

EXPERIMENTAL STUDY

Effect of combined medicated thread moxibustion plus needle picking therapy of Zhuang nationality medicine on antioxidant levels in a rat model of sciatica

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Abstract

OBJECTIVE: To investigate the effect and underlying mechanisms of combined medicated thread moxibustion therapy plus needle picking therapy of Zhuang nationality medicine on antioxidant levels in a rat model of sciatica.

METHODS: One hundred Wistar rats, of specific pathogen free level, were randomly divided into five groups: normal control group, model group, medicated thread moxibustion group, needle picking group, and combination group. Each group contained 20 rats. In the model, medicated thread moxibustion, needle picking, and combination groups, sciatica models were established through chronic

constriction injury of the sciatic nerve. After the model was established, the rats in the medicated thread moxibustion, needle picking, and combination groups were given the corresponding therapies for 21 days. The control and model groups received no treatment. Reactive oxygen species, superoxide dismutase, malondialdehyde, and total antioxidant capacity changes were determined. Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase subunit NADPH oxidases 4 (NOX4) mRNA expression and the morphology of cells were observed to detect apoptosis of gamma-aminobutyric acid ergic (GABAergic) neurons.

RESULTS: Compared with control group, reactive oxygen species and malondialdehyde levels rose significantly in the model group ($P < 0.01$), while superoxide dismutase and total antioxidant capacity levels were lowered ($P < 0.05$). Compared with the model group, reactive oxygen species and malondialdehyde decreased in the needle picking group ($P < 0.05$), while superoxide dismutase levels were increased ($P < 0.05$); reactive oxygen species and malondialdehyde significantly decreased in the combination group ($P < 0.01$). In addition, the model group had higher NOX4 mRNA expression than that of the control group ($P < 0.05$), and the combination group had lower expression levels than that of the model group ($P < 0.05$). Apoptosis of GABAergic neurons was observed in the model group, and was attenuated after combined therapy.

CONCLUSION: The medicated thread moxibustion therapy plus needle picking therapy of Zhuang nationality medicine can prevent oxidative damage in the rat model of sciatica via down-regulating NOX4 expression, improving antioxidant capacity, and in-

hibiting the oxidative damage pathway of GABAergic neurons.

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Key words: Chuang medicine; Zhuang thread moxibustion; Needles; Sciatica

INTRODUCTION

Sciatica refers to a chronic, recurrent pain in the dominant region along the sciatic nerve pathway. This pain can last for a few months to several years. It is worth exploring an effective and inexpensive therapy for sciatica. Minority medicines are of great importance to China's healthcare, and play a crucial role in the minority region. Zhuang nationality medicine, one of the minority medicines, has a long history in the Zhuang Autonomous Region in China. Among them, medicated thread moxibustion therapy and needle picking therapy are two dominant techniques for the treatment of chronic diseases or incurable diseases, such as chronic pain, senile disease, insomnia, asthma, and rheumatism, which have been confirmed in clinical trials and practice.^{1,2} Medicated thread moxibustion therapy and needle picking therapy are regarded as a simple, effective and cheap therapeutic strategy for sciatica. Previous studies emphasize the success of clinical trials,³ but little evidence is available on the treatment mechanism. A recent study showed that oxidative damage of gamma-aminobutyric acid (GABA) neurons is an important pathological mechanism of inducing sciatica.⁴ This study aimed to observe the effect on antioxidant levels in a rat model of sciatica of the medicated thread moxibustion therapy and plus needle picking therapy of Zhuang, and explore possible mechanisms of the action.

MATERIALS AND METHODS

Experimental animals and grouping

A total of one hundred male and female Wistar rats, of specific pathogen free grade, weighing (190 ± 11) g, were provided by Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China; license No. SCXK (Beijing) 2013-0006). Animals were housed in separate cages, and maintained at (23 ± 1) °C and 60%-70% humidity. Rats were fed with conventional forage and had free access to water for 3 days to adapt the environment, after which they were randomly divided into five groups according to random number table: control, model, medicated thread moxibustion, needle picking, and combination groups. Each group contained 20 rats. Experimental animals were treated in strict accordance with the Guidelines of the Use and Care of Laboratory Animals in 2006.

Reagents and instruments

Malondialdehyde (MDA) detection kit (lot No.

001302015), superoxide dismutase (SOD) detection kit (lot No. 00120354), reactive oxygen species (ROS) detection kit (lot No. 031306142), and total anti-oxidative capacity (TAOC) detection kit (lot No. 1305468) were provided by Shanghai Biochemistry Company (Shanghai, China). Chloral hydrate (batch No. 20130013; Beijing Chemical Reagent Company, Beijing, China); Hoechst dye and apoptosis detection kit (lot No. 13025645; Beyotime Institute of Biotechnology); Trizol (Gibco-BRL); M-MLV (Piomegan); Tag enzyme and DNAmak (CapitalBio Corporation, Beijing, China); Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase subunit NADPH oxidase-4 (NOX4) gene primers (Invitrogen); desktop high-speed refrigerated centrifuge (type Z360K; HERMLE, Leipzig, Germany); electronic balance (type AE160; Mettler, Switzerland); fluorescence spectrophotometer (type F-3000; Hitachi, Japan); electric glass homogenizer (type DY-89; Ningbo Scientz Biotechnology Co., Ltd., Ningbo, China); microplate reader (type MK3; Thermo Labsyste, Chengdu, China); dual-use computer rapid freezing paraffin microtome (KD-2258-VI; Zhejiang Cody's Import @ Export Co., Ltd., Hangzhou, China); fluorescence microscope (Olympus IX51, Tokyo, Japan); gel imaging system (chemidoc XRS, Bio-Rad, Los Angeles, CA, USA); PCR instrument (type 7900HT; ABI Company, Shanghai, China).

Model establishment and interventions

Chronic constriction injury of the sciatic nerve was performed according to a previous method with slight modifications.⁵ Eighty models were successfully divided into four groups, namely the model, medicated thread moxibustion, needle picking, and combination groups. The remaining rats served as the control group. In brief, after rats were anesthetized with 3 mL/kg of 10% (v/v) chloral hydrate, a 2-cm longitudinal incision was made on the left thigh, and then the semitendinosus and biceps femoris were bluntly dissected with hemostatic forceps, freeing and exposing the sciatic nerve between the muscular crack. Subsequently the nerve at the ligation site was wrapped with porous film and sutured with four surgical lines at an interval of 1 mm. The tightness of the ligation was adjusted to avoid affecting epineurial blood supply. The incision was sutured and sterilized. After the model was successfully established, each group received the following interventions.

Medicated thread moxibustion group: according to "Experimental Acupuncture", edited by Li *et al.*,⁶ the rats received the moxibustion at bilateral Zusanli (ST 36). The medicated thread, 0.7 mm in diameter, was kindled on the alcohol lamp or candle, and then the flame was extinguished, leaving a bead-like sparkle on the top of the medicated thread. The physician held the thread using their thumb and index finger, and directly pressed the sparkle at Zusanli (ST 36) in a rapid

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