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## Gastroprotective Effect of Pectin Preparations Against Indomethacin-induced Lesions in Rats

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**Abstract:** Occurrence of ulcerous lesions of gastric mucosa induced by the therapy with nonsteroidal anti-inflammatory drugs remains a widespread problem. Application of up-to-date drugs used for prevention of these disorders is often associated with undesirable side effects, so the new agents possessing gastroprotective effects should be found and studied. The aim of this study was to estimate the preventive influence of low esterified pectin and calcium pectate on indomethacin induced gastric mucous injury in rats. Both substances were given to animals for 8 days daily in the doses of 10, 25 and 50 mg kg<sup>-1</sup> before indomethacin administration. The results showed that pectin substances used in the doses 25 and 50 mg kg<sup>-1</sup> suppress formation of the gastric mucosa lesions. In groups of rats preliminary given low esterified pectin the total number of lesions was 52.5 and 43.9% lower then in the control group depending on the dose of polysaccharide. In rats given calcium pectate the total number of lesions was 45.2 and 51.6% lower, then in the control. In relation to the ulcer size, advance administration of polysaccharides particularly prevented formation of small spot ulcers. Therefore, we can conclude that pectin substances may be used as prophylactic agents for regular NSAID users.

**Key words:** Dietary fiber, pectin, gastric ulcer, indomethacin

### INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) remain the agents of choice in the treatment of many rheumatic and other inflammatory diseases because of their analgesic and anti-inflammatory properties. However, use of NSAIDs is also associated with an increased frequency of peptic ulcers and ulcer complications, such as major upper gastrointestinal bleeding and perforation (Hernandez-Díaz and García Rodríguez, 2000; García Rodríguez and Hernandez-Díaz, 2001; García Rodríguez *et al.*, 2001; Lim and Heatley, 2005). It has been estimated that in the United States, NSAID-induced gastrointestinal complications is a cause for at least 16 500 deaths and 107 000 hospitalizations each year (Wolfe *et al.*, 1999). Epidemiological studies have shown that the frequency of gastropathy and death is 3 to 10 fold higher (Langman *et al.*, 1994; Henry *et al.*, 1996) in patients regularly taking NSAIDs in comparison with those not taking these agents. Endoscopic studies have shown that occurrence of peptic ulcer among regular users of NSAIDs varies from 20 to 30 % (Del Valle *et al.*,

2003). The concern about NSAID-induced gastroduodenal damage is arisen because complications may often occur without any preceding signs of peptic ulcer disease (Chan and Leung, 2002). Moreover, recent epidemiological observations in combination with endoscopic findings suggest that NSAIDs might not only aggravate the course of preexisting peptic ulcer but also induce formation of the clinically relevant ones *de novo* (García Rodríguez and Hernandez-Díaz, 2004).

There are several mechanisms described, by which NSAIDs cause mucosal injury. Some drugs possess direct toxic influence on the gastric mucosa which is exacerbated by acidity, for acidity accelerates absorption of NSAIDs being in their non-ionized form. They also impair prostaglandin-dependent protective system of mucosa (Hawkey, 2000; Patel *et al.*, 2002). If the superficial cells are damaged by either of these mechanisms, a second wave of injury mediated by luminal acid often occurs and generates deeper ulcerative lesions (Elliott *et al.*, 1996).

Hitherto the general approach to prevention of the side effects of NSAIDs consists of the treatment with histamine-2 receptor antagonists such as ranitidine and

famotidine or proton pump inhibitors such as omeprazole and lansoprazole reducing hydrochloric acid secretion, administration of synthetic forms of prostaglandin E such as misoprostol substituting depleted endogenous prostaglandin activity as well as the prescription of locally acting agents and antacids (Hudson *et al.*, 1997; Hawkey *et al.*, 1998; Lai *et al.*, 2002; Chan and Graham, 2004). However, the H<sub>2</sub> receptor antagonist ranitidine and the locally acting agent sucralfate were shown to be not effective in prevention of gastric ulcers in long-term NSAID users (Yeomans *et al.*, 1998; Micklewright *et al.*, 2003). Other H<sub>2</sub> receptor antagonists and proton pump inhibitors are not very effective for healing and prevention of NSAID-associated gastric ulcers in a case of continuing therapy with NSAIDs, although they accelerate healing and help to prevent duodenal ulcers (Yeomans *et al.*, 1998; Chan and Graham, 2004). The use of prostaglandin analogues such as misoprostol is limited due to their gastrointestinal side effects such as diarrhea and abdominal cramps (Silverstein *et al.*, 1995).

Therefore, the new agents purposed for prevention of gastrointestinal complications induced by NSAID usage should be found. At present attention is given to a group of substances called dietary fibers, which were shown to exert beneficial effects on human body (American Dietetic Association, 1997). A few of experimental works and clinical observations showed that dietary fibers such as pectin may successfully cure gastric mucous lesions and reduce their formation *de novo* (Waterhouse *et al.*, 2000; Ferrucci *et al.*, 2003; Ryan-Harshman and Aldoori, 2004). Taking to account these findings we presumed that pectins, besides healing effects, may have preventive influence on gastric lesion development. The main goal in our present study was to investigate the effects of prophylactic usage of the two pectin preparations, differing in some physico-chemical properties, on development of experimental gastric mucosal lesions in rats. After advance administration of pectin samples we induced gastric mucosal injury by indomethacin and estimated the number and sizes of lesions formed.

## MATERIALS AND METHODS

**Pectin preparation:** Purified pectin samples were prepared at the Department of Pharmacy at Vladivostok State Medical University, Russia. Low esterified pectin was obtained from commercial high esterified citrus pectin (Copenhagen Pectin A/S, Lille Skensved, Denmark) using method of alkali de-esterification. Beforehand high esterified pectin was rinsed with 50% ethanol solution for

2 h, then separated with filtration, rinsed on the filter with 1.5 L of 50% ethanol solution and then with 1 L of 95% ethanol and dried at 70°C. De-esterification process was carried out as follows: 20 g of purified high esterified pectin was suspended in 200 mL of 50% ethanol and then 4 g of NaOH in 50% ethanol was added under continuing stirring. When the set degree of esterification of pectin (approximately 1%) was achieved the mix was stirred for 2 h and soured with 1 M HCl solution in 50% ethanol reaching the pH meanings 5-6. Obtained pectin was separated from water-ethanol solution using glass filter and rinsed consecutively with 300 mL of 50% ethanol, 150 mL of 95% ethanol and dried at 70°C.

**Pectin analysis:** Characterization of the pectin samples was carried out in the laboratory of pharmacology of Institute of Marine Biology, Far East Branch of Russian Academy of Sciences. The galacturonan content of the pectin preparations was determined colorimetrically by the *m*-hydroxydiphenyl method (Blumenkrantz and Asboe-Haunsen, 1973). The degree of esterification was characterized using titrimetric analysis (Afanasyev *et al.*, 1984). Intrinsic viscosity of pectin was determined in 0.05 M NaCl/0.005 M Na-oxalate at 25.0°C and pH 6.0 using an Ubbelohde viscosimeter. The intrinsic viscosity is related empirically to the molecular weight by the Mark-Howink relation (Anger and Berth, 1986).

**Test animals and diet:** Experiments using animals were carried on at the animal house of Scientific Research Institute of Pharmacology, Tomsk Research Center, Siberian Branch of Russian Academy of Sciences in November, 2005. In this study were used the healthy adult albino male rats (Wistar) weighing 220-280 g each. The animals were housed in stainless steel, wired cages (in groups of three-four per cage) and kept in an isolated room under standard conditions of temperature (20-22°C), ambient humidity (50-55%) and 12 h light-dark cycle (8.00 am-8.00 pm). The rats were allowed free access to food and water. The composition of the standard diet was as follows (g/100 g): casein, 21.0 cellulose, 5.3 sunflower oil, 7.0 cholesterol, 1.0 sucrose, 15.0 starch, 45.9 methionine, 0.3 minerals, 3.5 vitamin mixture 1.0. The use of animals in this study was in accord to the principles and guidelines of the Scientific Research Institute of Pharmacology.

**Experimental design:** The rats were randomized into seven groups of 9-10 animals. Animals of the control group were daily fed the standard diet and 1 h before feeding (8:00) given water (1.0 mL 100 g of body weight)

by gastric gavage for eight days. Within the same period of time animals of the three test groups were daily fed the standard diet and one hour before feeding (8:00) administered by gastric gavage a solution containing 10, 25 and 50 mg kg<sup>-1</sup> of dry low-esterified pectin in a volume of 1 mL per 100 g body weight. Animals of the another three test groups were administered orally through gavage calcium pectate suspension containing 10, 25 and 50 mg kg<sup>-1</sup> of dry calcium pectate, respectively, in a volume of 1 mL per 100 g body weight. On the 9th day the rats of the control and test groups were fasted for 20 h but provided water *ad libitum*. After that, the gastric mucosal lesions were induced by intragastric administration of 60 mg kg<sup>-1</sup> body weight of indomethacin suspended in 1 mL of physiological saline with a trace amount of Tween 80 (Bates *et al.*, 1979). The rats were killed 6 h after the administration of indomethacin using overdose of diethyl ether. The stomach was removed and incised along the greater curvature, then rinsed gently with physiological saline. The gastric mucous lesions visible with the naked eye were found in the gastric mucosa as small black-red spots (0.5-1 mm diameter), large black-red spots (1-2 mm diameter) and elongated black-red scratches (1-10 mm long by 0.5-1 mm wide) paralleling the long axis of the stomach.

The degree of gastric mucosa injury was estimated according to the number of small spots, large spots and scratches and the total number of lesions in relation to one animal. In addition, the length (mm) of each lesion was measured and the lesion index was expressed as the total sum of the length of all the lesions in the glandular region (Rifat-uz-Zaman *et al.*, 2004).

**Statistical analysis:** The data was expressed as mean±SEM (Standard Error of Mean). Results obtained at the end of the study were analyzed using one-way ANOVA using post hoc Tukey's test and p values were determined. Differences were considered significant at p<0.05 and highly significant at p<0.001. Statistical analysis was performed using software package SPSS (Statistical Package for Social Sciences) for Windows, version 11.0.

## RESULTS

The preparations studied were not differing in molecular weight, anhydrogalacturonic acid content and the degree of esterification. But the differences were in the calcium content and the water solubility (Table 1).

Advance administration of the both pectin preparations in a dose 10 mg kg<sup>-1</sup> were shown to be not effective regarding prevention of the indomethacin induced gastric mucous injury. At the same time, the oral administration of these pectin preparations in doses of 25 mg and 50 mg kg<sup>-1</sup> daily for 8 days contributed to a significant decrease of the gastric mucosal lesion development induced by indomethacin (Table 2). In particular, in rats given 25 and 50 mg kg<sup>-1</sup> of low-esterified pectin the number of small spot lesions of gastric mucosa was 5.3 and 6.7 times, respectively, lower than in the control group. In rats given 25 and 50 mg kg<sup>-1</sup> of calcium pectate the number of small spot lesions of gastric mucosa was approximately 4.5 and 16.7 times, respectively, lower than in rats of the control group. In the stomach of rats administered low-esterified pectin in a dose of 25 mg kg<sup>-1</sup> the number of large spot lesions was averagely 52.8% lower than in the control. The rest of animals given low esterified pectin in a dose of 50 mg kg<sup>-1</sup> or calcium pectate in the doses of 25 and 50 mg kg<sup>-1</sup> the number of large spot lesions was lower, then in the control group, but the differences were not considered significant. The number of scratches in rats administered 25 and 50 mg kg<sup>-1</sup> of low-esterified pectin was lower averagely by 40.5% and 35.1%, respectively, then in the

Table 1: Physico-chemical properties of tested pectin preparations

Property	Low-esterified pectin	Calcium pectate
Molecular weight	39300	39300
Anhydrogalacturonic acid	69.0%	67.3%
Degree of esterification	1.2%	1.2%
Calcium	n.d.	38 mg g <sup>-1</sup>
Protein	n.d.	n.d.
Methyl ester	n.d.	n.d.
Acetyl ester	n.d.	n.d.
Water solubility	Water soluble	Non soluble

nd-not determined

Table 2: Effect of pectin preparations on the indomethacin-induced gastric ulcerogenesis in rats

Group of animals	No. of rats with lesions found (%)	No. of small spots/rat	No. of large spots/rat	No. of scratches/rat	Total No. of lesions/rat	Lesion index (mm)
Control (vehicle), n = 9	100	10.0±1.8	12.3±1.5	25.9±1.7	48.2±2.1	77.6±4.7
Low-esterified pectin (25 mg kg <sup>-1</sup> ), n = 10	100	1.9±0.9 <sup>a</sup>	5.8±1.4 <sup>b</sup>	15.4±1.9 <sup>a</sup>	23.0±2.7 <sup>c</sup>	46.1±6.0 <sup>c</sup>
Low-esterified pectin (50 mg kg <sup>-1</sup> ), n = 10	100	1.5±0.5 <sup>a</sup>	8.7±1.2	16.8±3.0 <sup>a</sup>	27.0±3.5 <sup>c</sup>	32.1±4.8 <sup>c</sup>
Calcium pectate (25 mg kg <sup>-1</sup> ), n = 10	100	2.2±0.9 <sup>b</sup>	9.1±1.4	15.1±2.0 <sup>c</sup>	26.4±2.9 <sup>c</sup>	47.0±5.9 <sup>c</sup>
Calcium pectate (50 mg kg <sup>-1</sup> ), n = 10	100	0.6±0.3 <sup>c</sup>	9.1±1.2	13.6±2.2 <sup>c</sup>	23.3±2.6 <sup>c</sup>	47.2±6.4 <sup>b</sup>

Significant differences from control, <sup>a</sup>p<0.05, <sup>b</sup>p<0.01, <sup>c</sup>p<0.001

control group. The number of scratches in rats given calcium pectate in the doses of 25 and 50 mg kg<sup>-1</sup> was lower averagely by 41.7 and 47.5%, respectively, then in the control. According to the total number of gastric mucous lesions the differences were considered highly significant between groups of animals given 25 and 50 mg kg<sup>-1</sup> of low esterified pectin and calcium pectate and the rats of control group. The same highly significant differences were found between groups of rats given 25 and 50 mg kg<sup>-1</sup> of the pectin preparations and animals of the control group regarding lesion index.

## DISCUSSION

For the last two decades it is known that gastric ulcer and erosion formation is accompanied by progressive decrease in the prostaglandin synthesis (Reeves and Stables, 1985). Indomethacin as well as other NSAIDs has been known to be the potent inhibitor of cyclo-oxygenase enzyme (Levine, 2001). The selective inhibition of cyclo-oxygenase pathway decreases the levels of prostaglandin I<sub>2</sub>, prostaglandin E<sub>2</sub> and thromboxane A<sub>2</sub> and increases the level of leukotrienes in gastric mucosa. This has an impairing influence on mucosal vasculature resulting in inflammation and pain (Kapui *et al.*, 1993). It also further potentiates the effects of histamine on the secretion activity due to the inhibition of prostaglandin E<sub>2</sub> synthesis. It has been found that indomethacin and many other NSAIDs can cause apoptosis (Tabuchi *et al.*, 1994) and the damage in the stomach further activating acid secretion by a stimulatory pathway in addition to a prostaglandin, NO and Ca<sup>2+</sup> dependent inhibitory mechanism. Prostaglandins may have a dual role in the regulation of acid secretion in the damaged stomach i.e., the inhibitory effect at the parietal cells and the excitatory effect by enhanced release of mucosal histamine (Tabuchi *et al.*, 1994). Consequently, indomethacin raises a volume of gastric secretion and acid out put thus decreasing pH of gastric juices (Rifat-uz-Zaman *et al.*, 2006).

Medications recommended for prevention of the side effects of NSAIDs including synthetic prostaglandin analogues, proton pump inhibitors and histamine-2 receptors antagonists possess some undesirable effects. Therefore, investigation of properties and effects of other preparations suitable for prevention of NSAID-induced gastric mucous injury is of great interest.

Pectins are the ionic polysaccharides, which are used in the food industry because of their gelling and thickening properties (Thakur *et al.*, 1997). The main structural features of pectins are the linear chains containing more than one hundred (1-4)-linked  $\alpha$ -D-galacturonic acid residues, some of which are partially esterified with methanol defining the degree of

esterification of pectin (Schols and Voragen, 1996). A number of studies confirm that pectins exert beneficial health effects such as reduction of serum cholesterol levels (Gonzalez *et al.*, 1998), hepatoprotective activity (Khotimchenko *et al.*, 2004) as well as anti-ulcer effects (Ferrucci *et al.*, 2003; Waterhouse *et al.*, 2000). Pectins have a potential protective activity against a wide range of experimental gastric mucosal lesions and this may be caused by reinforcement of resistance of the mucosal barrier due to protective coating of pectin, antisecretion activity regarding hydrochloric acid and pepsinogen and the oxygen radical scavenging activity (Trommer and Neubert, 2005). In this study we have demonstrated that pectin have a capacity to prevent formation of ulcerous injury of gastric mucosa induced by indomethacin in rats after advance oral administration for 8 days. The exact mechanisms of preventive effects exerted by pectins are not clarified yet. Yoshikawa *et al.* (1993) have been reported that the lipid peroxidation induced by oxygen radicals plays an important role in the pathogenesis of indomethacin induced gastric mucosal changes as well as in gastric injuries. Previously we have found out that low-esterified pectin with the degree of esterification 1.2% exert preventive and healing effects in rats with modeled carbon tetrachloride induced liver injury. These effects manifested in reduced concentration of malone dealdehyde and conjugated diene levels in blood and liver (Khotimchenko *et al.*, 2004). Therefore, it may be suggested that the preventive influence of pectins regarding formation and development of indomethacin-induced gastric lesions may be partly related to inhibition of the lipid peroxidation.

In conclusion, the reported results have demonstrated the possibility that low-esterified pectin and calcium pectate prevent development of gastric mucosal lesions induced by indomethacin in rats. The mechanism of prophylaxis anti-ulcer activity of pectin preparations can not be elucidated on the base of the results obtained and requires further investigation.

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