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• *BRIEF REPORTS* •

Protective effect of Radix Acanthopanacis Senticosi capsule on colon of rat depression model

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Abstract

AIM: To investigate the abnormity of rat colon caused by depression and the ameliorative effects of Radix Acanthopanacis Senticosi (RAS) capsule on colon and their mechanisms in rat depression model.

METHODS: Chronic stress-induced model of depression of Wistar rats was produced. The experimental animals were randomly divided into model control, 5-aminosalicylic acid (5-ASA) therapy group and three RAS capsule therapy groups. These five groups were intracolonically treated daily (8:00 a.m.) for 2 wk with normal saline, 5-ASA (100 mg/kg) and RAS capsule at the doses of 300, 600 and 900 mg/kg, respectively. A normal control group of rats was also included in the study. Colonic activities of nitric oxide (NO) and superoxide dismutase (SOD), levels of malondialdehyde (MDA) and inducible nitric oxide synthase (iNOS) were determined by ultraviolet spectrophotometry. The expression of cyclooxygenase-2 (COX-2) in colonic tissue was detected by immunohistochemistry.

RESULTS: Enhanced colon inflammatory response and oxidative stress were observed in the chronic stress-induced rat depression model, which manifested as the significant increase of MDA, iNOS and NO levels, as well as the expressions of COX-2 in the colon tissue, but the colonic SOD activity was significantly decreased compared with the normal control (MDA: 10.34±2.77 *vs* 2.55±0.70; iNOS: 1.11±0.44 *vs* 0.25±0.16; COX-2: 53.26±8.16 *vs* 4.87±1.65; NO: 11.28±5.66 *vs* 4.76±1.55; SOD: 53.39±11.15 *vs* 84.45±22.31; $P<0.01$). However, these parameters were significantly ameliorated in rats treated locally with RAS capsule at the doses of 300, 600 and 900 mg/kg (iNOS: 0.65±0.31, 0.58±0.22 and 0.64±0.33; NO: 5.99±2.73, 6.87±1.96 and 6.50±1.58; MDA: 2.92±0.75, 3.19±1.08 and 3.26±1.24; SOD: 70.81±12.36, 73.30±15.30 and 69.09±11.03, respectively). The expressions of COX-2 in the colon were significantly ameliorated (28.83±9.48 and 27.04±9.56, respectively) when RAS capsule was administered at the doses of 600 and 900 mg/kg.

CONCLUSION: Administration of RAS capsule intracolonically may have significant therapeutic effects on the colon of rat depression model, which are probably due to its antioxidative action and inhibition of arachidonic acid metabolism.

Key words: RAS capsule; Arachidonic acid metabolism

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INTRODUCTION

In clinical studies, there is a strong relationship between gastrointestinal diseases, such as irritable bowel syndrome, inflammatory bowel disease, and psychological factors, especially anxiety and depression[1-3]. Several studies have shown that the prevalence of mental disorders in patients with gastrointestinal symptoms is about 60-85%[4-6].

Radix Acanthopanacis Senticosi (RAS) is a kind of traditional Chinese herbal medicine, and possesses a variety of pharmacological actions, including anti-oxidation, anti-inflammation, anti-tumor, promotion of immunoregulation, relief of anxiety and depression[7-10]. Based on manifold efficacies of RAS, and safety in use, we therefore assumed that RAS might contribute to the treatment of some gastrointestinal diseases through modulating emotion of patients. To our knowledge, there has been no report so far concerning the influence of psychological factors on colon. Therefore, we produced a rat depression model, and observed the injury of colon in rats caused by depression and the effect of RAS capsule on colon of rat depression model to test the hypothesis.

MATERIALS AND METHODS

Animals

Seventy healthy male Wistar rats, weighing 250±30 g, from the Animal Center, Academy of Hubei Preventive Medical

Sciences, were employed in the present study. The animals were fed standard rat chow, allowed access to tap water and acclimatized to the surroundings for 1 wk prior to the experiments. Then 60 rats were selected according to their open-field behaviors.

Reagents

RAS capsule was provided by Wusuli River Pharmaceutical Co. Ltd (Lot. 030201), 5-aminosalicylic acid (5-ASA) was purchased from Guoyi Pharmaceutical Ltd (Lot. 20029477). Monoclonal rabbit anti-rat- cyclooxygenase-2 (COX-2) and streptavidin-peroxidase (S-P) kits were obtained from Beijing Zhongshan Biological Technology Co. Ltd. Superoxide dismutase (SOD), malondialdehyde (MDA), inducible nitric oxide synthase (iNOS) and nitric oxide (NO) detection kits were purchased from Nanjing Jiancheng Bioengineering Institute. Other reagents used in the study were all of analytical grade.

Experimental protocols

A rat model of depression was established as previously described[11,12]. The rats received a variety of stressors for 21 d, including cold water swimming at 4 ℃ for 5 min, water deprivation for 24 h, food deprivation for 24 h, heat stress at 45 ℃ for 5 min, cage tilt at 45°, 12-h inverted light/dark cycle (7:00 a.m. lights off, 7:00 p.m. lights on), tail nip for 1 min, paw electric shock (electric current 1.0 mA×10 s, on every 1 min, lasted for 10 s, 30 times), *etc*. The animals were randomly divided into five groups (10 rats per group): model control, 5-ASA therapy group and three RAS capsule therapy groups. The animals were treated intracolonically with normal saline, 5-ASA (100 mg/kg) and RAS capsule at the doses of 300, 600 and 900 mg/kg, respectively, for 14 d after the depression model was produced. A normal control group of rats (10 rats) without receiving any stress was included and housed in a separate room; food and water were freely available in their home cages. The colonic tissue (8 cm proximal to the anus) was sampled for a variety of determinations after the animals were anesthetized with 200 d/L urethane.

Determination of colonic SOD, MDA, iNOS and NO

For the determination of colonic SOD, MDA, iNOS and NO, the colon tissue was rinsed and weighed, then put into tubes with 9 volumes of 9 g/L normal saline. The tubes were homogenized for 10 min. After centrifugation at 3 000 r/min for 10 min at 4 ℃, the supernatants were collected and stored at -20 ℃ until analysis with corresponding assay kits according to the manufacturer's instructions.

Detection of colonic COX-2 expression

The immunohistochemical methods for formalin-fixed and paraffin-embedded sections were described previously[13]. Colonic COX-2 expression was determined with S-P technique following the recommendations of assay kit manufacturer. Briefly, monoclonal rabbit anti-rat-COX-2 was diluted with PBS to 1:50, and used as the primary antibody in the corresponding detections. For the determination, dewaxed sections were incubated first with the primary antibody overnight at 4 ℃ after antigen retrieval. The binding of antibodies to their antigenic site in the tissue sections was further amplified with biotinylated goat anti-rabbit antibodies, followed by reaction with 3,3'-diaminobenzidine. Negative controls were established by substituting PBS for the primary antibody. COX-2 negatively expressed cells manifested as blue-stained nuclei while the positive cells as brown or dark brown cytoplasm and/or cell membrane. Expressions of COX-2 protein were semiquantitated respectively with automatic image analyzer (Nikon, Japan), in which the average value of absorbance (*A*) of positive staining in 10 randomly selected high-power fields (400×) for each section was used for comparison of the COX-2 protein expressions.

Statistical analysis

Experimental results were expressed as mean±SD. Statistical comparisons between groups were made by analysis of variance (ANOVA), followed by Student's *t* test. *P* value less than 0.05 was considered statistically significant.

RESULTS

Open-field behavior of depression model rats

The open-field test was designed to measure the reaction of rodents to a novel environment. In this test, animals were placed individually in the center of a square, wooden, white-colored open-field box with 36 squares measuring 10×10 cm^2 each. The activity was assessed for 5 min. The number of squares from which rats crawled out was the total number of crossing. The number of occasions on which the animals stood on their hind legs was the total number of rearings. Each rat was housed one per cage and fasted before sucrose intake test, then 10 g/L sucrose solution consumption in 24 h was examined. Crossing reflected the activity degree of animals, rearing reflected the curious degree to the novel surroundings, and sucrose intake test reflected animal's response to rewards[14,15]. The open-field behaviors, both crossing and rearing behaviors in 5 min of depression model rats, were significantly decreased compared with that of the normal control rats (Table 1, $P<0.01$). Also, the consuming of 10 g/L sucrose solution was significantly decreased compared with that of the normal control (Table 1, $P<0.01$).

Table 1 Open-field activities and sucrose intake test of rats (mean±SD)

Group	Cases *n*	Rearing /5 min	Crossing /5 min	Sucrose intake mL/24 h
Normal	10	32.20±11.14	82.50±29.62	42.70±10.24
Model	50	13.96±5.57[b]	37.80±12.32[b]	28.02±8.91[b]

[b]$P<0.01$ *vs* normal group.

Colonic oxidative and iNOS alterations

Prominent oxidative stress in colonic mucosa was induced in the depression model rats as shown by the significant elevation of colonic MDA and NO contents and decrease of colonic SOD activity compared with that of the normal

control rats (Tables 2, 3, $P<0.01$). iNOS increased significantly compared with that of the normal control rats (Table 3, $P<0.01$). These oxidative abnormalities and iNOS in colonic mucosa were obviously ameliorated by the treatment with RAS capsule at the doses of 300, 600 and 900 mg/kg, which manifested as the significant reduction of colonic MDA content and the increase of SOD activity compared with that of the model control rats (Table 2, $P<0.01$). Furthermore, a significant improvement of the elevated colonic iNOS and NO content was also observed in the animals treated with RAS capsule at the doses of 300, 600 and 900 mg/kg (Table 3, $P<0.05$-0.01).

Table 2 Effects of RAS capsule on SOD activity, MDA content and COX-2 expression in the colon tissue of depression model rats (n =10, mean±SD)

Group	Dose (mg/kg)	SOD (U/mg prot)	MDA (nmoL/mg prot)	COX-2 (A/%)
Normal	-	84.45±22.31	2.55±0.70	4.87±1.65
Model	-	53.39±11.15[d]	10.34±2.77[d]	53.26±8.16[d]
5-ASA	100	72.73±12.60[bc]	3.19±1.15[b]	24.59±9.14[b,d]
RAS	300	70.81±12.36[bc]	2.92±0.75[b]	50.40±10.66[d]
RAS	600	73.30±15.30[bc]	3.19±1.08[b]	28.83±9.48[b,d]
RAS	900	69.09±11.03[b,c]	3.26±1.24[b]	27.04±9.56[b,d]

[a]$P<0.05$, [b]$P<0.01$ *vs* model group; [c]$P<0.05$, [d]$P<0.01$ *vs* normal group.

Reduction of the expression of colonic COX-2 RAS capsule

The expression of colonic COX-2 was significantly increased in depression model animals compared with that of the normal controls ($P<0.01$), which was significantly ameliorated after the treatment with RAS capsule at the doses of 600 and 900 mg/kg (Table 2 and Figure 1, $P<0.01$).

Table 3 Effects of RAS capsule on the content of iNOS and NO in colon tissue of depression model rats (n =10, mean±SD)

Group	Dose (mg/kg)	iNOS (U/mg prot)	NO (μmoL/g prot)
Normal	-	0.25±0.16	4.76±1.55
Model	-	1.11±0.44[d]	11.28±5.66[d]
5-ASA	100	0.63±0.42[b,c]	5.89±2.10[d]
RAS	300	0.65±0.31[a,c]	5.99±2.73[b]
RAS	600	0.58±0.22[b,c]	6.87±1.96[a]
RAS	900	0.64±0.33[b,c]	6.50±1.58[a]

[a]$P<0.05$, [b]$P<0.01$ *vs* model group; [c]$P<0.05$, [d]$P<0.01$ *vs* normal group.

DISCUSSION

The depression model is a classic and mature model, in which rats receive a variety of mild stressors. The measurement most commonly used to track the effects is a decrease in consumption of a palatable sweet solution[12]. The model has a good predictive validity (behavioral changes are reversed by chronic treatment with a wide variety of antidepressants), face validity (almost all demonstrable symptoms of depression can be demonstrated), and construct validity (the chronic stressors cause a generalized decrease in responsiveness to rewards, comparable to anhedonia, the core symptom of the melancholic subtype of major depressive disorder)[16]. Overall, the model appears to be at least as valid as any other animal model of depression.

To our knowledge, the mechanisms of colon abnormity caused by depression in rats have not been clearly elucidated as yet. From the present study, we suspect the change of oxygen-free radical (OFR), NO and COX-2 in the colon of depression model rats might play important roles.

The antioxidant enzymatic system (SOD and MDA) is one of the most important free radical detoxification

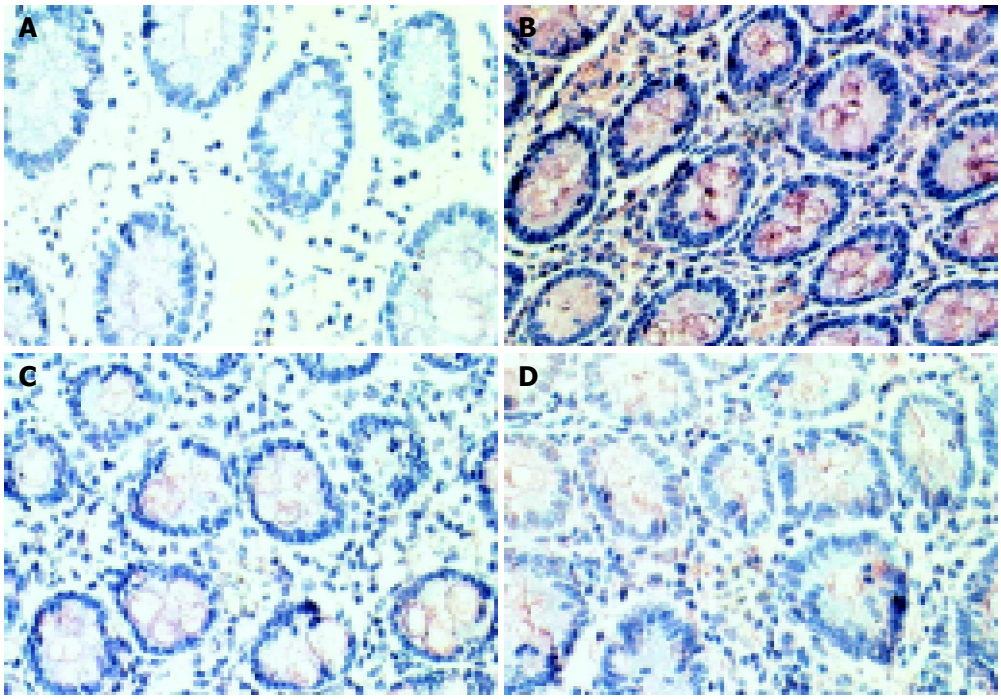

Figure 1 Expression of COX-2 in colonic tissue of rats (SP ×400). A: normal control; B: model control; C: 900 mg/kg RAS capsule therapy group; D: 600 mg/kg RAS capsule therapy group.

mechanisms. The enzymes act in equilibrium, and any imbalance of this system may provoke OFR generation[17]. To evaluate the free radical metabolism in depressed rat colon, we measured SOD (a part of the antioxidant system to protect membranes and essential proteins from the potentially damaging effects of reactive oxygen and lipid peroxides) and MDA (production of lipid peroxidation) as lipid peroxidation markers. The most remarkable findings of our study are the decrease of SOD activity and the increase of MDA concentration in depression model rats, indicating that the production of OFR is increased. OFR is an extremely reactive molecule that can disrupt lipid cell membranes, destroy cell enzyme functions, alter DNA, and lead to cell death[18,19]. The abnormity of antioxidant enzyme activities plays a role in injuries of rat colon caused by depression. The great improvement of SOD activity and significant decrease of MDA in depressed rat colon by clustering with RAS capsule indicate that RAS capsule could protect the colon from lipid peroxidation.

Pavlick *et al*[20] have revealed that the increase of oxidative stress and iNOS activity results in a pathological cascade of free radical reactions and further yields more oxidative free radicals, such as peroxynitrite ($ONOO^-$), to impair the structure and function of cells. Although resting cells do not express iNOS, their capacity of expressing this enzyme is present in several tissues. Indeed, cells such as macrophages, hepatocytes, vascular smooth muscle cells, endothelial cells have the ability to express iNOS under an appropriate stimulus[21]. During colonic injury, iNOS is induced[22], while the production of NO increases. Meanwhile, excessive NO dilates vasculature and enhances vasopermeability, as well as inactivates the activity of antioxidases such as SOD, by means of reacting with hydrosulfide group (-SH) in the enzymes[22,23]. Some oxidants modulate the expression of a variety of genes that are involved in the immune and inflammatory responses, which lead to apoptosis of intestinal epithelial cells, cascades of inflammatory response and the disruption of integrity and function of the intestinal mucosa[23-26]. RAS capsule at the doses of 300, 600 and 900 mg/kg could decrease the production of iNOS, thus decreasing NO content and protecting colon tissue from injury.

Cyclooxygenase is the key enzyme in arachidonate metabolism and catalyzes biosynthesis of prostaglandin H_2, which is the precursor for prostanoids. Two isoforms of COX have been identified: COX-1 is expressed constitutively in a number of cell types, which takes part in sustaining physiologic function of body. COX-2 is an inducing immediate-early gene, which is absent in normal tissue cells[27]. COX-2 is induced by a variety of cytokines, hormones, and tumor promoters, leading to the production of more prostaglandins. COX-2 could be activated to produce excessive prostaglandin E_2 and thromboxane B_2, two important inflammatory mediators, which contribute to the bowel hyperemia, edema and even dysfunction[28]. The significant increase of the expression of COX-2 in our study suggested that depression could induce inflammatory injury of colon in rats, while RAS capsule at the therapeutic doses of 600 and 900 mg/kg was as effective as 100 mg/kg 5-ASA on decreasing the expression of COX-2 in the colon of this rat model.

To elucidate the mechanisms underlying the therapeutic effects of RAS capsule, we observed simultaneously the changes of oxidative and inflammatory variables mentioned above in the colonic tissue after RAS capsule therapy. The results revealed that the SOD activity, MDA, iNOS and NO levels, as well as the expressions of COX-2 in the colonic mucosa were significantly ameliorated in the rats treated locally with RAS capsule at the given doses, compared with that in model control animals. Taken together, these findings suggest that the anti-oxidative stress and the anti-inflammatory response might be the fundamentals of RAS capsule action in ameliorating colon injury caused by depression.

In summary, brain-gut interaction and psychological factors alter not only the pathology of brain tissue, but also colon tissue. The results of our study show that depression can induce injury of colon through oxidative stress and inflammatory response. Intracolonic treatment with RAS capsule can ameliorate the pathological changes of colon in depressed rats, suggesting that RAS capsule can be used as an effective therapeutic agent for some colon diseases caused by psychological factors.

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