JTCM 中医杂志 Journal of Traditional Chinese Medicine

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J Tradit Chin Med 2016 December 15; 36(6): 779-783
ISSN 0255-2922

EXPERIMENTAL STUDY

Effects of electronically stimulating Tianshu (ST 25) and Dachangshu (BL 25), Quchi (LI 11) and Shangjuxu (ST 37) on the expressions of jejunum c-kit protein and c-kit mRNA in rats with functional diarrhea

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Supported by National Key Basic Research Program of China (973 Program): Bidirectional Regulation Effect and Mechanism of Acupuncture on Different Acupoints on Functional Bowel Disorders (No. 2011CB505204)

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Accepted: March 3, 2016

Abstract

OBJECTIVE: To investigate the effects of electronically stimulating Tianshu (ST 25) and Dachangshu (BL 25), Quchi (LI 11) and Shangjuxu (ST 37) on the jejunum c-kit protein and c-kit mRNA in rats with functional diarrhea (FD).

METHODS: FD models were established through intragastric administration with folium sennae. Experimental rats were then divided into 4 groups: blank group, model group, electroacupuncture group Ⅰ [Tianshu (ST 25) and Dachangshu (BL 25) of both sides] and electroacupuncture group Ⅱ [Quchi (Li 11) and Shangjuxu (ST 37) of both sides], 10 in each. After treatment with electroacupuncture for 10 days, The expressions of jejunum c-kit protein and c-kit mRNA in each group were detected with Western blot and Real-Time quantitative real-time polymerase chain reaction (PCR).

RESULTS: The expressions of c-kit protein and c-kit mRNA in the model group increased significantly compared to those in the blank group ($P < 0.01$); the expressions in electroacupuncture group Ⅰ significantly decreased compared to those in the model group ($P < 0.01$).

CONCLUSION: Our findings suggest that electronically stimulating both Tianshu (ST 25) and Dachangshu (BL 25) significantly increased the expressions of jejunum c-kit protein and c-kit mRNA in FD rats, which means the treatment might have better therapeutic effects on FD.

key words: Diarrhea; Interstitial cells of Cajal; Proto-oncogene proteins c-kit; Electroacupuncture

INTRODUCTION

FD is a common clinical disease mainly manifested by intestinal motility disorders and characterized by small intestine movement acceleration.[1] Its cause has not been elucidated and it is possibly related to genetic, environmental, psychological and mental factors.[2] It has been proved in clinical practice that certain acupoints were frequently chosen when treating with acupunc-

ture, such as the commonly-used shu-acupoint [Dachangshu (BL 25)], mu-acupoint [(Tianshu (ST 25)], he-acupoint [Quchi (LI 11)] and lower-he acupoint [(Shangjuxu (ST 37)], which could often get instant effects.[3-5] Currently, studies on the specific combination of acupoints for the treatment of functional Diarrhea (FD) were rarely reported and the therapeutic mechanism underlying the action is unclear. Therefore, in the present study, the regulating effects of electroacupuncture to the intestinal motility of FD model rats through stimulating the acupoint combinations of Tianshu (ST 25) and Dachangshu (BL 25), Quchi (LI 11) and Shangjuxu (ST 37) was respectively observed. Since the motility disorders of FD often happened to the jejunum, the present study mainly observed effects of different electroacupoint combinations on the expressions of jejunum c-kit protein and c-kit mRNA in FD model rats.

METERIALS AND METHODS

Folium Sennae was purchased from Quan'ao Company (Guangdong, China). 3-16 PKgeneral table type high-speed centrifuge was purchased from Sigma (Frankfurt, Germany). Smartspec 3000 UV-visible spectrophotometer was purchased from Bio-Rad (Los Angeles, CA, USA). NYCP-34ACG electrophoresis apparatus was purchased from Liuyi Biotechnology Company (Beijing, China). 90-2 constant temperature magnetic stirrer was purchased from Zhenrong Scientific Equipment Company (Shanghai, China). Real-time PCR apparatus was purchased from Bio-Rad (Los Angeles, CA, USA). C-kit primer, internal reference GAPDH primer, reverse transcription kit and Trizol were purchased from Invitrogen Laboratories Company (Carlsband, CA, USA). HANS-200A electric stimulator was purchased from Jisheng Medical Technology Company (Nanjing, China). 0.3 mm × 15 mm inch filiform needles were purchased from Huatuo Medical Instrument Company (Suzhou, China).

Intervention plan

Electroacupuncture group Ⅰ (EA Ⅰ) Location: according to the animal acupoint selection standards of Experimental Acupuncture Science edited by Prof. Li,[7] bilateral Tianshu and Dachangshu were located. "Tianshu (ST 25)": 5 mm to the navel; "Dachangshu (BL 25)": 5 mm to the processus spinosus of vertebra lumbalis Ⅳ. Manipulation: No.30 1-inch filiform needles ("Hua Tuo" stainless steel needle-Suzhou, Huatuo Medical Instrument Company, China) were prepared. After routine disinfection, filiform needles were punctured into corresponding points for 5 mm in depth. Electric stimulator: HANS- 200A electric stimulator (LH202H-Nanjing, Jisheng Medical Technology Company, China), disperse-dense wave, 2 Hz for disperse wave and 15 Hz for dense wave, slight local vibrating as the proper intensity (current intensity: 1.5 mA, voltage: 1-3 Ⅴ). Time: needle retention for 20 min, continuous electrical stimulation.

Electroacupuncture group Ⅱ (EA Ⅱ) location: according to the animal acupoint selection standards of ExperimentalAcupuncture Science edited by Prof. Li Zhongren,[7] bilateral Quchi and Shangjuxu were located. "Quchi (LI 11)": mid-concave of the front part of the lateral proximal joint of radius; "Shangjuxu (ST 37) ": 5mm down from Zusanli (ST 36) of hind leg of rats. Manipulation: No. 30 1-inch filiform needles ("Hua Tuo" stainless steel needle) werepunctured into corresponding points for 5 mm in depth. Electric stimulator: Korean electric stimulator (LH202H), dispersedense wave, 2 Hz for disperse wave and 15 Hz for dense wave, slight local vibrating as the proper intensity (current intensity: 1.5 mA, voltage: 1-3 V).Time: needle retention for 20 min, continuous electrical stimulation.

Blank control group and model group: without any intervention measures, rats in the two groups were seized and fixed once a day similarly like group Ⅰ and group Ⅱ.

Animals

Forty male rats, specefic pathogen free (SPF) level, 3.5 months old, mean body mass (200 ± 10) g, provided by the Experimental Animal Center of Xi'an Jiaotong University (Animal Certificate of Conformity: SCXK (Shaanxi) 2013.001). All rats were fed in separate cages and kept in appropriate environment (16-25 ℃, relative humidity of 50% to 70%, clean and quiet). This study was approved by the Animal Ethics Committee of Shaanxi University of Chinese Medicine.

Modeling and grouping

Rats in the model group, EA Ⅰ and EA Ⅱ: 90 g Fanxieye (*Folium Sennae*) was steeped with 6 times amount of water for 30 min, which was decocted for 10 min and then filtered through gauze. The filtered solution was decompressed and concentrated into 300 mL in therotary evaporator at 50 ℃, namely 0.3 g crude drug in 1 mL liquid, which was kept in refrigerator at − 20 ℃. When building models, rats were administered orally the liquid with the dose of 10 mL/kg once a day and continuously for 14 days altogether after heating the liquid to 25 ℃ through water bath.[6] For eliminating the influence of biological rhythms, experimental rats were conducted restraint stress at the same time of each day. For the blank group, rats were fed with ordinary dry forage (formulated by the SPF Laboratory Animal Center of Xi'an Jiaotong University) and for the same period of time as the other three groups.

After feeding for a week, all animals were divided into blank group, model group, EA Ⅰ and EA Ⅱ according to a random number table, 10 rats in each.