Inflammopharmacology 16 (2008) 162–167
0925-4692/08/040162-6
DOI 10.1007/s10787-007-0026-5

Inflammopharmacology

Research Article

Protective effect of polysaccharides from *Angelica sinensis* on ulcerative colitis in rats

V. K. C. Wong[1], L. Yu[2], C. H. Cho[2,*]

[1] Department of Pharmacology, Faculty of Medicine, The University of Hong Kong
[2] Department of Pharmacology, Faculty of Medicine, The Chinese University of Hong Kong, The Chinese University of Hong Kong. Shatin, New Territories. Hong Kong, China, e-mail: chcho@cuhk.edu.hk

Received 2 January 2007; revised 12 February 2007; accepted 12 February 2007

Abstract. Ulcerative colitis (UC) involves the dysregulation of intestinal mucosal immunity and imbalance between the reactive oxygen species (ROS) and the endogenous anti-oxidants. While the protective effects of *Angelica sinensis* (AS) polysaccharides on neutrophil-dependent gastric mucosal damage have been reported, similar protective effects on UC are still uncertain. Hence our study aimed to investigate the effects of AS polysaccharides on rats with acute UC induced by 2,4-dinitrobenzene sulphonic acid (DNBS) evaluated after 24 h. Intrarectal injection of DNBS significantly reduced the glutathione (GSH) content, increased malondialdehyde concentration and raised the amount of apoptotic cells in colon tissues, which were related to oxidative stress and attenuated by AS polysaccharides pretreatment (5 mg/ml and 10 mg/ml). These findings suggest that oxidative stress and GSH depletion are highly associated with the pathological mechanism of UC, and the protective effects of AS polysaccharides are closely related to the prevention of oxidative stress, which may occur during neutrophil infiltration in the pathological process of UC.

Key words: *Angelica sinensis* polysaccharides – ulcerative colitis

1. Introduction

Ulcerative colitis (UC) and Crohn's disease (CD) are known as inflammatory bowel disease (IBD) in which the pathogenic mechanisms are still ill defined. While IBD is more commonly found in western countries (Kirsner and Shorter, 1982), it is noted that there is an increasing trend of incidence in Asia (Yang et al., 2001). Despite the unknown mechanism of IBD, it is postulated that dysregulated and exaggerated local immune responses to commensal microbes in the gut, particularly in genetically susceptible individuals, are the key events in the pathogenesis of IBD (MacDermott, 1994). Indeed, the complex interaction between genetic factors and infectious agents can lead to dysregulation of intestinal mucosal immunity, resulting in activation of macrophages, enhanced endothelial cell adhesion molecules (ECAM) expression and infiltration of neutrophils (Galperin and Gershwin, 1997; Plevy et al., 1996; Thompson, 1994; Laroux and Grisham, 2001). The net result is the imbalance between pro-oxidant and anti-oxidant, which is exemplified by deterioration of anti-oxidative glutathione metabolism resulting in the production of harmful free radicals such as malondialdehyde (MDA) and 4-hydroxynoneal. Therefore, anti-inflammatory and immunosuppressing agents are the choices of drugs for IBD.

Angelica sinensis (AS), also known as Dong Guai, belongs to the species of Apiaceae. It is distributed worldwide, especially in Mainland China, Korea and Japan. Only the root of AS is used as a therapeutic agent renowned for tonifying role in the treatment of menstrual and puerperal disorders, and sterility in women for about two thousand years in China (Mckenna et al., 2002). Many chemical components of AS are found including carbohydrates, lipid compounds, organic acids, phenolic compounds and other constituents. One of the most active ingredients is polysaccharides. AS polysaccharides exert anti-inflammatory and protective action on the gastrointestinal mucosa through inhibition of neutrophil infiltration in the stomach (Cho et al., 2000). Besides, the direct healing effect of AS polysaccharides on gastric epithelial cells was found. Such healing action is directly associated with ornithine decarboxylase (ODC) activity and

* *Corresponding author*

c-myc protein expression. Further study demonstrated that this healing effect is partially due to epidermal growth factor (EGF)-mediated pathway (Ye et al., 2001a, b). However, application of AS polysaccharides in other form of inflammatory disorders has not been studied. Therefore, it is hypothesized that polysaccharides from AS also provide similar protective effects on rats with UC.

In the current study, we investigated whether AS polysaccharides could protect against UC if any through the reduction of oxidative stress as well as intestinal epithelial cell turnover in colitis animals.

2. Materials and methods

2.1. Extraction of AS polysaccharides

Roots of AS (Oliv.) Diels were bought from Minxian County, Gansu Province, China. The powder of AS polysaccharides was produced by water extraction and ethanol precipitation described by Cho et al. (2000). In brief, roots of AS were boiled in water for 4 h for 3 times. During the end of each four-hour boiling, the water extract was collected and the residue was reboiled in water for another four-hour period. Then all extracts were pooled and mixed with ethanol (final concentration 75% vol/vol) to precipitate the polysaccharide-enriched fraction. The AS polysaccharides solutions were made by reconstitution of the AS polysaccharides powder with water. This fraction consisted of 97% carbohydrates (about 3% of them uronic acids) and 3% proteins. These polysaccharides are probably of pectic nature (Cho et al., 2000). Two preparations, 5 mg/ml and 10 mg/ml in drinking water, were employed in this study.

2.2. Animals and experimental designs

Study of the effects of AS polysaccharides on UC was performed in five groups of male Sprague-Dawley rats (180–250 g). All animal protocols employed were in compliance with the Principles of Laboratory Animal Care and were approved by the Committee on the Use of Live Animals in Teaching and Research at the University of Hong Kong. Our pilot study showed that 5-day treatment with the current doses of AS polysaccharides before DNBS administration produced better responses on inflammation in respect to lesion area and myeloperoxidase activity in colon tissues. Therefore, this regimen of drug treatment was adopted in the present study. Animals were randomly assigned into four groups (9–10 rats in each group), which were subjected to 5-day treatment with AS polysaccharides and then given with 2,4-dinitrobenzene sulphonic acid (DNBS) (Fig. 1). Rats were sacrificed on the next day after DNBS administration by cervical dislocation and the distal colon tissues were taken out for biochemical and histological assessments.

2.3. Induction of ulcerative colitis

In the present study, DNBS was selected rather than 2,4,6-trinitrobenzene sulfonic acid as it produces colitis resembling UC and it also mimics UC conditions in humans (Hawkins et al., 1997). Animals were firstly anaesthetized with a mixture of ketamine and xylazine (2 ml/kg, i. p.). After that, colitis was induced by intracolonic administration of 0.25 ml of 50% ethanol (vol/vol) containing 30 mg of DNBS via a rubber cannula. The tip of the catheter was inserted in such a way that it was set at 8 cm proximal to the anus. After perfusing the DNBS solution, the cannula was kept inside the bodies of rats for thirty seconds to ensure that no leakage of the solution would occur. Finally, the cannula was removed and the rats were returned to cages. Same intracolonic treatment was applied to those rats in the normal control group except that distilled water was employed instead of DNBS solution.

2.4. Body weight measurement

Animals were weighed between 3:00 and 4:00 p. m. daily before DNBS treatment for 5 days. The change in body weight after DNBS-induction was calculated by subtracting the body weight just before DNBS administration from the body weight 24 h after colitis induction and expressed as percentage of change.

2.5. Measurement of colonic lesion area, microscopic lesion score and colon weight

The assessment of damage on the excised colon was determined by measuring the areas of colonic lesions and was expressed in mm^2. Concerning microscopic lesion score, the embedded and sectioned samples were mounted onto slides stained with haematoxylin and eosin, then the histological assessment was performed under light microscopy on coded

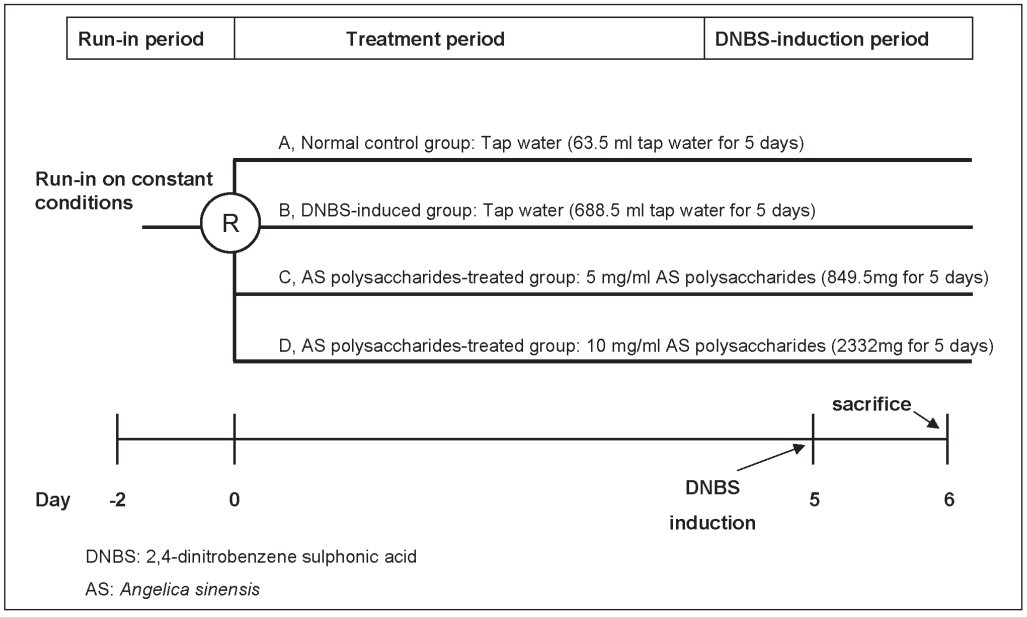

Fig. 1. Experimental design for *Angelica sinensis* (AS) treatment and ulcerative colitis induction by 2,4-dinitrobenzenesulphonic acid (DNBS).

slides using the scoring system suggested by Wallace and Keenan (1990) (Table 1).

2.6. Determination of intestinal glutathione (GSH) level

Colorimetric measurement was used to determine the content of GSH in the intestinal tissue. Briefly, the tissue was suspended in ice-cold phosphate buffer (pH 8.0) and homogenized with a homogenizer (Ultra-Turrax, T25, IKA, USA) for 30 s on ice. The extract was then centrifuged at 12,000 rpm for 30 min at 4 °C. Subsequently, supernatant was added to 4 % (vol/vol) 5-sulphosalicyclic acid for protein precipitation. Then the mixture was centrifuged at 3,000 g at 4 °C. Following this, deproteinized supernatant was added to 0.2 M phosphate buffer (pH 8.0), and 0.01 M 5,5′-dithiobis(2-nitrobenzoic acid) was further added. The solution was allowed to stand at room temperature to develop yellow colour. Then the optical density was measured at 412 nm by using reagent blank as control and the calibration curve of GSH was obtained by using different standard solutions. Lastly, together with the protein quantity measured by the Bradford assay, intestinal GSH content was calculated from the standard curve and normalized as μmol/L per μg of protein.

2.7. Determination of Malondialdehyde (MDA) level

MDA was measured using the method reported by Ohkawa et al. (1979). Colon tissue was homogenized with a homogenizer in ice-cold 1.15 % KCl aqueous solution. Then, aliquot of the homogenate was mixed with a mixture containing 0.8 % TBA, 20 % acetic acid, 8.1 % sodium dodecyl sulphate and distilled water. The whole mixture was incubated at 95 °C for 1 h and allowed to cool down in tap water. Samples were centrifuged at 4,000 rpm for 10 min. Finally, the optical density of the colour-complex in the supernatant was measured at 535 nm by a spectrophotometer (DU 650, Beckman, USA). 1,1,3,3-tetrathoxypropane was used as the standard. Meanwhile, portion of the crude extracts was also measured for protein content by using the Bradford assay. The amount of intestinal MDA was extrapolated from the standard curve and normalized as nmol MDA per μg of protein.

2.8. Colonic cellular apoptosis measurement

Immunohistochemical staining of the apoptotic cells in the distal colon was performed using the methods of terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) as reported by Strater et al. (1995). First, the samples were de-paraffinized and hydrated, followed by the treatment with proteinase K buffer at 37 °C. After that, the endogenous peroxidase activity was inactivated by using 3 % hydrogen peroxide in methanol at room temperature. Following this, tissue sections were incubated in the TdT buffer at 37 °C. At the same time, positive control was produced by incubating the slice with DNase 1 (1μg/ml) in the TdT buffer. After incubation, the reactions were quenched by adding terminating buffer (300 mM NaCl, 30 mM sodium citrate). Afterwards, tissue sections were blocked by the reaction with 2 % horse serum in PBS, which were then immersed in PBS with peroxidase conjugated streptavidin (DKAO) at 37 °C. The final step was to incubate slides with DAB/H_2O_2 solution at room temperature, counterstained with Mayer's hematoxylin, cleared in ethanol and xylene and then mounted onto slides. The number of apoptotic cells was recorded for 20 randomly selected complete crypts per tissue section.

2.9. Measurement of cell proliferation

Regarding the proliferative activity of colonic tissues, we employed proliferating cell nuclear antigen (PCNA) immunochemistry with modifications to quantify the number of PCNA-positive cells according to the methods described by Tarnawaski et al. (1992). Briefly, colonic samples were incubated with 0.01 M citric acid buffer (pH 6.0) at 80 °C. After incubation, pepsin digestion of the samples was performed at 37 °C and the slides were blocked by diluted serum (150 μl of normal serum in 10 ml of 0.05 M Tris-buffered saline, pH 7.8) at room temperature in a humidified container. Following this, slides were incubated with monoclonal antibody against rat PCNA (Santa Cruz Biotechnology, Santa Cruz, CA). After washing the slices with the Tris-buffered saline (TBS), slides were incubated with Link reagent (DAKO) at 25 °C. Afterwards, they were washed with TBS again and then incubated with streptavidin (DAKO). For the detection of PCNA-positive cells, further incubation of slides with H_2O_2-diaminobenzidine was performed. The final step was counterstaining the slides with Mayer's hematoxylin, washed with tap water, cleared by graded ethanol and then mounted. The counting of the colonic proliferating cells was the same as the method for apoptotic cell measurement.

Table 1. Criteria for microscopic damaging score of colonic ulceration and inflammation.

Score	Histological features
0	Normal
1	Damage limited to surface epithelium
2	Focal ulceration limited to mucosal
3	Focal, transmural inflammation, and ulceration
4	Extensive transmural ulceration and inflammation bordered by
5	normal mucosa
	Extensive transmural ulceration and inflammation involving entire section

2.10. Statistical methods

All data were given as mean ±S.E.M. Statistical analysis was conducted using the Student's two-tailed unpaired *t* test as well as Analysis of Variance (ANOVA), which was followed by the Dunnett's test. A *P* value less than 0.05 was regarded as statistically significant.

3. Results

3.1. Effects of Angelica sinensis polysaccharides on colonic lesion area, percentage of changes in body weight, microscopic lesion score and colon weight

The colonic lesion area in the DNBS control group (DNBS alone) was 208.1 ± 45.13 mm^2, which was significantly higher than that of the group without DNBS administration (0 mm^2, vehicle group) (Table 2). Tissue necrosis was observed in all the rats treated with DNBS. This suggests that DNBS treatment could exert damaging effect on colonic tissues. Pre-treatment with AS polysaccharides 5 mg/ml or 10 mg/ml prior to DNBS administration produced a marked reduction in colonic lesion areas and alleviated the macroscopic damaging effects generated by DNBS. The higher dose of AS provided the greater ameliorating action on the damage' (Table 2). A dose-dependent protective effect was observed in colonic lesion areas. Similar patterns were noted in the percentage of change in body weight of rats, in which there was a significant drop in body weight of rats in the DNBS-induced group (6.78 ± 0.01 %) when compared with the normal control group (3.47 ± 0.03 %). The lower dose of AS polysaccharides provided a significant attenuation of the body weight drop induced by DNBS treatment ($p < 0.05$).

Regarding the microscopic lesion score, DNBS treatment significantly increased the score (3.00 ± 0.28), when com-

Table 2. Effects of *Angelica sinensis* (AS) polysaccharide treatment given orally for 5 days before 2,4-dinitrobenzene sulphonic acid (DNBS) (0.25 ml given intracolonically) administration on colonic lesion area, microscopic damaging score, colon weigh and reduction in body weight in rats. CW/BW, colon weight per body weight indicates edema. The vehicle for AS polysaccharides was water. The data were expressed as means ±S.E.M. of 9–10 rats per group. * $p < 0.05$ compared with vehicle control without DNBS administration, † $p < 0.05$, †† $p < 0.01$ compared with DNBS induced group.

Group	Colonic lesion area (mm^2)	Microscopic lesion score	CW/BW (mg/g body weight)	Change in body weight (%)
Vehicle	0	0.33 ± 0.17	3.27 ± 0.34	3.47 ± 0.03
Vehicle + DNBS	208.1 ± 45.13*	3.00 ± 0.28*	5.63 ± 0.49*	–6.78 ± 0.01*
AS 5 mg/ml + DNBS	18.0 ± 4.67††	2.00 ± 0.15††	4.21 ± 0.62†	–3.78 ± 0.07†
AS 10 mg/ml + DNBS	7.2 ± 1.89†	2.10 ± 0.22†	5.47 ± 0.49	–6.02 ± 0.01

pared to the normal control group (0.33 ± 0.17). The two doses of AS polysaccharides provided a similar reduction in microscopic damaging scores. They demonstrated a significant reduction in the microscopic lesion score to 2.00 ± 0.15 and 2.10 ± 0.22 respectively, when compared with the DNBS-treated group.

In measuring the ratio of colon weight to body weight (CW/BW), DNBS significantly increased the CW/BW (5.63 ± 0.49 mg/g body weight) when compared with the normal control group (3.27 ± 0.34 mg/g body weight). Pre-treatment with the lower dose of AS polysaccharides significantly attenuated the increase of CW/BW caused by DNBS administration.

3.2. Effects of Angelica sinensis polysaccharides on the level of glutathione (GSH) and malondialdehyde (MDA)

Concerning the oxidative disturbances caused by DNBS, the agent significantly increased the level of MDA when compared with the basal level in the normal control group (Fig. 2). In the DNBS-treated group, the level of MDA was 322.69 ± 55.55 nmol/ μg protein, which was around 2 times that of the normal control group (163.73 ± 17.27 nmol/μg protein, $p < 0.05$). The 2 doses of AS polysaccharides produced a dose-dependent attenuation of the increment of MDA caused by DNBS. Moreover, significant result was detected at the higher dose of AS polysaccharides (175.73 ± 33.72 nmol MDA/μg protein). Concerning the result of glutathione level (GSH), both doses of AS polysaccharides alleviated the decrease of GSH level induced by DNBS (Fig. 3). Comparing with the normal control group of 0.66 ± 0.06 μM/mg protein, DNBS markedly decreased the GSH content to 0.45 ± 0.05 μM/mg protein ($p < 0.05$). AS polysaccharides provided a dose-dependent amelioration to the decrease in GSH level caused by DNBS and significant result was obtained at the higher dose of AS polysaccharides.

3.3. Effects of Angelica sinensis polysaccharides exposure on the DNBS-induced apoptotic cell death, cellular proliferation and the ratio of apoptosis/proliferation in colonic tissues

Similar to human ulcerative colitis, DNBS induced group raised the apoptotic cell counts when compared with the normal control group. AS polysaccharides provided a marked suppression on the increment of apoptotic cell counts caused by DNBS in a dose-dependent manner, and the higher dose of AS polysaccharides illustrated a significant effect in the reduction of apoptotic cell counts (Table 3). In cell proliferation, DNBS did not show significant effect on the proliferation of colonic cells; neither did both doses of AS polysaccharides. However, there was a trend for both doses of AS polysaccharides to increase the number of colonic proliferating cells.

Table 3. Effects of *Angelica sinensis* (AS) polysaccharide administered orally 5 days before 2,4-dinitrobenzene sulphonic acid (DNBS) (0.25 ml given intracolonically) administration on colonic apoptotic cell death, colonic proliferating cells and ratio of cellular apoptosis/proliferation in colonic mucosa. The vehicle for AS polysaccharides was water. The data were expressed as means ±S.E.M. of 9–10 rats per group. * $p < 0.05$ compared with vehicle control without DNBS administration, † $p < 0.05$ compared with the DNBS-treated group.

Group	Number of apoptotic cells	Number of proliferating cells	Apoptotic cells/ Proliferating cells (10^{-2})
Vehicle	35.1 ± 2.8	423 ± 45	8.3 ± 6.2
Vehicle + DNBS	63.2 ± 6.4*	475 ± 57	13.3 ± 11.2
AS 5 mg/ml + DNBS	57.9 ± 3.3	671 ± 35	8.6 ± 9.4
AS 10 mg/ml + DNBS	45.0 ± 3.9†	608 ± 62	7.4 ± 6.3

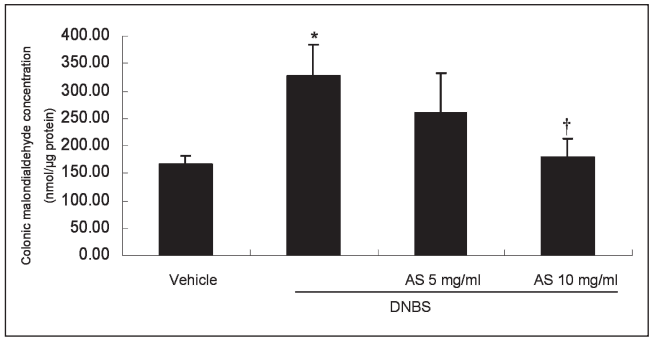

Fig. 2. Effects of *Angelica sinensis* (AS) polysaccharides on 2,4-dinitrobenzene sulphonic acid (DNBS)-induced colonic malondialdehyde concentration. The vehicle for AS polysaccharides was water. Results were expressed as means ±SEM of 9–10 rats. * $p < 0.05$ compared with vehicle control without DNBS administration; † $p < 0.05$ compared with DNBS-treated group.

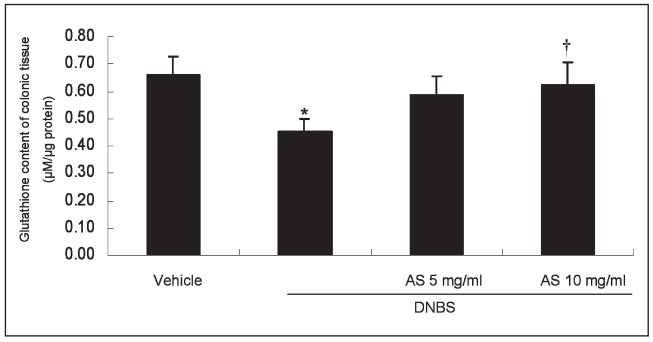

Fig. 3. Effects of *Angelica sinensis* (AS) polysaccharides on 2,4-dinitrobenzene sulphonic acid (DNBS)-induced colonic glutathione content. The vehicle for AS polysaccharides was water. Results were expressed as means ±SEM of 9–10 rats. * $p < 0.05$ compared with vehicle control without DNBS administration; † $p < 0.05$ compared with DNBS-treated group.

4. Discussion

AS polysaccharides pretreatment protected against acute mucosal damage induced by DNBS within 24 h in the colon. We studied further the underlying protective mechanism in animals. It has been reported that the occurrence of IBD is due to the imbalance between the pro-oxidant and anti-oxidant mechanisms (Grisham et al., 1991; Grisham, 1994). Besides, it is known that oxidative stress is highly associated with IBD patients and animal models of experimental colitis involving depletion of GSH level. The reactive oxygen species (ROS) react with intestinal macromolecules to elicit events like lipid peroxidation and finally cause intestinal cell apoptosis. Recently, it has been reported that the protective effect of polysaccharide-enriched fraction from AS on hepatic injury induced by acetaminophen was likely to be associated with the attenuation of GSH depletion (Ye et al., 2001c). Since GSH is a crucial determinant of tissue susceptibility to oxidative damage (Ischiropoulos et al., 1992), it is likely that the partial protection of AS polysaccharides on colitis in the current study is to maintain the GSH reserve in the colon (Fig. 3), which has been shown to act as a scavenger for free radical production during colitis formation. Furthermore, the increase in MDA concentration was completely blocked by AS polysaccharides pretreatment (Fig. 2). These findings reinforce the idea that AS polysaccharides could have significant anti-oxidant activity to inhibit lipid peroxidation in the colon. This could be the important mechanism to prevent colitis formation in the current study.

The protective effect of AS polysaccharides on apoptosis in intestinal cells was also studied. There was a pattern of decrease in apoptosis/proliferation ratio in the AS polysaccharides-treated groups in a dose-dependent manner (Table 3). It is believed that cytokines released from infiltrating immune cells and recruited to the inflammatory sites in ulcerative colitis, can alter intestinal epithelial cell turnover in such a way that higher apoptotic rate was observed (Fiocchi, 1998). Moreover, there is convincing evidence that TNF-α and interferon-α play an important role in UC and Crohn's disease. TNF-α, especially in combination with interferon-α, exerts potent direct effects on non-transformed human or rat intestinal epithelial cells turnover (Ruemmele et al., 1999). At the same time, Hui et al. (2006) reported that polysaccharides from AS have been shown to increase the turnover of gastrointestinal mucosal and hemopoietic stem cells. This report suggests that AS polysaccharides can produce immuno-stimulatory effects, which exert protective actions on cyclophophamide-induced leucopenia and proliferation arrest in the gastroduodenal mucosae. However, the association between the turnover of intestinal cells and AS polysaccharides treatment during ulcerative colitis is still unclear. Although the effect of AS polysaccharides on cell proliferation was less evident, the inhibitory effect on DNBS-induced apoptosis was noted. Since AS polysaccharides dose-dependently and significantly reduced the number of apoptotic cells in colonic mucosa, this effect could be due to the capacity of AS polysaccharides in attenuating oxidative stress, exemplified by increased GSH level and decreased MDA content in DNBS-treated mice. However, the direct anti-apoptotic effect by AS polysaccharides is still unknown. Nevertheless all these actions could in part protect against mucosal damage induced by DNBS in colonic tissues.

Subsequent to uncontrolled inflammatory response and oxidative stress, dramatic alternations in colon tissue morphology and intestinal microvascular function are also found both in experimental and human IBD (Laroux et al., 2001). The most notable histological changes associated with IBD are enhanced arteriolar blood flow, fluid exudation across intestinal capillaries, interstitial edema, thickening of the intestinal wall and colon inflammation, which are highly associated with many inflammatory mediators, including nitric oxide, bradykinin and prostaglandins. In fact, Hulten and co-workers (1977) showed that the blood flow in the colon of IBD patients with increases from two- to six-fold colon mass/body mass can be regarded as the extent of colon edema (Liu et al., 2003). Besides, according to Contran et al. (2005), UC is an ulcero-inflammatory disease with histopathological manifestation of mucosal destruction in which the disease may lead to outright ulceration and sometimes extends into the submucose and muscularis propria. Therefore, colon edema and lesion area can act as histological parameters for assessing the

severity of UC. In fact, these parameters are consistent with our present findings in which the macroscopic colonic lesion areas and microscopic lesion scores were dose-dependently reduced by both dosages of AS polysaccharides when compared with the DNBS control group (Table 2). This reduction of ulcer areas and decrease in colon architectural damages may be due to the anti-oxidant and anti-apoptotic effects of AS polysaccharides as mentioned above. Although lower dose of AS polysaccharides indicated significant attenuation of the drop in body mass and decreased the ratio of colon weight to body weight when compared with the DNBS-induced group, increasing the dose to 10 mg/ml did not further improve the clinical symptoms. In conclusion, our findings suggest that the protective effect of AS polysaccharides is highly associated with the decrease of oxidative stress and attenuation of apoptosis in colonic cells. Increases in oxidative stress and programmed cell death in the colon are the important pathogenic factors in the induction of UC.

References

Cho, C. H., Mei, Q. B., Shang, P. et al. (2000). Study of the gastrointestinal protective effects of polysaccharides from *Agnelica sinensis* in rats. *Planta Med*, **66**, 348–51.

Contran, R. S., Kumar, V. and Collins, T. (2005). *Robbins's pathological basis of disease*, *7th Edition*, Saunders, Philadelphia, 815.

Fiocchi, C. (1998). Inflammatory bowel disease: etiology and pathogenesis. *Gastroenterol*, **115**, 182–205.

Galperin, C., Gershwin, E: (1997). Immunopathogenesis of gastrointestinal and hepatobiliary diseases. *JAMA*, **278,** 1946–55.

Grisham, M. B., Volkmer, C., Tso, P. et al. (1991). Metabolism of trinitrobenzene sulfonic acid by rat colon produces reactive oxygen species. *Gastroenterol*, **101**, 540–7.

Grisham, M. B. (1994) Oxidants and free radicals in inflammatory bowel diseases. *Lancet*, **344**, 859–61.

Hawkins, J. V., Emmel, E. L., Feuer J. J. Nedelman, M. S. (1997). Protease activity in a hapten-induced model of ulcerative colitis in rats. *Dig. Dis. Sci*. **42**, 1969–80.

Hui, M. K. C., Wu, W. K. K., Shin, V. Y. et al. (2006). Polysaccharides from the root of *Angelica sinensis* protect bone marrow and gastrointestinal tissues against the cytotoxicity of cyclophosphamide in mice. *Int J Med Sci*, **3**, 1–6.

Hulten, L., Lindhagen, J., Lundgren, O. et al. (1977). Regional intestinal blood flow in ulcerative colitis and Chron's disease. *Gastroenterol*, **72**, 388–96.

Ischiropoulos, H., Zhu, L., Chen, J. et al. (1992). Peroxynitrite-mediated tyrosine nitration catalyzed by superoxide dismutase. *Arch Biochem Biophy*, **298**, 431–7.

Kirsner, J. B., Shorter, R. G. (1982). Recent developments in "non-specific" inflammatory bowel disease. *N Engl J Med*, **306**, 775–85.

Laroux, F. S., Grisham, M. B. (2001), Immunological basis of inflammatory bowel disease: role of the microcirculation. *Microcirculation* **8**, 283–301.

Liu, E. S. L., Ye, Y. N., Shin, V. Y. et al. (2003). Cigarette smoke exposure increases ulcerative colitis-associated colonic adenoma formation in mice. *Carcinogenesis*, **24**, 1407–13.

MacDermott, R. P. (1994). Alternations in the mucosal immune system in ulcer-active colitis and Chron disease. *Med Clin North Am*, **78**, 1207–31.

Mckenna, D. J., Jones, K., Hughes, K. (2002). *Botanical medicines: the desk reference for major herbal supplements, 2nd Edition*. Haworth Herbal Press, New York, 205–21.

Ohkawa, H., Ohishi, N., Yagi, K. (1979). Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* **95**, 351–8.

Plevy, S. E., Targan, S. R., Yang, H. et al. (1996). Tumor necrosis factor microsatellites define a Crohn's disease-associated haplotype on Chromosome 6. *Gastroenterol*, **110,** 1053–60.

Ruemmele, F. M., Dionne, S., Levy, E. et al. (1999). TNFα induced IEC-6 cell apoptosis requires activation of ICE-caspases whereas complete inhibition of the caspase-cascade led to necrotic cell death. *Biochem Biophys Res Commun*, **260**, 159–66.

Strater, J., Koretz, K., Gunthert, A. R. et al. (1995). In situ detection of enterocytic apoptosis in normal colonic mucosa and in familial adenomatous polyposis. *Gut* **37**, 819–25.

Tarnawski, A., Lu, S. Y., Stachura, J. et al. (1992). Adaptation of gastric mucosa to chronic alcohol administration is associated with increased mucosal expression of growth factors and their receptor. *Scand J Gastroenterol Suppl*, **193**, 19359–63.

Thompson, D. E. (1994). The role of mycobacteria in Crohn's disease. *J Med Microbiol*, **41**, 74–94.

Wallace, J. L., Keenan, C. M. (1990). An orally active inhibitor of leukotriene synthesis accelerates healing in a rat model of colitis. *Am J Ohysiol*, **258**, 527–34.

Yang, S. K., Loftus, E. V. Jr., Sandlborn, W. J. (2001). Epidemiology of inflammatory bowel disease in Asia. *Inflam Bowel Dis*, **7**, 206–70.

Ye, Y. N., Koo, M. W. L., Li, Y. et al. (2001a). *Angelica sinensis* modulates migration and proliferation of gastric epithelial cells. *Life Sciences*, **68**, 961–8.

Ye, Y. N., Liu, E. S., Shin, V. Y. et al. (2001b). A mechanistic study of proliferation induced by *Angelica sinensis* in a normal gastric epithelial cell line. *Biochem Pharmacol*, **61**, 1439–48.

Ye, Y. N., Liu, E. S. L., Li, Y. et al. (2001c). Protective effect of polysaccharides-enriched fraction from Angelica sinensis on hepatic injury. *Life Sciences*, **69**, 637–46.