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Experimental Research

Effect of moxibustion on the expression levels of proteins of neuron and neuropeptide in the intestinal tract of rats with Crohn's disease*

艾灸对克罗恩病大鼠肠道神经元及神经肽蛋白表达的影响*

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ABSTRACT

Objective To explore the regulating effect of moxibustion on the enteric nervous system of rats with Crohn's disease. **Methods** Ten SD rats were selected randomly from 40 rats as normal control (group A), and the other 30 rats were established into Crohn's disease rat models by adopting clysis method with TNBS. On the basis of modeling successfully, the model rats were randomly divided into model group (group B), herbs-partitioned moxibustion group (group C) and mild moxibustion group (group D) with 8 rats in each group (4 rats were dead during modeling. After modeling, 2 rats were selected from group A, and 2 rats were selected from models for determination, at last, 8 rats were included in each group). In group C and group D, herbs-partitioned moxibustion or mild moxibustion was applied on "Tiānshū (天枢 ST 25)" bilaterally, and the rats in group A and group B were fixed as in treatment groups. HE stain was conducted, and morphological observation was performed on the colonic tissue of rats; the expression levels of proteins of S-100, SP, NPY and their receptors were observed by adopting immunohistochemical method. **Results** Compared with group A, the expression levels of proteins of S-100, SP and its receptor NK1R, NPY and its receptors NPY1R and NPY2R in the intestinal tract of rats in model groups increased obviously, and the differences were statistically significant. ($P_{S\text{-}100}$<0.01, P_{SP}<0.05, P_{NK1R}<0.01, P_{NPY}<0.05, P_{NPY1R}<0.05, P_{NPY2R}<0.01), after treatment with drug-paste interposed moxibustion and mild moxibustion, the levels reduced significantly ($P_{S\text{-}100}$<0.05, P_{SP}<0.05, P_{NK1R}<0.01, P_{NPY}<0.05, P_{NPY1R}<0.05, P_{NPY2R}<0.01). **Conclusion** Moxibustion treatment may regulate the expression levels of proteins of S-100, SP, NK1R, NPY, NPY1R and NPY2R through warm stimulation, alleviate inflammatory response of colonic tissue, and repair impaired colonic mucosa, thus achieving the goal of treating Crohn's disease.

KEY WORDS: moxibustion; Crohn's disease; S-100; substance P; NK1R; neuropeptide Y; NPY1R; NPY2R

Crohn's disease (CD) is a kind of chronic nonspecific inflammatory bowel disease in the whole gastrointestinal tract with unclear pathogenesis. The disease distributes segmentally and rapidly with the main clinical manifestations of abdominal pain, diarrhea, abdominal mass, nausea, and other digestive tract symptoms, as well as fever, emaciation, anemia, and other constitutional symptoms, and possible manifestations of arthritis, acropachy, and other symptoms outside of the digestive tract. The disease progersses slowly and is easy to attack people repeatedly. CD patients' quality of life is obviously

lower than that of health people[1]. It was reported that the incidence of inflammatory bowel disease (IBD) was highest in Northern America, Europe, and other western countries[2]. Over the past decade, the incidence of CD in China has increased year by year, it was shown from a retrospective study in the region of Ningxia in China that the incidence of CD increased by 5.57 times from 2002 to 2014[3]. At present, there is no effective cure method, and the main measure is to control the disease, prevent and treat complications, and improve the patients' quality of life[4]. The pathogenesis of CD is still unclear, but more and more people have paid attention to the important role of enteric nervous system in the pathogenesis of IBD. It has been indicated from many modern clinical studies that acupuncture-moxibustion has a good curative effect in improving the clinical symptoms of CD patients, controlling the disease activity, and maintaining remission[5-6]. Therefore, in this paper, the effect of enteric nervous system in the incidence of CD and the mechanism of moxibustion in treatment of CD were discussed.

MATERIALS AND METHODS

Laboratory animals

Forty clean male Sprauge-Dawley (SD) rats with the weight of (120±20) g were selected, which were provided by Experimental Animal Center of Shanghai Institute of Traditional Chinese Medicine. Feeding condition: room temperature of (22±2) ℃, and humidity of (60±5) %. After adaptive feeding for five days, the study was conducted after the diet and behaviors of rats were normal. The use of all animals was strictly in accordance with the national provisions of the laboratory animal health. The experimental process was in line with the ethical standard of animals.

Modeling method

After weighing, 10 SD rats were selected randomly from 40 rats as normal control (group A), and the other 30 rats were established into models of Crohn's disease by adopting 2, 4, 6-trinitrobenzene sulfonic acid (TNBS, with the article No. of P2297, purchased from U.S. Sigma Company)[7]. All the rats were fasting for 24 h with drinking water freely before clysis. After weighing, 2% pentobarbital sodium (with the article No. of P3761, purchased from U.S. Sigma Company) was injected intraperitoneally, with the dosage of 20 mg/kg, for anesthesia. Preparation of TNBS enema solution: double distilled water was added into absolute ethyl alcohol (with the article No. of B00001302, purchased from Best Reagent Company) for preparing 50% ethyl alcohol, then 5% TNBS was mixed with 50% ethyl alcohol in the proportion of 2∶1 into TNBS enema solution. Clysis: 0.5 mL of TNBS enema solution was used for clysis in the rats in model group; infusion catheter, with the length of 12 cm, was inserted into the rectum for 6–8 cm, and TNBS enema solution was injected with 1 mL injection syringe. The rats' heads were downward, and they were inverted for 1 min after the catheters were pulled out in order to avoid the overflow of liquid. The mental state, eating, activity, and the stool property, etc. were daily observed. Clysis was conducted once every 10 days, and a total of 4 times were needed. In order to determine whether the models were established successfully, after modeling, 2 rats were randomly selected from group A, and 2 rats were selected from models for morphological observation of colonic tissue. Four rats were dead in the whole process of modeling with unknown cause, intestinal perforation was not found after autopsy.

Grouping and treatment

On the basis of modeling successfully, the model rats were randomly divided into model group (group B), herbs-partitioned moxibustion group (group C) and mild moxibustion group (group D) with 8 rats in each group.

Group A: without treatment, the rats were fixed as those in the treatment groups.

Group B: without treatment, the rats were fixed as those in the treatment groups.

Group C: "Tiānshū (天枢 ST 25)" points were used bilaterally. Formula of drug-paste: Fùzǐ (附子 *Radix Aconiti Lateralis Praeparata*), Ròuguì (肉桂 *Cortex Cinnamomi*), and Dānshēn (丹参 *Radix et Rhizoma Salviae Miltiorrhizae*), etc. (provided by the Department of Pharmacy of Yueyang Hospital of Integrated Traditional Chinese and Western Medicine, Shanghai University of Traditional Chinese Medicine) were ground into fine powder, and then the powder was concocted into drug-paste (with the diameter of 0.5 cm, and thickness of 0.3 cm) with yellow wine. The specification of moxa cone was about 90 mg, and two moxa cones were performed at each acupoint for each time; the treatment was conducted once a day, and a total of 7 times were needed (determined by the past experiment experience). of Locating of "ST 25": 5 mm lateral to the left and right sides of the upper 2/3 and lower 1/3 intersection ［i.e. "Shénquè (神阙 CV 8)"］ on the connecting line of the xiphoid process and superior margin of pubic symphysis on the abdominal median line of rats.