PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 November 7; 12(41): 6646-6651
World Journal of Gastroenterology ISSN 1007-9327

BASIC RESEARCH

Preventive effect of a pectic polysaccharide of the common cranberry *Vaccinium oxycoccos* L. on acetic acid-induced colitis in mice

Sergey V Popov, Pavel A Markov, Ida R Nikitina, Sergey Petrishev, Vasily Smirnov, Yury S Ovodov

Sergey V Popov, Pavel A Markov, Ida R Nikitina, Sergey Petrishev, Vasily Smirnov, Yury S Ovodov, Department of Molecular Immunology and Biotechnology, Institute of Physiology, Komi Science Centre, The Urals Branch of the Russian Academy of Sciences, 50 Pervomaiskaya str., Syktyvkar 167982, Russia
Supported by the Program of Presidium of the Russian Acad. Sci. «Molecular and cellular biology», by the Russian Fund for Basic Research, No. 06-04-48079 and by the Program for Leading Scientific Schools, No. 5796.2006.4
Correspondence to: Dr. Sergey V Popov, Institute of Physiology, Komi Science Centre, the Urals Branch of the Russian Academy of Sciences, 50 Pervomaiskaya str., Syktyvkar 167982, Russia. popov@physiol.komisc.ru
Telephone: +7-8212-241001 Fax: +7-8212-241001
Received: 2006-07-20 Accepted: 2006-09-21

Abstract

AIM: To study isolation and chemical characterization of pectin derived from the common cranberry *Vaccinium oxycoccos* L. (oxycoccusan OP) and the testing of its preventive effect on experimental colitis.

METHODS: Mice were administrated orally with OP two days prior to a rectal injection of 5% acetic acid and examined for colonic damage 24 h later. Colonic inflammation was characterized by macroscopical injury and enhanced levels of myeloperoxidase activity measured spectrophotometrically with o-phenylene diamine as the substrate. The mucus contents of the colon were determined by the Alcian blue dye binding method. Vascular permeability was estimated using 4% Evans blue passage after i.p. injection of 0.05 mol/L acetic acid.

RESULTS: In the mice treated with OP, colonic macroscopic scores (1.1 ± 0.4 *vs* 2.7, $P < 0.01$) and the total square area of damage (10 ± 2 *vs* 21 ± 7, $P < 0.01$) were significantly reduced when compared with the vehicle-treated colitis group. OP was shown to decrease the tissue myeloperoxidase activity in colons (42 ± 11 *vs* 112 ± 40, $P < 0.01$) and enhance the amount of mucus of colitis mice (0.9 ± 0.1 *vs* 0.4 ± 0.1, $P < 0.01$). The level of colonic malondialdehyde was noted to decrease in OP-pretreated mice (3.6 ± 0.7 *vs* 5.1 ± 0.8, $P < 0.01$). OP was found to decrease the inflammatory status of mice as was determined by reduction of vascular permeability (161 ± 34 *vs* 241 ± 21, $P < 0.01$). Adhesion of peritoneal neutrophils and macrophages was also shown to decrease after administration of OP (141 ± 50 *vs* 235 ± 37, $P < 0.05$).

CONCLUSION: Thus, a preventive effect of pectin from the common cranberry, namely oxycoccusan OP, on acetic acid-induced colitis in mice was detected. A reduction of neutrophil infiltration and antioxidant action may be implicated in the protective effect of oxycoccusan.

Key words: Common cranberry *Vaccinium oxycoccos* L; Pectin; Colitis; Anti-inflammatory; Mice

Popov SV, Markov PA, Nikitina IR, Petrishev S, Smirnov V, Ovodov YS. Preventive effect of a pectic polysaccharide of the common cranberry *Vaccinium oxycoccos* L. on acetic acid-induced colitis in mice. *World J Gastroenterol* 2006; 12(41): 6646-6651

http://www.wjgnet.com/1007-9327/12/6646.asp

INTRODUCTION

Inflammatory bowel disease (IBD), including Crohn's disease and ulcerative colitis (UC), are characterized by chronic and spontaneously relapsing inflammation resulting in tissue destruction[1]. At present, there is no radical treatment for UC. Steroids and salicylic acid preparations are usually used to control and suppress the inflammation. However, adverse reactions and a high relapse rate are problems arising from these therapies[2]. Acute intestinal inflammation is associated with the infiltration of a large number of leukocytes into the bowel mucosa. A decrease in leukocyte number and function has been shown to prevent bowel inflammation[3]. Therefore, leukocytes are assumed to be a potential target of colitis drug research.

Pectic polysaccharides are well known to be a component of dietary fiber and to possess immunomodulating capacity with a predominant effect on neutrophils and macrophages. Feeding with pectins has been found to reduce a degree of experimental bowel injury induced by acetic acid[4] and by dextran sulfate

sodium[5]. Oral pretreatment with comaruman, a pectin from cinquefoil *Comarum palustre* L., has been shown to prevent the development of experimental colitis in mice[6].

It is well known that physiological effects are determined by the pectin structure[7]. Pectins used as food additives consist mostly of linear chains of galacturonic acid residues[8], whereas pectins of fruits, vegetables and herbs are characterized by an abundance of diversity of the macromolecular structure[7]. Therefore, the search and chemical characterization of new pectins with colon-protecting capacity is of great interest.

Vaccinium sp. (Ericaceae) is widespread throughout the cool temperate Northern Hemisphere, including the northern parts of Europe, Asia and North America. Cranberries are widely used in food as well as in ethnomedicine[9]. Regular drinking of cranberry juice has been shown to reduce the recurrence of urinary tract infections due to its action against coliform bacteria[10]. Cranberries were found to contain fructose[11] and a high molecular weight constituent of an unknown nature[12] that inhibits the adherence of bacteria to eukaryotic cells. The non-dialyzable material prepared from the cranberry juice concentrate has been shown to decrease the pro-inflammatory cytokine response of macrophages induced by LPS[13]. Cranberries are among the richest food sources of procyanidins, which are polyphenols possessing a potent anti-oxidant capacity[14]. Phenolic compounds of cranberries have been found to inhibit the growth of human gastrointestinal pathogens[15].

Cranberries have been far reported to contain a high amount of pectic polysaccharides[16,17]. However, the structure and anti-inflammatory activity of pectin derived from cranberries have not been studied until now.

The present work is devoted to testing the preventive effect of the pectin from the common cranberry *Vaccinium oxycoccos* L. on experimental colitis.

MATERIALS AND METHODS

Materials

Oxycoccusan OP was extracted from the ripe berries of the common cranberry *Vaccinium oxycoccos* L. (*Oxycoccus palustre* Pers.) with aqueous ammonium oxalate as described previously[18]. OP was shown to consist of primarily of galacturonic acid residues (82%) together with the minor residues of rhamnose (1.5%), arabinose (8%), glucose (5%) and galactose (3%) (Mw = 100-300 kDa). The apple pectin (MP Biomedicals, Inc.) was determined to consist of the galacturonic acid residues (70%) together with residues of glucose (29%) and traces of rhamnose, arabinose and galactose residues (Mw = 200-400 kDa). Prednisolone (Akrihin), *o*-phenylene diamine, horseradish peroxidase (Roanal), alcian blue, fetal calf serum, thiobarbituric acid (Sigma), and 96-well flat-bottom tissue culture plates (Linbro®, MP Biomedicals, Inc.) were used in this study.

Animals

The structure of this study and the animal experimental procedures were approved by the Ethical Committee of the Komi Science Center of the Russian Academy of Sciences on Animal Care and Use.

Male A/HeJ mice weighing 20-25 g were used. They were housed in standard environmental conditions and fed with rodent diet and water ad libitum. The mice were fasted for a night before the induction of colitis but had free access to drinking tap water. Colitis was then induced and its severity was evaluated morphologically in three experiments. One of the biochemical parameters, including myeloperoxidase (MPO), malondialdehyde (MDA) or mucus content, was measured in the individual experiment after morphological observation. Each experimental group consisted of 7 animals.

Application of OP and induction of experimental colitis

The mice were lightly anesthetized with ether. A plastic catheter (2 cm long, external diameter 1 mm) was inserted rectally into the colon so that the tip was 3 cm proximal to the anus. Acetic acid (5%, pH 2.5, 0.15 mL,) was instilled into the colon lumen through the catheter, and saline was instilled as a control[19].

Animals were treated orally with OP at the dose of 25-100 mg/kg (dissolved in water) by using a flexible rubber catheter two days before the induction of colitis. The control mice received the same amount (0.2 mL) of water. The positive control and reference groups received prednisolone (5 mg/kg) and commercial apple pectin (100 mg/kg), respectively.

Assessment of colitis severity

Mice were killed by cervical dislocation one day after the administration of acetic acid. The entire colon was isolated and opened longitudinally, and rinsed with phosphate buffered saline (PBS). The macroscopic scoring of the colon damage was performed using the following criteria: 0, no macroscopic change; (1) mucosal erythema alone; (2) mild mucosal oedema, slight bleeding or small erosion; (3) moderate oedema, bleeding ulcers or erosion, oedema and tissue necrosis[20]. For each mouse, the ulcer area was determined by summing the sizes of lesions measured macroscopically by two blinded observers. The total area of damage was expressed as the relative percentage (%) of the total surface area of the colon.

Determination of colonic myeloperoxidase (MPO) activity

After the macroscopic measurements, the excised colons (100-150 mg) were homogenized in PBS (pH 7.4) and centrifuged at 10 000 × *g* for 20 min at 4℃. MPO activity in the supernatants was then assayed by mixing the supernatant with citric phosphate buffer (pH 5.0) containing 0.4 mg/ml of *o*-phenylene diamine and 0.015% hydrogen peroxide. The change in absorbance (A) at 492 nm was measured spectrophotometrically and compared with a standard dilution of horseradish peroxidase[21]. MPO activity was expressed as units/mg of tissue.

Measurement of the levels of lipid peroxidation

Malondialdehyde (MDA) formation was used to quantify lipid peroxidation in the intestinal wall and was measured as thiobarbituric acid-reactive material[22]. The intestinal samples were homogenized (100 mg/mL) in 1.2% KCl buffer. Two hundred microliters of the homogenates were then added to a reaction mixture consisting of 0.8%

aqueous thiobarbituric acid (0.75 mL), 8.1% sodium dodecyl sulfate (0.1 mL), 0.75 mL of 20% acetic acid (pH 3.5), and distilled water (0.3 mL). The mixture was heated at 90℃ for 45 min. After cooling to room temperature, 2.0 mL of a mixture of *N*-butanol and pyridine (15:1, v/v) was added to 2.0 mL of the samples. The mixture was shaken vigorously. After centrifugation at 4000 r/min for 10 min, the absorbance of the organic layer was measured at 540 nm. The MDA level is expressed as nanomoles per milligram of protein[23].

Evaluation of the adherent colonic mucus

The mucus content of the colon was determined spectrophotometrically by the Alcian blue dye binding method[24]. The colon was excised and immersed for 2 h in 0.1% Alcian blue in 0.16 mol/L sucrose solution buffered with 0.05 mol/L aqueous sodium acetate. The unbound dye was then removed by two subsequent washings for 15 and 45 min in 0.25 mol/L sucrose solution and the mucus-bound dye was eluted by immersing the colon in a 0.5 mol/L $MgCl_2$ solution for 2 h. The solution obtained was centrifuged at 4000 r/min and *A* of supernatant was read at 650 nm. The amount of Alcian blue extracted per one gram of the wet colonic sample was then calculated from standard curves.

Measurement of vascular permeability

Vascular permeability was measured in the healthy mice two days after a single oral administration of OP or indomethacin as a positive control. Mice were injected intravenously with 4% Evans blue (0.01 mL/g body weight). After injection of the dye, 0.05 mol/L acetic acid (0.01 mL/g body weight) was injected intraperitoneally. Fifteen minutes later, the mice were killed by an overdose of ether and the viscera were exposed after a 1 min period to allow blood to drain away from the abdominal wall. The animal was held by a flap of the abdominal wall and the viscera were irrigated with saline over a Petri dish. The washing was filtered through glass wool and transferred to a test tube. To each tube was added 0.1 mL of 0.05 mol/L NaOH to clear any turbidity due to protein, and *A* was read at 590 nm[25].

Adhesion of peritoneal leukocytes

The peritoneal cells were harvested from mice by peritoneal lavage with phosphate buffered saline (5 mL). The cells were washed by centrifugation (1500 r/min, 10 min) and then suspended in Hank's medium supplemented with 10% fetal calf serum. The cell suspensions (0.1 mL) were incubated in a 96-well flat-bottom tissue culture plate in the presence of phorbol-12-myristate-13-acetate (PMA, 0.025 mg/L) as a stimulating agent at 37℃ for 15 min. The wells were washed to remove the nonadherent cells and the adherent cells were fixed in ethanol and stained with azure-eosin. The colored material was solubilized in MeOH and *A* of the solution obtained was measured at 650 nm[26].

Statistical analysis

All data were expressed as the as mean ± SD. Nonparametric statistics were used to evaluate the results. Comparisons between groups were made with the Mann Whitney *U*-test (package "MedCalc" v.9.1.0.1, Mariakerke, Belgium). A value of $P < 0.05$ was considered significant.

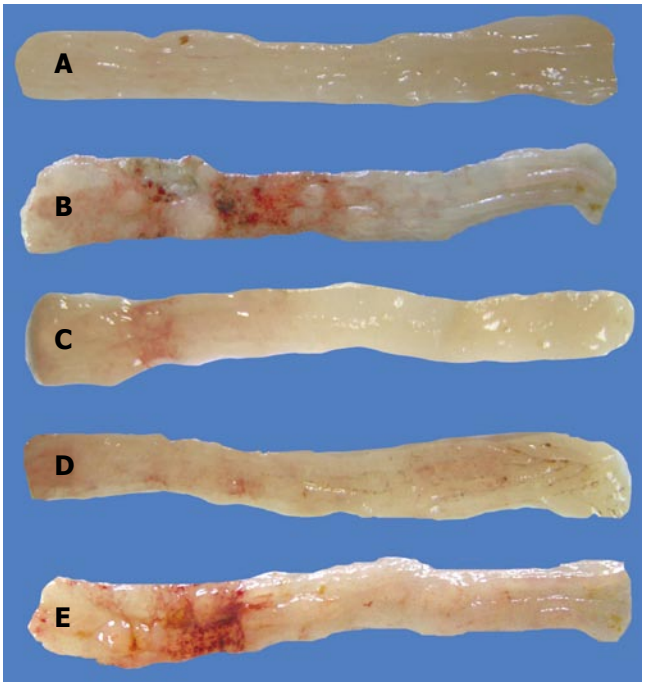

Figure 1 Macroscopic examination of the colonic mucosa before (**A**) and 24 h after acetic acid injection (**B-E**). Mice were treated two days previously with (**B**) saline (damage score 4, square area of injury 40%); (**C**) prednisolone 5 mg/kg (damage score 2, square area of injury 1%); (**D**) OP, 100 mg/kg (damage score 2, square area of injury 3%); (**E**) apple pectin, 100 mg/kg (damage score 3, square area of injury 25%). The colon without the cecum was removed and opened along the mesenteric border.

RESULTS

Effect of OP on colitis severity

All of the mice were found to be sick after a rectal injection of acetic acid and to have diarrhea with blood in the stool and abdominal distention. In comparison with healthy mice (Figure 1A), the colonic samples from mice with colitis were shown to have severe mucosal damage with edema, deep ulcerations and hemorrhages 24 h after the rectal injection of acetic acid (Figure 1B). A colonic inflammation was biochemically monitored by measuring the level of MPO activity in the colonic tissue taken from the site of inflammation. MPO activity was found to increase from 46 ± 8 to 112 ± 40 U/mg ($P < 0.01$, $n = 7$). Acetic acid infused rectally was shown to reduce the colon-bound mucus from 1.2 ± 0.4 to 0.4 ± 0.17 μg/mL ($P < 0.01$, $n = 7$). Damage of the colon reached the maximum level 24 h after the rectal injection of acetic acid. Signs of healing and regeneration of the mucosa were observed on the 5th d and the mucosa became almost normal on the 8th d after injection of acetic acid.

Oral administration of oxycoccusan OP two days before infusion of acetic acid into the colon was found to prevent the progression of colitis (Figure 1C). In the mice treated with OP, colonic macroscopic scores and the total square area of damage were significantly reduced when

Table 1 Preventive effect on colon injury of oxycoccusan OP administered orally two days before infusion of acetic acid into the rectum of mice (means ± SD)

Pretreatment	Macroscopic damage score	Total square of injury, %
Vehicle-treated (Colitis)	2.7 ± 0.8	21 ± 7
Oxycoccusan OP		
25 mg/kg	2.0 ± 0.4	17 ± 9
50 mg/kg	2.0 ± 0.8	20 ± 9
100 mg/kg	1.1 ± 0.4[b]	10 ± 2[b]
Prednisolone 5 mg/kg	1.3 ± 0.7[b]	8 ± 3[b]
Apple pectin 100 mg/kg	2.6 ± 0.7	23 ± 9

[b]$P < 0.01$ *vs* the vehicle-treated colitis group ('Colitis'). $n = 7$.

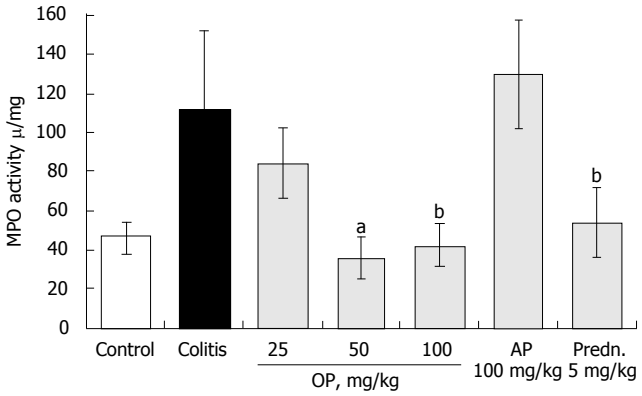

Figure 2 Effect of oxycoccusan OP on myeloperoxidase (MPO) activity in colonic tissue of mice with acetic acid-induced colitis. Values are means ± SD ($n = 7$), [a]$P < 0.05$, [b]$P < 0.01$ *vs* the colitis group.

compared with the vehicle-treated colitis group (Table 1). The preventive effect of OP was dose-related and comparable with that of prednisolone in a dose of 5 mg/kg, administered p.o. (Figure 1D). The apple pectin used as a reference pectic substance failed to influence the colitis (Figure 1E).

The MPO activities were estimated in the colonic samples as a marker of granulocyte and monocyte influx into the tissue. A peroral pretreatment of mice with OP orally was shown to attenuate the tissue MPO activity in colons as compared with the colitis group (Figure 2).

In the next experiment, the macroscopic score (1.8 ± 0.4 *vs* 2.7 ± 0.6, $P < 0.05$) and square area of damage (7 ± 3 *vs* 28% ± 10%, $P < 0.01$) were also decreased in colitis mice receiving OP pretreatment. Corresponding to morphological alterations, MDA levels were increased in the colonic samples of colitis mice in comparison to healthy mice. MDA levels in the samples obtained from mice that received OP were found to decrease compared to those in the colitis group (Table 2).

In the third experiment, the production of mucus was measured in the colonic samples of healthy, colitis-affected (macroscopic score 2.8 ± 0.9; square area of damage 21% ± 7%) and OP-administered (macroscopic score 1.7 ± 0.5; damage square 8% ± 6%) mice. OP was found to stimulate the production of mucus by colons of colitis-affected mice. The evaluation of Alcian blue recovery from colon-bound mucus in the OP-treated animals with colitis showed that the levels of adherent mucus were comparable with those detected in native mice (Figure 3).

Table 2 Preventive effects on colonic MDA levels of oxycoccusan OP administered orally two days before infusion of acetic acid into the rectum of mice (means ± SD)

Pretreatment	nmol/mg of protein
Healthy	3.6 ± 0.8
Vehicle-treated (Colitis)	5.1 ± 0.8
Oxycoccusan OP 100 mg/kg	3.6 ± 0.7[b]
Apple pectin 100 mg/kg	4.2 ± 1.4

[b]$P < 0.01$ *vs* vehicle-treated colitis group ('Colitis'). $n = 7$.

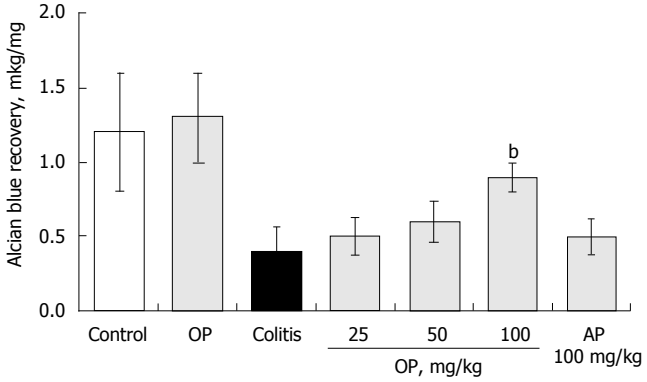

Figure 3 Effect of oxycoccusan OP (100 mg/kg) on the levels of bound mucus in the colon of mice. Values are means ± SD ($n = 7$), [b]$P < 0.01$ *vs* the appropriate control.

Effect of OP on healthy mice

Vascular permeability, the adhesion of peritoneal leukocytes, and colon-bound mucus were measured two days after oral administration of oxycoccusan to native mice as the initial step to elucidate the mechanism of the preventive effect of oxycoccusan. Oral administration of OP failed to influence the colon mucosa of healthy mice (Figure 3). OP was found to decrease the inflammatory status of mice as was detected by a reduction of vascular permeability (Figure 4). The adhesion of peritoneal neutrophils and macrophages was shown to slightly decrease after the administration of OP (Figure 5). The apple pectin, in comparison with oxycoccusan, failed to possess anti-inflammatory activity and failed to enhance the mucosal layer.

DISCUSSION

Plant polysaccharides have been previously demonstrated to reduce colon damage in experimental colitis[4,5,27]. The described investigations were performed at an extended exposure (6-21 d) to polysaccharides starting after the induction of colitis. Various effects of polysaccharides are likely to interfuse during this long exposure. In the present study, pectin OP was found to prevent acetic acid-

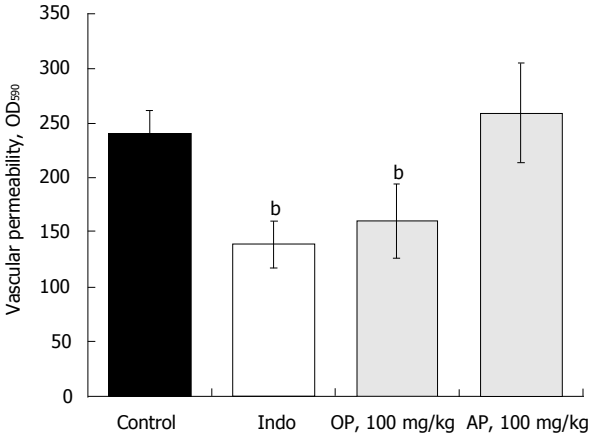

Figure 4 Effect of oxycoccusan OP on the vascular permeability of the healthy mice. Values are means ± SD (n = 7), $^{b}P < 0.01$ *vs* the control.

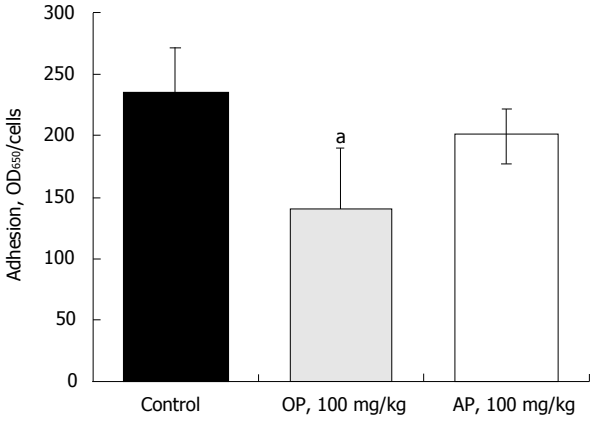

Figure 5 Effect of oxycoccusan OP on the adhesion of peritoneal leukocytes of the healthy mice. Values are means ± SD (n = 7), $^{a}P < 0.05$ *vs* the control.

induced colitis when given once beforehand at doses of 50-100 mg/kg. The doses of pectin used were chosen to approximate the estimated human intake as dietary fiber[28]. The protective effect of OP was comparable with that of prednisolone, which is a conventional medicine for the treatment of acute episodes of UC.

The exact mechanism of the preventive effect of OP on colitis remains unclear. The suppression of MPO activity by OP indicates that pectin inhibits the accumulation of neutrophils in the colonic mucosa. Indeed, MPO activity has been previously shown to be proportional to a number of infiltrating granulocytes, primarily neutrophils in the model of colonic inflammation[29]. Increasing amounts of transmigrating neutrophils induce significant epithelial disruptions resulting in epithelial discontinuities and erosions[30]. Therefore, it has been suggested that a delay in the influx of neutrophils into the intestinal wall is implicated in the preventive effect of cranberry pectin. A migration of neutrophils from the microvasculature is due to the influx of luminal proinflammatory bacterial products into the *lamina propria,* which results from disruption of the gastrointestinal barrier by oxygen radicals[31]. The role of reactive oxygen species has been stressed in recent years. Increased lipid peroxidation has been identified in the colons of patients with UC[32] as well as in experimental colitis induced by acetic acid[22]. Oxycoccusan was found to prevent an increase in MDA concentration. Plant polysaccharides, including pectins, are assumed to be capable of minimizing free radical-induced damage. A polysaccharide from *Angelica sinensis* has been found to possess a protective effect on immunological colon injury, which is probably due to the mechanism of antioxidation[33]. Ko *et al*[34] showed that colonic damage was significantly reduced by pretreatment of mice with an *Astragalus membranaceus* extract that contains polysaccharides and saponins. A polysaccharide from *Rheum tanguticum* has been shown to protect intestinal epithelial cells against oxidative stress[35]. A significant reduction in mucosal damage has been noted when pectin was administered before the perfusion of rat jejunum with peroxyl and hydroxyl radicals[36]. Therefore, it was proposed that the protective effect of OP against colitis was related to its antioxidant action. However, the presence of flavonoids in the OP preparations should be confirmed before elucidation of the mechanism of antioxidant action of polysaccharides *per se*.

Leukocyte adhesion represents one of the first steps in initiation of the inflammatory response and it is essential for the accumulation of active immune cells at sites of inflammation[37]. In light of this connection, the ability of OP to influence the adhesion of peritoneal leukocytes was determined in order to elucidate the involvement of leukocytes in the mechanism of the antiinflammatory effect. The data on leukocyte adhesion reduction may partially explain the preventive effect of OP on the accumulation of neutrophils in the intestinal wall.

A reduction of vascular permeability by OP indicates the suppression of the release of vasoactive mediators (histamine, serotonin) by competent cells. Sulfated glycosaminoglycan heparin has been shown to inhibit exocytosis in mast cells[38]. Oxycoccusan, as a pectin, possess structural similarity with heparin, which comprises a polyanionic macromolecule with a negative charge density. A possible effect of OP on the activity of basophils and mast cells is under investigation. It is possible that OP exerts its preventive effect on colitis by affecting plasma factors that regulate vascular permeability and cellular trafficking during the process of inflammation.

Thus, a preventive effect of pectin from the common cranberry, namely oxycoccusan OP, on acetic acid-induced colitis in mice was detected. Neutrophil infiltration reduction and antioxidant action may be implicated in the protective effect of oxycoccusan.

REFERENCES

1 **Hendrickson BA**, Gokhale R, Cho JH. Clinical aspects and pathophysiology of inflammatory bowel disease. *Clin Microbiol Rev* 2002; **15**: 79-94

2 **Xu CT**, Meng SY, Pan BR. Drug therapy for ulcerative colitis. *World J Gastroenterol* 2004; **10**: 2311-2317

3 **Rampton DS**, Phil D. New treatments for inflammatory bowel disease. *World J Gastroenterol* 1998; **4**: 369-376

4 **Rolandelli RH**, Saul SH, Settle RG, Jacobs DO, Trerotola SO, Rombeau JL. Comparison of parenteral nutrition and enteral

feeding with pectin in experimental colitis in the rat. *Am J Clin Nutr* 1988; **47**: 715-721
5 **Lim BO**, Lee SH, Park DK, Choue RW. Effect of dietary pectin on the production of immunoglobulins and cytokines by mesenteric lymph node lymphocytes in mouse colitis induced with dextran sulfate sodium. *Biosci Biotechnol Biochem* 2003; **67**: 1706-1712
6 **Popov SV**, Ovodova RG, Markov PA, Nikitina IR, Ovodov YS. Protective Effect of Comaruman, a Pectin of Cinquefoil Comarum palustre L., on Acetic Acid-Induced Colitis in Mice. *Dig Dis Sci* 2006; **51**: 1532-1537
7 **Ovodov IuS**. Polysaccharides of flower plants: structure and physiological activity. *Bioorg Khim* 1998; **24**: 423-439
8 **Thakur BR**, Singh RK, Handa AK. Chemistry and uses of pectin--a review. *Crit Rev Food Sci Nutr* 1997; **37**: 47-73
9 **Borchers AT**, Keen CL, Stern JS, Gershwin ME. Inflammation and Native American medicine: the role of botanicals. *Am J Clin Nutr* 2000; **72**: 339-347
10 **Kontiokari T**, Sundqvist K, Nuutinen M, Pokka T, Koskela M, Uhari M. Randomised trial of cranberry-lingonberry juice and Lactobacillus GG drink for the prevention of urinary tract infections in women. *BMJ* 2001; **322**: 1571
11 **Zafriri D**, Ofek I, Adar R, Pocino M, Sharon N. Inhibitory activity of cranberry juice on adherence of type 1 and type P fimbriated Escherichia coli to eucaryotic cells. *Antimicrob Agents Chemother* 1989; **33**: 92-98
12 **Shmuely H**, Burger O, Neeman I, Yahav J, Samra Z, Niv Y, Sharon N, Weiss E, Athamna A, Tabak M, Ofek I. Susceptibility of Helicobacter pylori isolates to the antiadhesion activity of a high-molecular-weight constituent of cranberry. *Diagn Microbiol Infect Dis* 2004; **50**: 231-235
13 **Bodet C**, Chandad F, Grenier D. Anti-inflammatory activity of a high-molecular-weight cranberry fraction on macrophages stimulated by lipopolysaccharides from periodontopathogens. *J Dent Res* 2006; **85**: 235-239
14 **Gu L**, Kelm MA, Hammerstone JF, Beecher G, Holden J, Haytowitz D, Gebhardt S, Prior RL. Concentrations of proanthocyanidins in common foods and estimations of normal consumption. *J Nutr* 2004; **134**: 613–617
15 **Puupponen-Pimia R**, Nohynek L, Alakomi HL, Oksman-Caldentey KM. The action of berry phenolics against human intestinal pathogens. *Biofactors* 2005; **23**: 243-251
16 **Clague JA**, Fellers CR. Relation of benzonic acid content and other constituents of cranberries to keeping quality. *Plant Physiol* 1934; **9**: 631-636
17 **Elwell WE**, Dehn WM. Pectic content of plant material. *Plant Physiol* 1939; **14**: 809-816
18 **Polle AY**, Ovodova RG, Chizhov AO, Shashkov AS, Ovodov YS. Structure of tanacetan, a pectic polysaccharide from tansy Tanacetum vulgare L. *Biochemistry* (Mosc) 2002; **67**: 1371-1376
19 **Itoh H**, Kataoka H. Roles of hepatocyte growth factor activator (HGFA) and its inhibitor HAI-1 in the regeneration of injured gastrointestinal mucosa. *J Gastroenterol* 2002; **37** Suppl 14: 15-21
20 **Mahgoub AA**, El-Medany AA, Hager HH, Mustafa AA, El-Sabah DM. Evaluating the prophylactic potential of zafirlukast against the toxic effects of acetic acid on the rat colon. *Toxicol Lett* 2003; **145**: 79-87
21 **Evans SM**, Laszlo F, Whittle BJ. Site-specific lesion formation, inflammation and inducible nitric oxide synthase expression by indomethacin in the rat intestine. *Eur J Pharmacol* 2000; **388**: 281-285
22 **Cetinkaya A**, Bulbuloglu E, Kantarceken B, Ciralik H, Kurutas EB, Buyukbese MA, Gumusalan Y. Effects of L-carnitine on oxidant/antioxidant status in acetic acid-induced colitis. *Dig Dis Sci* 2006; **51**: 488-494
23 **Lowry OH**, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951; **193**: 265-75
24 **Blandizzi C**, Natale G, Gherardi G, Lazzeri G, Marveggio C, Colucci R, Carignani D, Del Tacca M. Acid-independent gastroprotective effects of lansoprazole in experimental mucosal injury. *Dig Dis Sci* 1999; **44**: 2039-2050
25 **Pereira BM**, da Silva BP, Pereira NA, Parente JP. Anti-inflammatory and immunologically active polysaccharides of Periandra mediterranea. *Phytochemistry* 2000; **54**: 409-413
26 **Fraser I**, Hughes D, Gordon S. Divalent cation-independent macrophage adhesion inhibited by monoclonal antibody to murine scavenger receptor. *Nature* 1993; **364**: 343-346
27 **Liu L**, Wang ZP, Xu CT, Pan BR, Mei QB, Long Y, Liu JY, Zhou SY. Effects of Rheum tanguticum polysaccharide on TNBS -induced colitis and CD4+T cells in rats. *World J Gastroenterol* 2003; **9**: 2284-2288
28 **Englyst HN**, Quigley ME, Hudson GJ. Definition and measurement of dietary fibre. *Eur J Clin Nutr* 1995; **49** Suppl 3: S48-S62
29 **Krawisz JE**, Sharon P, Stenson WF. Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity. Assessment of inflammation in rat and hamster models. *Gastroenterology* 1984; **87**: 1344-1350
30 **Nusrat A**, Parkos CA, Liang TW, Carnes DK, Madara JL. Neutrophil migration across model intestinal epithelia: monolayer disruption and subsequent events in epithelial repair. *Gastroenterology* 1997; **113**: 1489-1500
31 **Pravda J**. Radical induction theory of ulcerative colitis. *World J Gastroenterol* 2005; **11**: 2371-2384
32 **Koch TR**, Yuan LX, Stryker SJ, Ratliff P, Telford GL, Opara EC. Total antioxidant capacity of colon in patients with chronic ulcerative colitis. *Dig Dis Sci* 2000; **45**: 1814-1819
33 **Liu SP**, Dong WG, Wu DF, Luo HS, Yu JP. Protective effect of angelica sinensis polysaccharide on experimental immunological colon injury in rats. *World J Gastroenterol* 2003; **9**: 2786-2790
34 **Ko JK**, Lam FY, Cheung AP. Amelioration of experimental colitis by Astragalus membranaceus through anti-oxidation and inhibition of adhesion molecule synthesis. *World J Gastroenterol* 2005; **11**: 5787-5794
35 **Liu LN**, Mei QB, Liu L, Zhang F, Liu ZG, Wang ZP, Wang RT. Protective effects of *Rheum tanguticum* polysaccharide against hydrogen peroxide-induced intestinal epithelial cell injury. *World J Gastroenterol* 2005; **11**: 1503-1507
36 **Kohen R**, Shadmi V, Kakunda A, Rubinstein A. Prevention of oxidative damage in the rat jejunal mucosa by pectin. *Br J Nutr* 1993; **69**: 789-800
37 **Wagner JG**, Roth RA. Neutrophil migration during endotoxemia. *J Leukoc Biol* 1999; **66**: 10-24
38 **Inase N**, Schreck RE, Lazarus SC. Heparin inhibits histamine release from canine mast cells. *Am J Physiol* 1993; **264**: L387-L390

S- Editor Wang J **L- Editor** Rippe RA **E- Editor** Bai SH