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EXPERIMENTAL STUDY

Heat induces adenosine triphosphate release from mast cells *in vitro*: a putative mechanism for moxibustion

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Abstract

OBJECTIVE: To investigate the role of adenosine triphosphate (ATP) purinergic signaling in mast cells (MCs) modulated by heat to further understand the molecular mechanisms of moxibustion.

METHODS: Skin temperatures induced by monkshood cake moxibustion were evaluated by measuring the Neiguan acupoint (PC 6) from 31 participants with a digital thermocouple thermometer. Temperatures of 43 ℃ and 52 ℃ were applied to cultured human leukemia mast cell line HMC-1 in vitro. Calcium fluorescence was applied to detect intracellular Ca^{2+} ($[Ca^{2+}]_i$). Extracellular ATP contents were measured by luciferin-luciferase assay.

RESULTS: Maximum skin temperatures mostly ranged from 40-45 ℃, but some reached up to 50 ℃. Both 43 ℃ and 52 ℃ induced MC degranulation, which was accompanied by an increase in $[Ca^{2+}]_i$ and ATP release. Complexing extracellular Ca^{2+} with 5 mM ethylene glycol-bis (β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA) inhibited the noxious heat-induced elevation of $[Ca^{2+}]_i$ and prevented the enhanced ATP secretion by those cells at 52 ℃, but not 43 ℃.

CONCLUSION: Monkshood cake moxibustion can generate heat sufficient to trigger cellular events of MCs, including degranulation, $[Ca^{2+}]_i$ elevation, and ATP release, suggesting that purinergic signals originating from MCs are possibly the initiating response of acupoints to moxibustion.

Key words: Fire toxin syndrome; Mast cells; Cell degranulation; Calcium; Adenosine triphosphate; Moxibustion

INTRODUCTION

Moxibustion is a common therapy in Traditional Chinese Medicine (TCM). Its main purpose is to stimulate acupoints with heat. The thermal properties of different kinds of moxibustion have been evaluated in several previous studies. For moxibustion with warming needles, finite element analysis showed that the maximum temperature that the skin reaches is in excess of 50 ℃.[1,2] This indirect analysis was further confirmed by direct temperature measurements of the warming needles.[1] For direct moxibustion, the highest temperatures of the skin were > 45 ℃ with a distance of < 3 cm between the moxa and acupoint.[3] For indirect moxibustion, monkshood cake moxibustion is mostly applied in clinics. The characteristic maximum temperature of 57 ℃, measured at the bottom of mediator cakes, has been found for monkshood cake moxibustion. However, the actual skin temperature generated by this treatment remains unknown.

Mast cells (MCs) have been shown to represent a component of morphological characteristics of acupuncture points.[4] With respect to their functional specificity, MCs participate in the analgesic effects in response to needling acupuncture,[5] moxibustion,[6] and laser acupuncture[7] in vivo. Transient receptor potential vanilloid 2 (TRPV2)-mediated degranulation, increase of whole-cell membrane currents, and elevation of intracellular calcium ($[Ca^{2+}]_i$) activity were induced by a temperature of 52 ℃ in cultured MCs in vitro.[8] Aside from TRPV2, MCs also express TRPV1 channels[8] that are activated by temperatures higher than 43 ℃.[9] Considering the temperature characteristics of moxibustion described above, both TRPV1 and TRPV2 expressed on MCs are likely activated during heat treatment.

Increasing evidence suggests that extracellular adenosine triphosphate (ATP) and purinergic signaling participate in various physiological and pathophysiological processes.[10] The purinergic signaling pathway was hypothesized to be involved in the mechanism of pain relief induced by acupuncture.[11] It was recently demonstrated that adenosine, a metabolic product of ATP, participates in the analgesic effect of needling acupuncture by binding to the A1 purinergic receptor at acupuncture points.[12] MCs express purinergic receptors[13] and are capable of releasing ATP after physical stimuli.[14] However, whether the ATP purinergic signal of MCs is involved in moxibustion remains unknown. The interaction between TRPV channels and purinergic signals was reported in previous studies.[15,16] TRPV3 in keratinocytes transmits thermal information to neurons by releasing ATP.[16] It is unclear whether high temperatures can activate TRPV1 or TRPV2 channels to trigger ATP purinergic signals in MCs.

The aim of this study was to understand the role of ATP purinergic signaling of MCs during moxibustion. We measured the temperature of human skin on Neiguan (PC 6) generated by monkshood cake moxibustion and found that maximum temperatures were usually in the range of 40-45 ℃, and occasionally exceeded 50 ℃. Temperatures of 43 ℃and 52 ℃ were applied to cultured human leukemia mast cell line HMC-1 *in vitro* to detect their responses in morphology and function. The two temperatures induced degranulation of HMC-1 cells, elevated the intracellular calcium, and potentiated ATP release.

METHODS AND MATERIALS

Human tests

Thirty-one healthy undergraduate students, 17 males and 14 females, were recruited, aged 22-24. Each subject's left Neiguan (PC 6) was heated by monkshood cake moxibustion. Commercial moxa-cigars ($\Phi = 24$ mm) (Hanyi, Hanyi Airong Limited Company, Nanyang, China) were cut into 2 g cakes at 8 mm thick. The cut monkshood was fired from the top during moxibustion. The digital thermocouple thermometer (model: UT-325, UNI-T, Shanghai Uni-Trend Electronics Company, Shanghai, China) was placed on the left Neiguan (PC 6) and was completely covered by the monkshood cake. The thermometer was connected to a computer and data were recorded every 10 s with UT32X software (Shanghai Uni-Trend Electronics Company, Shanghai, China). Participants were given informed consent and the study was performed in accordance with the guidelines of the Clinic Trail Committee of Shanghai University of TCM (Certificate No. ChiECRCT-20110022).

Cell culture

Human mast cell line 1 (HMC-1) was kindly provided by Dr. J.H. Butterfield (Mayo Clinic, Rochester, MN, USA). Cultivation was performed as previously described.[17] In brief, the cells were incubated in Iscove's modified Dulbecco's medium (IMDM) (Gibco Life Technologies, Grand Island, NE, USA) without phenol red, supplemented with 2 mM L-glutamine, 25 mM 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES) (Sigma, Sigma-Aldrich, St. Louis, MO, USA), 10% (v/v) fetal bovine serum (Gibco, Life techonolgies, Grand Island, NE, USA), 1% penicillin and streptomycin (Gibco, Life techonolgies, Grand Island, NE, USA), in a 95% humidity-controlled incubator (Model: 310, Thermo, Thermo Electron, Waltham, MA, USA) with 5% CO_2 at 37 ℃. Cell density was about 1×10^5/mL.

Reagents and solutions

The bath solution (BS) for HMC-1 cells was composed of the following (in mM): 150 NaCl, 5 KCl, 2 $CaCl_2$, 5 $MgCl_2$, 4 D-sorbitol, 10 HEPES, and pH 7.4 (adjusted with NaOH). Osmotic pressure of the solutions was 310 mOsm/L (Model: 3300, Micro Osmometer, Advanced Instruments Inc., Norwood, MA,