with steroid-induced gastric ulcers, prednisolone (20 mg/kg) was dissolved in 80% ethanol (8 ml/kg) and administered into the stomach (single treatment). Sea-buckthorn oil (2.5 mg/kg) and calcium pectate (25 and 50 mg/kg) were administered one time through the probe 3 h after ulcerogenic treatment [4]. H. Shay ulceration is associated with combined effect of neurodystrophic and acid-peptic aggressive factors of gastric juice. White line laparotomy was performed under light ether anesthesia. The pylorus was ligated. The tissues were sutured layer-by-layer. Ethanol ulcers were induced by single administration of 1 ml 96° ethanol per 200 g body weight [8]. The animals were euthanized by ether overdose (1 h after ethanol administration, 4 h after surgery, 24 h after prednisolone treatment). The number and area of gastric mucosal injuries were estimated under a microscope. They were classified into pinpoint, large, and band-like injuries. The number and mean area of ulcers were estimated. The degree of gastric mucosal injury was evaluated from the total area of ulcers in each rat. The severity of ulceration was expressed as a ratio of the degree of gastric mucosal injury in treated and control rats (%).

The significance of differences was estimated by nonparametric Mann—Whitney *U* test. The differences were significant at $p<0.05$.

RESULTS

Ethanol-induced damage to the gastric mucosa in control rats was manifested in the formation of deep and extended hemorrhagic erosions and ulcers, circulatory dysregulation, and severe spasm of submucosal venules (Table 1). Famotidine significantly decreased the mean area of pinpoint ulcers and prevented the formation of large lesions, but increased the area of band-like ulcers ($p<0.01$ compared to control specimens). The degree and severity of gastric mucosal injury increased in famotidine-treated rats (by 3.4 times and 241%, respectively; Table 1). Low effectiveness of selective H_2 receptor antagonist reflects insufficient effect on the acid-peptic factor on this model of ulcerogenesis.

Evaluation of the gastroprotective effect of calcium pectate revealed antiulcer activity of nonstarch polysaccharide in doses of 25 and 50 mg/kg (compared to the reference drug). Calcium pectate significantly decreased the mean area of lesions. The degree

TABLE 1. Effects of Calcium Pectate and Reference Drugs on Ulcerative Lesions of the Gastric Mucosa in Rats with Ethanol-Induced Ulcers, Prednisolone-Induced Ulcers, and H. Shay Ulceration

Group, dose	Mean area of lesions, mm²			Area of injury to the gastric mucosa, mm²	Severity of injury to the gastric mucosa, %
	pinpoint	band-like	large		
Ethanol-induced injury (male rats)					
Control (*n*=10)	2.82±0.52	106.5±55.0	1.26±0.96	110.6±54.9	100
Famotidine, 5 mg/kg (*n*=10)	1.12±0.57*	376.0±100.4*	0	377.1±100.0	341
Pectate, 25 mg/kg (*n*=10)	5.49±1.02*+	82.4±22.1+	0	87.8±22.0+	79
Pectate, 50 mg/kg (*n*=10)	3.39±0.46+	78.7±18.5+	0.32±0.32	82.5±18.6+	75
Prednisolone-induced injury (female rats)					
Control (*n*=10)	3.80±1.81	82.28±58.61	5.50±1.48	91.6±57.6	100
Sea-buckthorn oil, 2.5 ml/kg (*n*=10)	2.48±0.93	63.85±47.15	4.84±2.55	71.2±47.2	78
Pectate, 25 mg/kg (*n*=10)	3.08±0.45	5.75±2.61*	0.31±0.31**	9.1±2.6*xx	10
Pectate, 50 mg/kg (*n*=10)	3.29±0.83	3.34±2.24*x	1.02±1.02	7.7±2.2*xx	8
H. Shay ulceration (female rats)					
Control (*n*=9)	3.22±0.83	10.22±3.23	4.45±2.45	17.89±3.57	100
Maalox, 317 mg/kg (*n*=7)	0.50±0.19**	5.00±1.87	4.03±2.17	9.54±2.67	53
Pectate, 25 mg/kg (*n*=9)	1.72±0.64	6.60±3.02	2.44±1.18	10.76±3.76*	59
Pectate, 50 mg/kg (*n*=9)	2.00±0.76	2.56±1.36**	1.74±1.06	6.30±1.45*	35

Note. *n*, number of animals per group. *$p<0.01$ and **$p<0.05$ compared to the control; +$p<0.01$ compared to famotidine; x$p<0.01$ and xx$p<0.05$ compared to sea-buckthorn oil.

and severity of injuries to the gastric mucosa decreased by 4.3 and 4.6 times, respectively ($p<0.01$).

An important problem of prevention and therapy for ulcer disease of the stomach and duodenum is ulcerogenic activity of glucocorticoids. These drugs are widely used in cardiac and antirheumatic therapy [1,7]. Hyperemia, surface hemorrhages, high vascularization, and severe erosions and ulcers of the gastric mucosa were revealed in untreated animals with steroid-induced gastric ulcers. Mild hyperemia of the gastric mucosa was also found in rats receiving sea-buckthorn oil. The animals of this group were characterized by moderate hemorrhage, vascularization, and mild erosions and ulcers. The area of lesions tended to decrease in these animals (Table 1). The pharmacotherapeutic effect of sea-buckthorn oil manifested in a 22% decrease in the severity of gastric mucosal injury in rats.

The rats receiving calcium pectate in doses of 25 and 50 mg/kg had rose-colored gastric mucosa and small hemorrhages. Erosions and ulcers were rarely revealed. Single treatment with polysaccharide reduced the severity of injury in animals with steroid-induced ulcers. Calcium pectate in a dose of 25 mg/kg decreased the area of band-like and large ulcers by 14.3 and 17.7 times, respectively ($p<0.05$). These parameters decreased by 24.6 ($p<0.01$) and 5.4 times ($p>0.05$), respectively, after administration of calcium pectate in a dose of 50 mg/kg. The degree and severity of gastric mucosal injury in calcium pectate-treated rats were lower compared to the control ($p<0.01$) and sea-buckthorn oil group ($p<0.05$).

An important aggressive factors during ulceration is increased effect of the acid-peptic factor due to enhanced production of hydrochloric acid and pepsin.

Study of H. Shay ulceration showed that this treatment contributes to the formation of all types of ulcerative lesions in 100% control rats (Table 1). The protective effect of the reference drug Maalox manifested in a 6.4-fold decrease in the area of pinpoint lesions ($p<0.05$). Maalox tended to decrease the area of band-like lesions and degree of gastric mucosal injury (by 2 and 1.9 times, respectively, $p>0.05$). The severity of ulceration in Maalox-treated rats was 47% lower than in control specimens. The course of pretreatment with calcium pectate in doses of 25 and 50 mg/kg reduced the severity of ulceration in rats after pyloric ligation. The degree and severity of gastric mucosal injury in animals of the calcium pectate group significantly differed from the control, but were similar to those in Maalox-treated specimens (Table 1). Non-starch polysaccharide are characterized by high gastroprotective activity on various models of ulceration.

Calcium pectate produced a combined pharmacological effect during prednisolone-induced ulcers, ethanol-induced ulcers, and H. Shay ulceration. It can be related to the influence of calcium pectate on various pathogenetic stages, including a decrease in aggressiveness of the acid-peptic factor (cytoprotective activity of gel), antiinflammatory and reparative effect of monocarboxylic acids, increase in the resistance of the gastric mucosa due to antioxidant properties of polysaccharide, and improvement of energy production and microcirculation in the wall of the gastroduodenal mucosa [9,10, 11,13].

The antiulcer effect of calcium pectate is probably associated with gel formation. This ability differs in pectins with various degrees of esterification. Cation-binding activity of pectins is related to the presence of nonesterified carboxylic groups in galacturonic acid residues [5,6]. In the presence of calcium, demethylated pectins form the gel due to ionic cross-links between homogalacturonan chains. The mechanism of gel formation is poorly understood. The egg-box model was described [12]. This model suggests the formation of cross-linked contact zones between calcium and at least 6 adjacent nonesterified residues of galacturonic acid. Polymer mesh is capable of retaining the molecules of water. Gel-forming activity and physicochemical properties of pectins are determined by the degree of esterification and interaction of carboxylic groups in galacturonic acid with bivalent electrons (primarily with calcium).

Our results indicate that course prophylactic treatment and single therapeutic administration of calcium pectate significantly increase the resistance of the gastroduodenal mucosa to aggressive factors of ulcerogenesis (ethanol-induced ulcers, prednisolone-induced ulcers, and H. Shay ulceration). Gastroprotective activity and mechanism for the effect of nonstarch polysaccharide with a certain structure require further investigations.

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