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Experimental Research

Mild moxibustion at different intervention times on the levels of ET-1 and NO in the uterine tissues of rats with cold-damp coagulation and stagnation type dysmenorrhea☆

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ABSTRACT

Objective: To observe the differences in analgesic effect of moxibustion at different intervention times on dysmenorrhea rats and explore its effect mechanism.
Methods: Forty-five female Wistar rats were randomly divided into blank control group (group A), model group (group B), pre-moxibustion group (group C), instant moxibustion group (group D) and pre-instant moxibustion group (group E), with 9 rats in each group. Cold-damp coagulation and stagnation type dysmenorrhea models were established. In group C, mild moxibustion on "Shénquè (神阙 CV 8) " and "Guānyuán (关元 CV 4)" was carried out from the time after modeling on the 8th day for 3 consecutive days. In group D, mild moxibustion was given as the same methods with group C after injection with oxytocin on the 11th day. In group E, mild moxibustion was given as the same methods from the time after modeling on the 8th day to that after injection with oxytocin on the 11th day for 4 consecutive days. The writhing behavior and the changes in levels of ET-1 and NO in uterine tissues of rats with dysmenorrhea in each group were observed.
Results: Comparison of the latent period: compared with (4.38 ± 1.06) min in group B, the latent period of rats in group C (9.67 ± 1.32) min, group D (11.78 ± 1.30) min and group E (15.00 ± 1.22) min obviously prolonged (all $p < 0.01$). Compared with group C, the latent period of group E obviously prolonged ($p < 0.01$). Compared with group D, the latent period of group E obviously prolonged ($p < 0.01$).Comparison of the writhing times: compared with (4.38 ± 1.06) in group B, the writhing times of rats in group C (9.67 ± 1.32), group D (11.78 ± 1.30) and group E (15.00 ± 1.22) reduced (all $p < 0.01$). Compared with group C, the writhing times of rats in group D and group E reduced (both $p < 0.01$). Compared with group D, the writhing times in group E reduced ($p < 0.05$). Comparison of the total writhing score: compared with (4.38 ± 1.06) in group B, the total writhing score of rats in group C (9.67 ± 1.32), group D (11.78 ± 1.30) and group E (15.00 ± 1.22) decreased (all $p < 0.01$). Compared with group C, the total writhing score of rats in group D and group E decreased (both $p < 0.01$). Compared with group D, the total writhing score of rats in group E decreased ($p < 0.05$). Comparison of ET-1 level: compared with (4.80 ± 0.47) in group A, the ET-1 level in uterine tissues of rats in group B (7.57 ± 0.69) significantly increased ($P < 0.01$). Compared with group B, the ET-1 level in uterine tissues of rats in group C (6.20 ± 0.50), group D (5.67 ± 0.29) and group E (5.16 ± 0.33) obviously decreased (all $p < 0.01$). Compared with group C, the ET-1 level in uterine tissues of rats in group D and group E obviously decreased ($p < 0.05$, $p < 0.01$). Compared with group D, the ET-1 level in uterine tissues of rats in group Eob-

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viously decreased ($p < 0.05$). Comparison of NO level: compared with (6.63 ± 1.83) in group A, the NO level in uterine tissues of rats in group B (1.62 ± 0.58) significantly decreased ($p < 0.01$). Compared with group B, the NO level in uterine tissues of rats in group C (3.60 ± 0.59), group D (4.77 ± 0.67) and group E (5.99 ± 0.63) obviously increased (all $p < 0.01$). Compared with group C, the NO level in uterine tissues of rats in group Dand group E obviously increased ($p < 0.05$, $p < 0.01$). Compared with group D, the NO level in uterine tissues of rats in group E obviously increased ($p < 0.01$).

Conclusion: The analgesic effect of mild moxibustion at different intervention times on cold-damp coagulation and stagnation type dysmenorrhea rats was different, which was the most significant in pre-instant moxibustion group. One of the mechanisms of action may be related with the adjustment of abnormal levels of ET-1 and NO.

Primary dysmenorrhea is one of the common diseases and frequently-occurring diseases among adolescent women. It is believed in modern medicine that its occurrence is closely related to such factors as neuroendocrine, heredity, immunity, metabolism and environment [1]. Moxibustion, as a traditional therapy, is effective and easy to operate in the treatment of primary dysmenorrhea [2–4]. Most patients with dysmenorrhea can be classified as cold-damp coagulation and stagnation type according to previous clinical treatment. Moxibustion therapy on Shénquè (神阙CV 8) and Guānyuán (关元CV 4) has been used to treat several cases of primary dysmenorrhea with satisfactory curative effect [5–7]. It has been found during clinical treatment that the intervention time of moxibustion has a certain influence on the clinical efficacy. But there are few reports about the difference in analgesic effect between moxibustion at different intervention times on primary dysmenorrhea. On the basis of the establishment of rat models of cold-damp coagulation and stagnation type primary dysmenorrhea in this study, the changes of endothelin (ET-1) and nitric oxide (NO) levels in uterine tissues were observed through mild moxibustion intervention on CV 8 and CV 4 at different times, in order to explore the difference in analgesic effect between moxibustion at different intervention times on primary dysmenorrhea and its possible mechanism.

Materials and methods

Laboratory animals

Sixty clean, healthy, mature and unmated Wistar female rats, with the age of 8–10 weeks and body mass of (200 ± 20) g, were provided by the animal experiment center of Hebei Medical University (certification No. 1509045). The rats were fed in the clean animal laboratory with the room temperature of (23 ± 2) °C, humidity of (45 ± 5)%, and light and dark duration of 12 h, respectively, and they were permitted to drink and eat freely at liberty. The disposal of laboratory animals was reviewed and approved by the Ethics Committee of Hebei University of Chinese Medicine.

Major reagents and instruments

Iodine [^{125}I] endothelin radioimmunoassay kit, Beijing North Institute of Biological Technology, batch No. 20160124. NO kit, Nanjing Jiancheng Bioengineering Institute, batch No. 20160123. Estradiol benzoate injection, Chifeng Boen Pharmaceutical Co. Ltd., batch No. 150401. Oxytocin injection, Chifeng Boen Pharmaceutical Co. Ltd., batch No: 140901. Moxa stick of 7 mm, Nanyang Hanyi Moxa Co. Ltd., batch No. 150324. TD10001 type electronic balance, Tianjin Balance Instrument Co. Ltd. DP73 type digital microscope, Olympus Optical Co. Ltd. HMIAS-2000 type microscopic image analysis system, Wuhan Tongji Medical University. TDL-5-A type centrifuge, Shanghai Anting Scientific Instrument Factory. SHB-D type water circulating vacuum pump, Zhengzhou Greatwall Scientific Industrial and Trade Co. Ltd. FJ-2021 type Gamma radioimmunoassay counter, Xi'an 262 Factory.

Modeling and grouping

After adaptive feed for 7d, the 60 Wistar female rats were screened for 4d by adopting vaginal smear test [8,9]. The rats without anestrus or in the same period all the time were eliminated, and 45 rats were selected finally. According to the random number table method, the 45 rats were randomly divided into blank control group (group A), model group (group B), pre-moxibustion group (group C), instant moxibustion group (group D) and pre-instant moxibustion group (group E), with 9 rats in each group. Normal saline was injected subcutaneously in the thigh of rats in group A for once a day for 10 consecutive days (0.5 mL/rat on the 1st day and the 10th day, and 0.2 mL/rat on the 2nd day to the 9th day). Cold-damp coagulation and stagnation type dysmenorrhea models were established by adopting (0 ± 1) °C ice water bath method combined with estradiol benzoate injection method [10,11] in other groups. The posterior limbs and lower abdomen of rats were immersed into (0 ± 1) °C ice-water mixture (with room temperature of 23 ± 2 °C) for cold stimulus for once a day, and 20 min per time. In addition, subcutaneous injection with estradiol benzoate in the thigh of rats was carried out for once a day for 10 consecutive days (0.5 mg/rat on the 1st day and the 10th day, and 0.2 mg/rat on the 2nd day to the 9th day). Such manifestations as shivering, hollow back and pilo-erection, sneezing, cowering and hypokinesia, low spirits, loose stools, less eating and drinking, and pale mouth, lip, ear, nose, claw, nail and tail conformed to the symptoms of cold-damp coagulation and stagnation. Intraperitoneal injection with oxytocin in the dosage of 2 U/rat was carried out in the 11th day. When such symptoms as contraction and indent in the abdomen, tetanic extension of the body and posterior limbs, and internal rotation of the hip and limbs in one side occurred, it indicated that the models were established successfully [12].

Treatment methods

In group C, "CV 8" (on the medioventral line, the intersection of upper 2/3 and lower 1/3 in the connective suture of xiphoid process and the superior margin of symphysis pubis [13]) and "CV 4″ (on the medioventral line, the midpoint of the connective suture of the roots of both posterior limbs of rats [14]) were selected and located through staining. The rats were placed into the fixed bags 75 min after modeling on the 8th day, and 7 mm moxa sticks were held to perform mild moxibustion on both the two acupoints for 10 min/time. The manipulations were conducted for once a day, lasting for 3 days.

In group E, intervention as above-mentioned was carried out after injection with oxytocin from the 8th day to the 11th day

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