

Effects of Pleuran (β -Glucan Isolated from *Pleurotus ostreatus*) on Experimental Colitis in Rats

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Summary

The effects of pleuran, β -1,3 glucan isolated from *Pleurotus ostreatus*, were studied in a model of acute colitis induced by intracolonic administration of acetic acid. There was a reduction of the colonic damage score, colonic wet weight and wet/dry weight ratio 48 h after single luminal 2 % pleuran suspension pretreatment. Similar results were obtained after repeated intraperitoneal administration of pleuran in doses of 30 and 100 mg/kg. Pleuran given orally as a 10 % food component over 4 weeks was effective in reducing the extent of mucosal damage, but did not prevent the increase of myeloperoxidase in the injured colonic segment. In the segment without macroscopic evidence of inflammation, myeloperoxidase activity was significantly lower as documented by histological examination. The results indicate a possible role of this immunomodulator in the treatment of ulcerative colitis.

Key words

Pleuran • Glucan • Acetic acid induced colitis • Rats

Introduction

Ulcerative colitis is a chronically recurrent inflammatory bowel disease of unknown origin. Among various etiopathogenic mechanisms, immunological processes and reactive oxygen metabolites have been proposed to contribute considerably to the development of tissue injury (Keshavarzian *et al.* 1990, Grisham 1994, Fiocchi 1998). Glucans, a structurally diverse group of glucose polymers isolated from microorganisms, are known to exhibit properties of "biological response modifiers" (Bohn and BeMiller 1995). Their immunomodulating effects include the capacity to render hosts more resistant to infections, neoplasms and

radiation (Chihara *et al.* 1982, Di Luzio 1983, Di Luzio *et al.* 1985). On analyzing their radioprotective effect, glucans were also found to act as effective free radical scavengers (Patchen *et al.* 1987). The aim of this study was to determine whether pleuran, the β -1,3 glucan isolated from *Pleurotus ostreatus* (Kuniak *et al.* 1992), could protect the colonic mucosa in a model of experimental colonic inflammation. Preliminary results of these studies were reported in abstract form (Bobek *et al.* 1999, Galbavý *et al.* 1999, Nosálová *et al.* 1999).

Material and Methods

Animals and diet

Male Wistar rats from monitored conventional breeding (Inst. Exp. Pharmacology SAS at Dobrá Voda), weighing 200-280 g, were used. Animals were randomly distributed into four groups. The first and second group were fed a pelleted standard rodent diet (KKZ-P-M, Dobrá Voda). The third group was given a semisynthetic diet of the following composition (in %): starch 56.85, casein 18, pork fat 10, cellulose 10, mineral and vitamin mixture 4 and 1, choline chloride 0.15 (cellulose diet) (Yamashita et al. 1980). In the fourth group, pleuran was substituted for cellulose (pleuran diet). The rats were maintained for 4 weeks under standard conditions without modification of the light regime, with access to tap water and the diets *ad libitum*.

Chemicals

Pleuran (β -1,3-D glucan) was kindly supplied by Ing. Kuniak from the Slovak Technical University, Faculty of Chemical Technology, Bratislava. It is a polyglucose derivative isolated from fresh *Pleurotus ostreatus* fruiting bodies by extraction with 0.15 M NaOH. The product was re-extracted by acetone and dried at 60 °C (Karácsonyi and Kuniak 1994). Pleuran is insoluble in water and organic solvents.

Agents for myeloperoxidase (MPO) assay were from Sigma (St. Louis, MO, USA), other chemicals were of analytical grade.

Induction of colitis

The rats were anesthetized by 50 mg/kg thiopental i.p. Colitis was induced by a modified method of Fedorak et al. (1992), (Nosáľová et al. 1999). After laparotomy, a reversible ligature was tied at the junction of the caecum and colon ascendens. The colon was cleansed of its luminal content with warm saline (37 °C) and the residual fluid was manually pressed out. Distal to the ligature 2 ml of 4 % acetic acid (AA) were administered into the colon and after 40 s exposure the luminal content was expelled by instillation of 10 ml of air. The ligature was removed and the abdomen was sutured. Control rats were sham operated.

Pleuran was administered as follows

Groups fed a standard rodent diet:

Group 1: single intracolonic (i.c.) administration of 2 ml 2% pleuran suspension in saline 30 min before exposure to AA; control rats received saline in the same volume;

Group 2: repeated intraperitoneal (i.p.) pleuran administration in doses of 10, 30 and 100 mg/kg in a volume of 0.5 ml/100 g 48 h and 24 h before AA plus 2 ml 2 %

pleuran suspension administered i.c. 30 min before AA exposure; control rats received the vehicle only.

Groups fed a semisynthetic diet:

Group 3: rats fed a cellulose diet for 4 weeks;

Group 4: oral administration of pleuran as a 10 % food component for 4 weeks.

At the end of the experiment, colitis in groups 3 and 4 was induced by i.c. administration of 4 % AA, as described above; control rats were sham-operated.

All rats were allowed to recover with food and water *ad libitum* and the resulting injury was assessed after 48 h.

Assessment of colonic damage

The rats were weighed, inspected for the presence of diarrhoea and sacrificed by decapitation. The colons were excised and opened longitudinally, rinsed with cold saline and observed under a dissecting microscope. Using a slightly modified scoring system of Wallace et al. (1989), colonic damage was evaluated by an observer uninformed about the treatment. The following criteria for scoring the colonic damage were used: 0 – normal appearance, 1 – hyperemia, 2 – hemorrhage, 3 – one ulcer, 4 – two or more sites of ulceration, 5 – ulceration extending >1 cm along the length of the colon, 6-10 – if damage covered >2 cm along the length of the colon, the score was increased by one point for each additional cm of involvement. The wet and dry weight of colon was recorded and the colonic wet/dry weight ratio was calculated.

Colonic myeloperoxidase activity

Myeloperoxidase (MPO) activity was determined in colonic wall samples 48 h after the induction of colitis. The samples were taken from the inflammatory site (or from the colonic segment usually predisposed to damage) and from the site without evidence of macroscopic damage. MPO activity was assayed spectrophotometrically by determining the decomposition of hydrogen peroxide using o-dianisidine as the hydrogen donor (Bradley et al. 1982). Tissue samples of approximately 50 mg were taken, weighed and homogenized three times for 30 s at 4 °C in 1 ml of ice-cold 0.5 % hexadecyltrimethylammonium bromide in 50 mmol/l phosphate buffer (pH 6). The homogenate was subjected to three freeze/thaw cycles and centrifuged for 15 min at 40 000xg. MPO activity was determined by the addition of 0.1 ml of the supernatant to 2.9 ml of 50 mmol/l phosphate buffer containing 0.167 mg/ml o-

dianisidine dihydrochloride and 0.0005 % hydrogen peroxide. The change in absorbance at 460 nm over a 5 min period was measured at 25 °C. The data are expressed as the change in absorbance/min/g wet weight.

Morphological analysis

Colonic tissues were dissected and preserved in a formalin solution. Histological examinations were performed on paraffin cross-sections stained with hematoxylin and eosin and by the chloracetate esterase technique. Using a semiquantitative scale, the extent of ulcerations, crypt abscesses, inflammatory infiltration, vessel dilatation and necrosis thickness were determined. Morphometric examination of neutrophil infiltration was performed by the Impor system (Kvant, SR).

The following scales were used:

Ulcerations: 0 – none, 1 – erosion or ulceration not crossing lamina muscularis mucosae, 2 – multiple ulcerations, 3 – ulceration expanding into the submucosa.

Crypt abscesses: 0 – none, 1 – 1-3 abscesses/slide, 2 – 4-9, 3 – 10 and more abscesses.

Inflammatory infiltration: 0 – none, 1 – mild, 2 – medium, 3 – pronounced.

Vessel dilatation: 0 – none, 1 – mild, 2 – medium, 3 – pronounced.

Necrosis thickness: 0 – none, 1 – up to 0.1 mm, 2 – 0.2 mm, 3 – 0.3 mm.

Statistical analysis

The data are expressed as means $\pm$ S.E.M. Student's t-test was used for statistical analysis, P values < 0.05 were considered significant. Non-parametric data were analyzed by the Mann-Whitney U test.

Results

Intracolonic administration of 4 % acetic acid induced an acute inflammatory reaction in control untreated rats. Diffuse hyperemia, bleeding with erosions, ulcerations and increased colonic wet weight and the wet/dry weight ratio were present in the colons 48 h after AA injection. As shown in Table 1, repeated i.p. pleuran administration in doses of 30 and 100 mg/kg significantly reduced the values of colonic inflammatory parameters. Comparable findings were obtained with single i.c. pleuran administration, resulting in a significant decrease of colonic damage score, wet weight and the wet/dry

weight ratio in colons with AA induced inflammation (Table 2). Chronic 4-week oral pleuran administration was effective in diminishing the score of colonic damage. The difference in colonic wet weight increase induced by AA in rats fed the cellulose diet or pleuran diet was not significant (Table 3).

Table 1. Effect of pleuran administered intraperitoneally on parameters of colonic injury in acetic acid induced colitis in rats.

	Pleuran dose mg/kg	Colonic damage score	Colonic wet weight mg/cm	Wet/dry weight ratio
<i>Saline</i>				
+ AA		3.8 $\pm$ 0.3	94.3 $\pm$ 1.6	5.2 $\pm$ 0.1
<i>Pleuran</i>				
+ AA	10	2.7 $\pm$ 1.2	87.5 $\pm$ 11.2	4.4 $\pm$ 0.2
<i>Saline</i>				
+ AA		6.0 $\pm$ 0.0	107.5 $\pm$ 2.6	5.4 $\pm$ 0.1
<i>Pleuran</i>				
+ AA	30	1.5 $\pm$ 0.5*	70.9 $\pm$ 6.7*	4.6 $\pm$ 0.3
<i>Saline</i>				
+ AA		7.3 $\pm$ 0.7	128.8 $\pm$ 6.4	5.3 $\pm$ 0.1
<i>Pleuran</i>				
+ AA	100	0.7 $\pm$ 0.2*	65.8 $\pm$ 3.9*	4.3 $\pm$ 0.1*

Means $\pm$ S.E.M., n=5 rats in each group, *P<0.05 acetic acid (AA) plus pleuran-treated rats versus acetic acid plus saline-treated rats.

Table 2. Effect of pleuran administered intracolonicly on parameters of colonic injury in acetic acid-induced colitis in rats.

Treatment	Colonic damage score	Colonic wet weight mg/cm	Wet/dry weight ratio
AA + cellulose	6.0 $\pm$ 0.6	97.9 $\pm$ 5.8	4.8 $\pm$ 0.1
AA + pleuran	1.5 $\pm$ 0.5**	71.9 $\pm$ 2.5**	4.2 $\pm$ 0.1**

Means $\pm$ S.E.M., n=6-9 rats in each group, **P<0.01 acetic acid (AA) plus pleuran-treated rats versus acetic acid plus cellulose-treated rats.

Table 3. Effect of pleuran administered orally on parameters of colonic injury in acetic acid-induced colitis in rats.

Treatment	Colonic damage score	Colonic wet weight mg/cm
Cellulose sham	0	83.3±2.9
Cellulose + AA	3.45±0.26	114.6±6.3*
Pleuran sham	0	79.3±4.1
Pleuran + AA	2.11±0.38 ⁺	101.6±4.7*

Means ± S.E.M., each group consists of at least 8 rats, ⁺*P*<0.05 acetic acid (AA) plus pleuran-treated rats vs acetic acid plus cellulose-treated rats, **P*<0.05 sham versus acetic acid.

The activity of colonic MPO was increased by inflammation. Pleuran oral pretreatment significantly decreased MPO in control sham-operated rats as compared to pleuran untreated rats but failed to prevent

the MPO increase after acetic acid in the colonic segment predisposed to damage (Fig. 1). However, the MPO activity in the segment was usually without evidence of macroscopic damage and was significantly lower in the pleuran group than in the untreated (cellulose) group (Fig. 2).

The results of histological and morphometric analysis are summarized in Table 4. The administration of acetic acid induced ulcerations and crypt abscesses to the same extent in both groups receiving the cellulose or pleuran diet. There were no significant differences in vasodilatation found between the groups studied. The thickness of epithelial necrosis was smaller in the pleuran diet group compared to the cellulose one, but the difference was not significant. Acetic acid increased inflammatory infiltration of the colonic wall. The number of neutrophils in the vicinity of ulceration was not increased significantly in the pleuran group. Nevertheless, in the segment without signs of inflammation there was a significant decrease of neutrophil infiltration in this group, indicating neutrophil migration by pleuran to the injured mucosa.

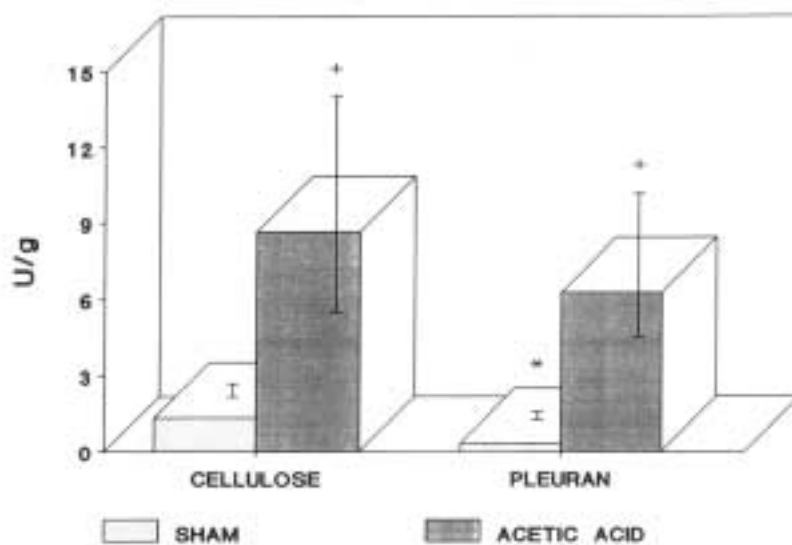


Fig. 1. Effect of chronic oral pleuran treatment on myeloperoxidase (MPO) activity measured in colonic segment predisposed to inflammatory damage and/or damaged 48 h after the induction of colitis by intracolonic administration of 4 % acetic acid. Control rats were sham-operated. Pleuran was given as a 10 % food component for 4 weeks. Values are means ± S.E.M., * *P*<0.05 pleuran-treated rats versus cellulose-treated rats, + *P*<0.05 acetic acid versus sham-operated rats.

Discussion

Our results have shown that pleuran locally administered, with or without concomitant parenteral pretreatment, was effective in reducing colonic damage induced by acetic acid. Higa *et al.* (1993) also found attenuation of epithelial injury in acute experimental colitis by immunomodulators. A similar protective effect of pleuran was observed in models of experimental

infections (Ciba Geigy Report 1991). Pleuran demonstrated a dose-dependent ability to promote the survival of mice susceptible to systemic *Listeria monocytogenes* infection, when administered prophylactically by the intraperitoneal but not the oral route. The doses above 10 mg/kg given 72 h and 24 h before infection were maximally effective. Similarly, an apparent dose-dependent promotion of survival of mice susceptible to pulmonary *Haemophilus influenzae*

infections occurred when pleuran was administered i.p. 72 h and 24 h prior to the infection, with the optimal dose of 3 mg/kg. In this experiment, pleuran was also effective

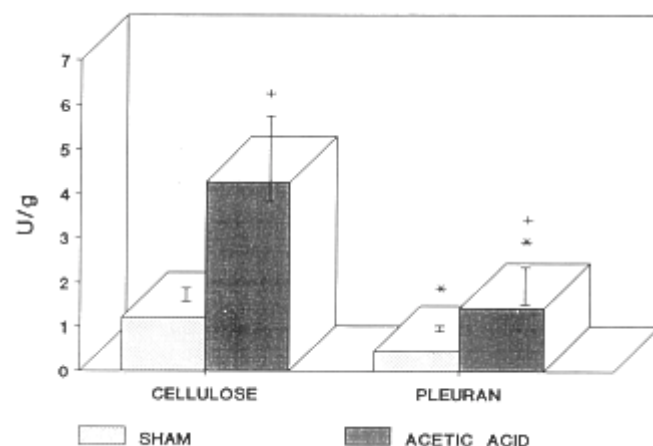
after oral administration in a dose of 3 mg/kg. In our experiment, a reduction of colonic damage score was recorded after chronic oral pleuran administration.

Table 4. Effect of pleuran on morphological parameters of colonic injury induced by acetic acid in rats.

	Controls Cellulose	Acetic acid Cellulose	Controls Pleuran	Acetic acid Pleuran
<i>Ulcerations</i>	0	1.8±0.5	0	1.6±0.4
<i>Crypt abscesses</i>	0	1.1±0.3	0	1.0±0.3
<i>Vessel dilatation</i>	0.5±0.2	0.7±0.2	0.7±0.3	0.7±0.2
<i>Necrosis thickness</i>	0	0.4±0.3	0	0.2±0.2
<i>Inflammatory infiltration</i>	0	1.8±0.4	0	1.4±0.3
<i>Neutrophil number</i>				
<i>close to ulceration</i>		51.1±17.1		110.5±36.9
<i>Neutrophil number</i>				
<i>distant from ulceration</i>		43.2±5.9		27.4±1.8

Means ± S.E.M., **P*<0.05 acetic acid plus pleuran-treated versus acetic acid plus cellulose-treated rats.

Fig. 2. Effect of chronic oral pleuran treatment on myeloperoxidase (MPO) activity measured in colonic segment non-predisposed to damage 48 h after induction of colitis by intracolonic administration of 4 % acetic acid. Control rats were sham-operated. Pleuran was given as a 10 % food component for 4 weeks. Values are means ± S.E.M., * *P*<0.05 pleuran-treated versus cellulose-treated rats, + *P*<0.05 acetic acid versus sham-operated rats.



In accordance with the effect of structurally identical β -glucans from baker's yeast or from other microbial and fungal sources, pleuran shows an immunostimulatory effect mediated by the activation of neutrophils, macrophages, monocytes and NK cells through specific receptors CR3 (CD11b/CD18) (Větvíčka *et al.* 1996) and β -glucan receptor (Thorton *et al.* 1996). This is accompanied by stimulation of cytokine production, such as TNF- α , interleukin 1, etc., resulting in increased immunological surveillance.

The ability of pleuran to activate nonspecific defence mechanisms is probably responsible for lower

MPO levels caused by anesthesia and stress of sham operation in pleuran-treated rats. The difference in the effect of pleuran on MPO activity in the colonic segments predisposed or non-predisposed to damage may be explained by the influence of pleuran on the migration and accumulation of polymorphonuclear leukocytes to the sites of inflammatory injury, as evidenced by histological and morphometric analysis. Neutrophils participate in the pathogenesis of inflammatory bowel diseases as a main source of reactive oxygen species (Grisham and Granger 1988), the role of which in ulcerative colitis has been generally accepted. An

increased content of ROS found in the colonic mucosa of patients with ulcerative colitis or Crohn's disease correlated positively with the intensity of the disease (Simmonds *et al.* 1992).

Our results on the protective effect of orally applied pleuran in the prevention or dietetic therapy of inflammatory colon disease are promising. Moreover, the content of pleuran is 10 times higher in the oyster

mushroom compared to the β -glucan content in *Saccharomyces cerevisiae*, making the production of this substance more economical.

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Reprint requests

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