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EXPERIMENTAL STUDY

Role of transient receptor potential vanilloid subetype 1 in the increase of thermal pain threshold by moxibustion

Li Jiejun, Jiang Jingfeng

Li Jiejun, Jiang Jingfeng, Key Laboratory of Acupuncture Combined with Medication, Nanjing University of Traditional Chinese Medicine, Nanjing 210029, China.

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Correspondence to: Assistant-Prof. Jiang Jingfeng, Acupuncture Department of Nanjing University of TCM, Nanjing 210029, China. Dr.acujf@njutcm.edu.cn

Telephone: +86-13951717991

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Abstract

OBTECTIVE: To explore the role of transient receptor potential vanilloid subetype 1 (TRPV1) in the increase of the thermal pain threshold by moxibustion.

METHODS: Forty Kunming mice (20 ± 2) g were randomized into control group,capsaicin group, capsazepine group, moxibustion group and moxibustion + capsazepine (MC) group with 8 mice in each, and 16 C57BL/6 wild-type mice (18 ± 2) g were randomized into wild-type (WT) control group and WT moxibustion group with 8 mice in each, and 14 TRPV1 knockout mice (18 ± 2) g were randomized into knockout (KO) control group and KO moxibustion group with 7 in each. Each mouse in the capsaicin group was subcutaneously injected with the amount of 0.1 mL/10 g into L5 and L6 spinal cords; each mouse in the capsazepine group was intraperitoneally injected with the amount of 0.1 mL/10 g. Similarly, each mouse in the moxibustion group was given a suspended moxibustion with specially-made moxa-stick for 20 min on L5 and L6 spinal cords. Each mouse in MC group was intraperitoneally injected with the amount of 0.1 mL/10 g first, then after 15 min was given a suspended moxibustion for 20 min on L5 and L6 spinal cords. Each mouse in WT moxibustion group and KO moxibustion group was given a suspended moxibustion with specially-made moxa-stick for 20 min on L5 and L6 spinal cords. The control group, WT control group and KO control group were of no treatment in any way. After all treatments were completed, the digital-display measurement instrument for thermal pain was used to measure the threshold of thermal pain in each group respectively.

RESULTS: Compared with the control group, the thresholds of thermal pain in the moxibustion group and MC group were significantly increased (P <0.01); no significant changes in the thresholds in the capsaicin group and the capsazepine group (P > 0.05); compared with moxibustion group, he threshold of thermal in MC group was obviously decreased (P < 0.01). Compared with WT control group, the threshold of thermal pain in WT moxibustion group was significantly increased (P < 0.01); compared with KO control group, no changes in the threshold in KO moxibustion group (P > 0.05).

CONCLUSION: TRPV1 participated in the process of increasing the threshold of thermal pain by stimulating L5 and L6 of mice spinal cord with burning mosa-stick.

Key words: Moxa stick moxibustion; Pain threshold; Heat stimulation; Transient Receptor Potential Vanilloid subetype 1

INTRODUCTION

The transient receptor potential vanilloid (TRPV) 1 receptor is a nonselective cation channel, which is expressed on peripheral and central terminals of small and mediumsized primary sensory neurons.[1] Since the receptor could be activated by capsaicin of specificity, it is also known as capsaicin receptor,[2] which could also be activated by a wide range of noxious stimulations including protons and heat etc. When the temperature is higher than 43 ℃, TRPV1 will be activated as is recognised as one of the thermo-sensitive cation channels.[3] TRPV1 can also be sensitized by different stimulations,[1] and its sensitization is crucial in the development of pain,[4] which is an important property of pain signaling,[5] playing a key role in pain transmission and modulation.[6] Under certain nerve injury or inflammatory disease conditions, TRPV1 is up-regulated.[7] TRPV1 is a noxious signaling, which becomes the potential therapeutic target for developing analgesics.[4] Many studies show that, the expression of TRPV1 could be inhibited by virtue of TRPV1 antagonist to attenuate pain and heat hyperalgesia.[8-11] However, topical application of capsaicin is clinically effective in treating a variety of chronic painful conditions, including postherpetic neuralgia and HIV-associated peripheral neuropathies. Its analgesic action is considered a consequence of inhibition of TRPV1 function by desensitization.[7]

Moxibustion therapy is an important part of the acupuncture and moxibustion therapy in Traditional Chinese Medicine. It is a kind of external treatment using folium artemisiae argyi or moxa as the principal material to cauterize or warm and iron at the acupoint or diseased region on body surface after ignition for the purpose of prevention, healthcare and cure. The heat stimulation of ignited moxa will directly or indirectly effects at specific areas of human body surface, generate effects on local and distant regions through meridians, and it is also the key property and the one of the causes for curative effect.[12] Pain relief is one of the main effects of moxibustion therapy[12] which has been existed for over two thousand years with a wide range of indications, applicable for deficiency, excess and acute diseases.[13] There is a research shown that, moxibustion may increase the expressions of POMC mRNA and PDYN mRNA in hypothalamus of rats,as well as promote the analgesic potency of organism, suggesting the moxibustion has analgesic effect.[14]

TRPV1 is a temperature sensor which is a member of the TRP family, and may be in connection with the activation mechanism of warming and promoting effect from moxibustion. TRPV1 can be activated by heat stimulation of > 43 ℃, which is similar to the local skin temperature caused by moxibustion.[15] TRPV1 plays a basic role in the signal transduction of tissue injuries and pain reponses, regaeding the analgesic effect of moxibustion, therefore, whether TRPV1 participates in the analgesic process of moxibustion heat effect? This study shall try to discuss the role of TRPV1 in the increase of the thermal pain threshold by moxibustion.

MATERIALS AND METHODS

Experimental animals

Fifty Kunming (KM) male mice of 18-22g (Certificate No. of SCXK (Shanghai): 2007-0005, provided by Shanghai Slac Laboratory Animal Co., Ltd.,), 24 C57BL/6 wild-type male mice of 16-20 g [Certificate No. of SCXK (Jiangsu): 2010-0001, provided by Laboratory Animal Center of Nanjing University], and 21 J003770 TRPV1 knockout male mice of 17-20 g [Certificate No. SCXK (Jiangsu): 2010-0001, provided by Laboratory Animal Center of Nanjing University].

Reagent preparation

Capsaicin (50 mg),Capsazepine (25 mg), 500 mL absolute ethyl alcohol, Polyoxyethylene Sorbitan (Tween 80) (500 mL) and Dimethyl Sulfoxide (50 mL) were purchased from Sigma (St. Louis, MO, USA). Normal Saline (250 mL) was purchased from Guangdong Litai pharmaceutical Limited by Share Ltd. (Guangdong, China). Capsaicin in powder 50mg is dissolved in 10% polyoxyethylene sorbitan (Tween 80) and 10% ethanol before being mixed with 80% normal saline to obtain a capsaicin solution of 0.01 M;[16] Capsazepine in powder 25 mg is dissolved in 25 mL dimethyl sulphoxide and then diluted in normal saline to obtain a capsazepine solution of 0.01 M.[17]

Experimental apparatus

JL-F Digital-display type measurement instrument for thermal pain was purchased from Shanghai Precision Instrument Co., Ltd., (Shanghai, China), Special-made moxa-stick (5 mm × 200 mm) was purchased from Nanyang Hanyi Moxibustion Technology Development Co., Ltd., (Nanyang, China), 1 mL Disposable sterilized syringe was purchased from Changzhou Medical Appliances General Factory Co., Ltd., (Changzhou, China)

Experiment Ⅰ

Grouping: 40 KM mice (20 ± 2) g are randomized into 5 groups by random number table method: control group, capsaicin group, capsazepine group, moxibustion group and moxibustion + capsazepine (MC) group with 8 mice in each, and the rest 10 mice are backups without any treatment.

Group treatment: Each mouse in the capsaicin group is subcutaneously injected with the amount of 0.1 mL/10 g into the L5, L6 spinal cords;[18] Each mouse in the capsazepine group is intraperitoneally injected with the amount of 0.1 mL/10 g. Similarly, each mouse in the moxibustion group is given a suspended moxibustion with specially-made moxa-stick for 20 min on L5 and L6 spinal cords. Each mouse in MC group is intraperitoneally injected with the amount of 0.1 mL/10 g first, then after 15 min is given a suspended moxibustion for 20 min on L5 and L6 spinal cords. The control group was of no treatment in any way.

Pain threshold measurement: after all treatments are completed, use the digital-display measurement instrument for thermal pain to respectively measure the threshold of thermal pain in each group. Record the displaying value on the instrument when the tails of mice go up for heat.

***Experiment* Ⅱ**

Grouping: 16 C57BL/6 wild-type mice [(18 ± 2) g] are randomized into 2 groups by random number table method: wild-type (WT) control group and WT moxibustion group with 8 in each and the rest 8 are backups without any treatment. 14 TRPV1 knockout mice [(18 ± 2) g] are randomized into 2 groups by random number table method: knockout (KO) control group and KO moxibustion group with 7 in each and the rest 7 are backups without any treatment.

Group treatment: each mouse in WT moxibustion group and KO moxibustion group is given a suspended moxibustion with specially-made moxa-stick for 20 min on L5 and L6 spinal cords. The WT control group and KO control group are of no treatment in any way.

Pain threshold measurement: After all treatments were completed, the digital-display measurement instrument for thermal pain was used to measure the threshold of thermal pain in each group respectively.

Data analysis

All data were presented as mean ± standard deviation statistics, tested by one-way analysis of variance, analysis with SPSS 17.0 (SPSS, Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

RESULTS

The digital-display measurement instrument for thermal pain presents the threshold level in the length of time it recorded. The higher of heat tolerance, the longer of the time recorded, indicating the higher threshold and larger threshold value of thermal pain. In our study, tail-flick time(s) of mice was taken as the value of thermal pain threshold.

Comparison of threshold of thermal pain for KM mice among the groups

In experiment Ⅰ, compared with the control group [(15.4 ± 4.5) s], no significant changes in the thermal pain thresholds in the capsaicin group [(12.7 ± 2.8) s] and the capsazepine group [(16.7 ± 2.6) s] ($P > 0.05$), which was of the similar levels with the control group. The thermal pain threshold in the moxibustion group [(44.3 ± 6.5 s)] and MC group [(31.6 ± 2.4) s] were significantly increased ($P <0.01$); compared with the moxibustion group [(44.3 ± 6.5) s], the threshold in MC group [(31.6 ± 2.4) s] was obviously decreased with significance ($P < 0.01$), indicating moxibustion played a role in increasing the thermal pain threshold, while capsazepine might play the role of partial antagonism against the analgesic effect of moxibustion.

Comparison of threshold of thermal pain of mice in wild-type groups and TRPV1 knockout groups

In Experiment Ⅱ, compared with WT control group [(16.7 ± 2.5) s], the thermal pain threshold in WT moxibustion group [(49.1 ± 8.6) s] had been increased significantly ($P < 0.01$); compared with KO control group [(53.1 ± 9.3) s], no changes in the threshold in KO moxibustion group [(54.0 ± 9.6) s] ($P > 0.05$), suggesting that TRPV1 knockout mice is less sensitive to noxious heat stimulation,with threshold of thermal pain not obviously changed, which means TRPV1 was essential element in causing hyperalgesia responses to noxious thermal stimulation.

DISCUSSION

Pain is the instantaneous unpleasant sensation subsequent to a noxious or potentially injurious stimulus, as a warning system for tissue protection against injuries.[19] "Pain Threshold" refers to the minimum amount of stimulations causing pain, which is widely used as the indicator for measuring analgesic effect[14] that, the higher of threshold of pain, the better the analgesic effect shall be, conversely, the lower the threshold of pain, the poorer the analgesic effect.

TRPV1 is a member of the transient receptor potential family, which is a heat- sensitive ligand-gated Ca^{2+} -permeable ion channel.[4] TRPV1 channels are found throughout the body in epithelial cells and in peripheral and central terminals in neurons that can be activated by capsaicin, low pH, proton and heat stimulation of > 43 ℃. TRPV1,a noxious stimuli molecular integrator, exerts variety of functions ranging from nociception and pain.[20] Hence TRPV1 is regarded as the most hopeful therapeutic target in pain treatment.

Moxibustion therapy is an important part of the acupuncture and moxibustion therapy in Traditional Chinese Medicine, and a kind of external treatment for the purpose of prevention, healthcare and cure. Moxibustion therapy is capable of curing pain that, the heat stimulation of moxibustion might be the premise and foundation of analgesic effect playing.[14]

Our study has found that the threshold of thermal pain in the moxibustion group was significantly increased compared with it in the control group, suggest-

ing that moxibustion was capable of increasing the thermal pain threshold. The threshold of thermal pain in the WT moxibustion group was significantly increased compared with it in the WT control group; and no significant difference was found between the threshold of thermal pain in the KO moxibustion group and it in the KO control group, which is consistent with the study results of predecessors. The wild-type mice exhibited decreased thresholds or latencies of withdrawal in response to thermal stimuli, in contrast, mice lacking functional TRPV1 only displayed less sensitive to painful thermal stimuli.[5] After heat stimulation of moxibustion, the thermal pain threshold in WT moxibustion group is increased significantly, while no changes in it of the KO moxibustion group. It could be concluded from the above results that, the moxibustion increasing threshold of thermal pain is correlative to TRPV1.

Previous studies have shown that after a long-time or high-dose administration of TRPV1 agonist, the thermal pain threshold could be increased, thus generating an analgesic effect,[21] whereas a single administration of lower doses may cause activation of TRPV1.[22] In this experiment, the thermal pain threshold of capsaicin group was slightly decreased compared with it of the control group, indicating that capsaicin played no role in increasing the thermal pain threshold. The reason might be the single administration of capsaicin *via* local subcutaneous injection in capsaicin group was in a lower dose thereby activating TRPV1 causing decrease of the threshold; while the longer time of heat stimulation increased the threshold in the moxibustion group.

Single administration of capsazepine is incapable of increasing the thermal pain threshold,[17] which has been verified in our experiment. Thermal pain threshold in the capazepine group was close to it in the control group, indicating that capsazepine was incapable of increasing the thermal pain threshold under the condition of non-activated TRPV1. The thermal pain threshold in the MC group was significantly differentfrom it in the control group; and it was significantly decreased compared with the moxibustion group, indicating that capsazepine might play the role of partial antagonism against the analgesic effect of moxibustion. Research of Jiyeon Kwak showed that hyperpolarization-activated cation currents [I (h)] contributed to pain, particularly in the generation of chronic neuropathic pain abnormalities. Capsaicin (1 μM) inhibited I (h), but the capsaicin-induced inhibition of I (h) was prevented by the TRPV1 antagonist, capsazepine.[23] The burning quality of capsaicin-induced pain suggests that capsaicin and heat might evoke painful responses through a common molecular pathway,[24] and for the reason, capsazepine might be able to interdict the response caused by heat as it interdicts capsaicin, which might be the reason for lower thermal pain threshold in the MC group than it in the moxibustion group.

TRPV1 is the target in controlling chronic and acute pain and the key of analgesia.[20,25] Studys found that capsaicin and other vanilloid receptors selectively activate TRPV1 (nociceptive receptors) to desensitize it, promoting the analgesic (therapeutic) effect of compounds of capsaicin and other vanilloid receptors. Presented repeated TRPV1 agonists application to the skin produces a degeneration of epidermal nerve fibers that may underlie this type of analgesia.[26] Schumacher M A had revealed several overlapping and complementary mechanisms of capsaicin analgesia including receptor desensitization, nociceptor dysfunction, neuropeptide depletion, and nerve terminal destruction, where the destruction of nociceptor terminals may play the greatest role in the effect of analgesia/treatment.[21]

In conclusion, moxibustion increasing thermal pain threshold was correlative to TRPV1, and the increase in Thermal pain threshold might be achieved *via* desensitization of TRPV1 or damage of sensory terminals[27] by the heat stimulations of moxibustion, where the mechanism should be further explored. Without obvious side effects observed, moxibustion can be a safe intervention for pain relief.

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