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Gastroprotective effect of zosterin, a pectin from seagrass *ZOSTERA MARINA L.*

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SUMMARY

Zosterin is a pectin from a seagrasses of the family *Zosteraceae*. Zosterin was given to rats intragastrically once 1h before the emotional stress or injection of indomethacin, or administration of 2, 4-D solution daily for seven days at dose of 100 mg/kg. The data obtained demonstrate that zosterin enhances resistance of the stomach tissue to various ulcerogenic factors (emotional stress, indomethacin, pesticide 2, 4-D). It was shown to possess a gastroprotective effect, which is accompanied by diminution of the number and sizes of destructive regions in the gastric mucosa during the ulcer affection, as well as reduction of ATP and glycogen deficit, decrease of lactate excess, and normalization of the energy balance in the gastric mucosa. According to its antiulcer effect, zosterin may be recommended for application in prevention and treatment of stomach diseases together with the basic therapy.

Key words: Zosterin; Pectin; Emotional stress; Indomethacin; Pesticide 2, 4-D; Gastric mucosa damage

INTRODUCTION

Gastric ulcer is widespread disease dominating in gastroenterologic pathology. Numerous clinical and experimental observations revealed some etiological factors promoting the development and progress of the ulcer disease, such as neuropsychologic, medicinal chemical and other ones (Dixon, 1994; Jorens *et al.*, 1995; Roth, 1996; Spirt, 2004). At present, numerous positive data concerning the application of pectins in prophylaxis and complex therapy of ulcer disease are accumulated (Cummings *et al.*, 1992; Thakur *et al.*, 1997; Nie *et al.*, 1999; Galati *et al.*, 2002). They possess mainly enveloping and adsorbing action, normalize stomach motility, improve blood supply of the stomach mucous membrane and reduce the degree of in- fluence of gastric chyme to the mucous membrane of the gastrointestinal system (Sun *et al.*, 1991, 1992; Figler *et al.*, 1999; Venner *et al.*, 1999).

A pectin from a seagrasses of the family *Zosteraceae* named "zosterin" attracted our attention, as a gastroprotective remedy. Its structure, physical-chemical properties and pharmacokinetics are well studied (Popov *et al.*, 1990; Ovodov, 1998). Like other pectins, zosterin is characterized by the presence in the chemical structure of polysaccharide chains composed of residue of galacturonic acid connected by α-1→4-glycoside bonds. One of the specific features of this pectin is the low degree of esterification in its molecule (Ovodov *et al.*, 1975). The carbohydrate chain of zosterin was found to contain the residues of D-galacturonic

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acid (60-65%) and D-apiose (20-25%) as the main sugar constituents, as well as the residues of D-galactose, L-arabinose, D-xylose and 2-O-methyl-D-xylose in comparatively small amounts.

Experimental investigations with zosterin revealed such pharmacological effects as antiviral, antibacterial, antioxidant, actoprotective (increasing physical capacity of work), hypolipedemic, antitumor, immunomodulatory, as well as intensification of synthesis of nucleic acids (Loyenko *et al.*, 1997; Khasina and Tiupeleev, 1999; Khasina *et al.*, 2001; Sgrebneva *et al.*, 2002; Khasina *et al.*, 2003). Clinical trial has shown its efficiency in a complex treatment of gastroduodenal pathology (Miroshnitchenko *et al.*, 1998). In preventive and clinical medicine zosterin has found application as an antidote at a lead intoxication (Loyenko *et al.*, 1997).

In this paper we studied the gastroprotective effect of zosterin on selected models of the acute and chronic damage of rat stomach.

MATERIALS AND METHODS

Pectin medicine

The zosterin, low-esterified pectin from the seagrass *Zostera marina L.*, was obtained from Vostokpharm Co Ltd, Vladivostok, Russia. Physical-chemical properties of pectin were as follows: concentration of galacturonic acid was 74.8%, degree of esterification - 5.7%, and characteristic viscosity - 340 ml/g of galacturonan. The apple pectin "Medetopect" (SANOFI-WINHROP, France) was used as a medicine for comparison. Zosterin and medetopect were given to animals at a dose of 100 mg/kg.

Animals and diet

The experiments were performed on 77 male Wistar rats with body weights of 160-170 g (≪Stolbovaya≫ Nursery of the Ministry of Health of the Russian Federation). Animals were divided randomly into eleven experimental groups and housed by seven per plastic cage (45×35×20 cm) under the controlled conditions at 19-21℃, 80± 10% humidity, and 12 h light/dark cycle. The standard vivarium ration and water were freely available, ad libitum. The maintenance of animals corresponded to the principles of The Council of European Communities (86/609 EEC).

Experimental design

Gastroprotective effect of zosterin was studied using three selected methods of stomach damage. Control 1 group rats received physiological solution. Rats were deprived of food and had free access to water 24 h before the neurogenic and indomethacinic treatments. Zosterin was given to animals intragastrically once 1 h before the emotional stress or injection of the indomethacin, or administration of the 2, 4-D solution daily for seven days.

Neurogenic destruction of the gastric mucosa (GM) by means of emotional stress was performed by immersion of rats in individual perforative plexiglass boxes limiting movement, to the level of xiphoid appendix, into water at 23℃ for 2 h. 21 animals were divided into three groups: Control 1 group, Control 2 (emotional stress) group and Emotional stress + zosterin group. Control 1 group was given physiological solution; Control 2 group was given physiological solution and exposed to emotional stress; Emotional stress + zosterin group received zosterin and exposed to emotional stress.

Medicinal destruction of the stomach was performed by intragastric introduction of indomethacin (Sopharma, Bulgaria) in the form of aqueous suspension at dose of 25 mg/kg. There were 28 rats in four groups: Control 1 group, Control 2 (indomethacin) group, Indomethacin + zosterin group, Indomethacin + medetopect group.

Ulcerogenic effect of pesticide, 2, 4-dichlorophenoxyacetic acid (2, 4-D), was observed after intragastric introduction of 2, 4-D (an aqueous

concentrate contained 500 g/l of the 2, 4-D; 4th danger class, Registration No. 582313-1, BASF-AG) on an empty stomah at dose of 90 mg/kg for seven days. 28 rats were divided into four groups: Control 1 group, Control 2 (2, 4-D) group, 2, 4-D + zosterin group, 2, 4-D + medetopect group.

In the end of the experiments rats were killed by decapitation under the light ether anesthesia. In the experiments animals were killed: at emotional stress immediately after taking out of water, after 4 h indomethacin injection and a day after the last 2, 4-D administration. The stomach was removed, cut along the greater curvature, washed with physiological solution and macroscopically investigated under a binocular loop. The number of animals with GM damage, the ulceration rate (the number of damages per animal), the total length of damages (sum of all spot hemorrhages, erosions, and strip-like ulcerations in millimeters) were estimated and index of Pauls (IP, an integral index of destructions in the stomach) was calculated by the formula: IP = (ulceration rate × percentage of animals with damages) /100 (Pauls *et al.*, 1947). Gastroprotective activity of zosterin and medetopect was calculated as a ratio of IP of Control 2 groups (without the medicine) to IP of tested groups (with the medicine).

Biochemical analysis

The corticosterone contents in plasma of blood were measured by fluorimetrical method on spectrofluorimeter Hitachi MPF-4 (De Moore *et al.*,1960). In the region with the most destruction of GM the amounts of glycogen were estimated using anthron reagent (Seifter *et al.*, 1950), the adenosine triphosphate (ATP; Lampecht and Trautschold, 1970) and lactate (Hohorst, 1970) contents were established spectrophotometrically by measuring absorbance at 340 nm.

Statistical analysis

Values are presented as mean ± standard error of mean. Statistical analysis of the data was carried out with STATISTICA for Windows r.5.1b (Statsoft, Inc.) program; the significance of differences was evaluated using Student's *t*-test. Differences at $P < 0.05$ were considered statistically significant.

RESULTS

Gastroprotective activity of zosterin on emotional stress induced stomach damage

A severe 2-h water immersion under the conditions when animals were kept in unnatural vertical position with limited movements caused emotional (neu- rogenic) tension in rats (Table 1). Treatment with zosterin decreased stress reaction of rats and, hence, the degree of stomach damage. Against this background, the content of corticosterone in Emotional stress + zosterin group was only 15% whereas in Control 2 group it was 27% higher than in Control 1 group. The number of structural lesions and the total length of damages in the GM of Emotional stress + zosterin group were 46 and 58% less than in Control 2 group. After the injection of zosterin IP was 2.2 times lower than in Control 2 group. Simultaneously, normalizing effect of zosterin on the content of energy maintenance metabolites in the GM was observed. For example, the levels of APT and glycogen were 90 and 86% in Emotional stress + zosterin group, whereas in Control 2 group these values were 58 and 68% as against Control 1 group. At the same time, the amounts of lactate in the GM of Emotional stress + zosterin group decreased by 16% relative to Control 2 group.

Antiulcerogenic effect of zosterin and medetopect on indomethacin induced damage of the gastric mucosa

A marked gastroprotective effect of zosterin was also observed in the indomethacin model of stomach lesions (Table 2). The number of lesions, total length of damages and IP were diminished

Table 1. Gastroprotective activity of zosterin on emotional stress induced stomach damage(%)

	Groups		
	Control 1	Control 2 (emotional stress)	Emotional stress + zosterin
Number of animals with GM damage(%)		100	85.7
Ulceration rate		10.6 ± 0.85	5.7 ± 0.22**
Total length of damages (mm)		13.7 ± 0.98	5.7 ± 0.32**
Index of Pauls		10.6	4.9
ATP (μMol/g)	0.90 ± 0.06	0.52 ± 0.04*	0.81 ± 0.06**
Glycogen (μMol/g)	15.6 ± 1.05	10.7 ± 0.98*	13.4 ± 0.69**
Lactate (μMol/g)	1.02 ± 0.07	1.32 ± 0.09*	1.15 ± 0.08
Serum corticosterone (μMol/l)	0.40 ± 0.03	0.51 ± 0.04*	0.46 ± 0.04

Values are means ± standard error of the mean (n = 7 rats/group).
*P < 0.05, Control 2 versus Control 1; **P < 0.05, Emotional stress + zosterin versus Control 2; ATP, adenosine triphosphate; GM, gastric mucosa.

Table 2. Antiulcerogenic effect of zosterin and medetopect on indomethacin induced damage(%) of the gastric mucosa

	Group			
	Control 1	Control 2 (indomethacin)	Indomethacin + zosterin	Indomethacin + medetopect
Number of animals with GM damage(%)		100	57,1	71,4
Ulceration rate		15.6 ± 0.52	9.4 ± 0.55**	10.4 ± 0.75**
Total length of damages (mm)		28.7 ± 1.75	18.4 ± 0.95**	23.2 ± 1.50**
Index of Pauls		15.6	5.4	7.4
Gastroprotective activity			2.9	2.1
ATP (μMol/g)	0.96 ± 0.06	0.65 ± 0.05*	0.88 ± 0.04**	0.85 ± 0.05**
Glycogen (μMol/g)	14.2 ± 0.76	9.3 ± 0.41*	13.0 ± 0.65**	12.4 ± 0.70**
Lactate (μMol/g)	0.98 ± 0.04	1.28 ± 0.06*	1.10 ± 0.07	1.00 ± 0.05**
Corticosterone (μMol/l)	0.45 ± 0.02	0.57 ± 0.04*	0.44 ± 0.03**	0.50 ± 0.02

Values are means ± standard error of the mean (n = 7 rats/group).
*P < 0.05, Control 2 versus Control 1; **P < 0.05, Control 2 versus Indomethacin + zosterin, Indomethacin + medetopect; ATP, adenosine triphosphate; GM, gastric mucosa.

Table 3. Protective effect of zosterin and medetopect on 2, 4-D induced damage(%) of the gastric mucosa

	Groups			
	Control 1	Control 2 (2, 4-D)	2, 4-D + zosterin	2, 4-D + medetopect
Number of animals with GM damage(%)		85.7	71.4	71.4
Ulceration rate		11.2 ± 0.72	4.7 ± 0.30**	6.3 ± 0.55**
Total length of damages (mm)		12.6 ± 0.82	7.4 ± 0.57**	5.5 ± 0.45**
Index of Pauls		9.6	3.4	4.5
Gastroprotective activity			2.8	2.1
ATP (μMol/g)	1.02 ± 0.07	0.72 ± 0.05*	0.88 ± 0.06	0.88 ± 0.05**
Glycogen (μMol/g)	13.5 ± 1.02	9.8 ± 0.62*	12.6 ± 0.71**	12.0 ± 0.94
Lactate (μMol/g)	0.90 ± 0.06	1.18 ± 0.07*	0.94 ± 0.06**	0.95 ± 0.07**
Corticosterone (μMol/l)	0.40 ± 0.03	0.54 ± 0.05*	0.44 ± 0.03	0.45 ± 0.04

Values are means ± standard error of the mean (n = 7 rats/group).
*P < 0.05, Control 2 versus Control 1; **P < 0.05, Control 2 versus 2, 4-D + zosterin, 2, 4-D + medetopect; ATP, adenosine triphosphate; GM, gastric mucosa.

by 40, 36, and 65%, respectively, due to zosterin treatin comparison with Control 2 group. Protective effect of zosterin was expressed as less substantial deficit of ATP (8%) and glycogen (9%), and less lactate excess (12%) in the GM compared with Control 2 group (32, 35, and 31%, respectively). Zosterin prevents the development of stress induced by indomethacin: the amount of corticosterone in plasma was 98%, whereas in Control 2 group it was 127% as against Control 1 group. Comparison of gastroprotective effect of polysaccharide medicines-zosterin and medetopect, showed similarity of their protective effects. Medetopect prevented the ulcerogenic effect of indomethacin. Against the background of medetopect treatment, the number of lesions, IP, and contents of energy substrates in the GM significantly differed from the values of Control 2 group. At the same time, gastroprotective effect of medetopect was slightly lower than that of zosterin: these effects were 2.1 and 2.9, respectively.

Protective effect of zosterin and medetopect on 2, 4-D induced damage of the gastric mucosa

In the case of intragastric injections of 2, 4-D damage of GM was observed as small, bloody erosions and disturbance in tissue metabolism (Table 3). Application of zosterin enhanced the resistance of GM to the destructive action of 2,4-D: ulceration rate, total length of damages and IP were 58, 41, and 65%, respectively, which was lower than that of Control 2 group. The energy stock of GM (ATP, glycogen) is maintained on a higher level after the injection of zosterin: the amounts of ATP and glycogen were 86 and 93%, whereas in Control 2 group they were 70 and 72% respectively, as against Control 1 group. At the same time, acidosis in GM was less expressed: the amounts of lactate were 27% lower than in untreated animals (Control 2). Animals were stressed to lesser extent with 2, 4-D + zosterin treatment: in this case, the corticosterone level in plasma was 25% lower than the corresponding

parameter of the animals from Control 2 group. For comparison, this model of the stomach destruction was used for investigation of gastroprotective activity of medetopect. In this case, the number of erosive, hemorrhagic damages was reduced; the stocks of ATP and glycogen in GM were less exhausted. At the same time, gastroprotective effect of zosterin yields a little to that of medetopect.

DISCUSSION

The obtained data demonstrate that zosterin enhances the resistance of the stomach tissue to various ulcerogenic factors. In zosterin-treated rats, the disturbance of trophic and methabolic processes, that are of great importance in the pathogenesis of stomach disorders, was substantially less expressed than in the animals of Control 2 group. Diminution of IP values, total length of damages and ulceration rate were significant, and testified to the ability of zosterin to have protective effect on GM at pathogenesis of various etiology. It is well established that polysaccharides not only show antipeptic, antacid, and antibacterial properties in the complex therapy of ulcer, but also promote the production of viscous glycoproteins covering the mucosa, bind bile acids, and normalize local immune processes in the alimentary canal (Thakur *et al.*, 1997; Figler *et al.*, 1999). For the treatment of gastroduodenal pathology of children, a 1% gel of zosterin in a dose of 25 mg per kilogram of body weight three times a day an hour before meal during 12-16 days is successfully used. It increases the viscosity of mucus, enhances the sorption of toxins and microorganisms, prevents the release of H^+ into the stomach aperture, normalizes the regeneration and nonspecific protection of GM, reduces the colonization of *Helicobacter pylori*, and prevents inflammation in the stomach (Miroshnitchenko *et al.*,1998). Studies of pectic polysaccharides from the roots of *Bupleurum falcatum L.* and the leaves of *Panax ginseng C.A. Meyer* demonstrated the same positive effect in various models of stomach damage (Sun *et al.*,1991; 1992). One of the mechanisms explaining reduction of ulceration rate in zosterin-treated rats at 2, 4-D administration is the capacity of pectins to bind chlororganic pesticides in stomach (Ta *et al.*, 1999).

Now trophic processes are understood as biochemical processes, directed to maintenance of structural integrity and constancy of cells and tissues function. In the result of macroergic substances deficiency (like ATP) a shift of energy metabolism towards anaerobic one, energetically less favorable, takes place. Redirection in energy supply of cells breaks plastic processes, changes tissue metabolism that finally leads to infringement of structurally functional integrity of a cell. The necessary condition of erosive lesions and gastric ulcers development is a fast and deep reduction of energetic resources of the mucous membrane. In case of development of stressful ulcers on the background of hemorrhagic shock (bloodletting) decrease of ATP stocks in GM was observed (Mengey and Masters, 1974). In the present study, zosterin administration prior ulcerogenic agents exhibited protective effect that was expressed in diminution of the number and sizes of destructive regions in GM, as well as in reduction of ATP and glycogen deficit, decrease in the lactate contents, and normalization of the energy balance in GM. There is still a large gap in knowledge about pectin influence on energy stock of GM. As is known, synthesis of nucleic acids directly and stechiometrically depends upon ATP content in a tissue. At the same time, it has been reported that pectin increases total DNA content of cecal, colonic, jejunum and ileum mucosa (Oku, 1995; Fukunaga *et al.*, 2003).

Now does not cause doubt the role of stress as the main etiologic factor of diseases of the gastrointestinal tract. The level of corticosterone in

plasma of blood was a marker of stress-reaction of rats to water immersion, indomethacin and pesticide 2, 4-D in our research. On the background of zosterin stress-reaction of animals to ulcerogenic factors was less expressed: the corticosterone content in plasma of blood was significantly lower than in untreated rats. This data show enhancement of nonspecific resistance of an organism by zosterin, as it was received on other experimental models (Khasina *et al.*, 2001; 2003). The mechanisms of gastroprotective action of zosterin need further investigation.

According to its antiulcer effect, zosterin may be recommended for application in the prevention and treatment of stomach diseases together with the basic therapy.

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