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The effect of electroacupuncture at ST36 on severe thermal injury-induced remote acute lung injury in rats

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ABSTRACT

Objective: Acupuncture at ST36 can produce anti-inflammatory effects, which might be associated with vagus nerve activity. This study explored the effects of electroacupuncture (EA) at ST36 on severe thermal injury-induced remote acute lung injury in rats.

Interventions: Forty male Sprague-Dawley (SD) rats were randomly divided into five groups: (1) the sham (S) group, (2) the thermal injury (TEM) group subjected to 30% total body surface area (30% TBSA) third-degree scald, (3) the EA at ST36 group subjected to EA stimulation at ST36 (3 V, 2 ms, and 3 Hz) after 30% TBSA scald, (4) the EA at non-acupoint group subjected to EA stimulation at non-acupoint after 30% TBSA scald, and (5) the α-bungarotoxin (α7 nicotinic acetylcholine receptor subunit antagonist) group administered 1.0 μg kg^{-1} α-bungarotoxin before EA at ST36.

Measurements and main results: Thermal injury of 30% TBSA induced leukocytosis in the alveolar space, interstitial edema, and the pro-inflammatory cytokines interleukin (IL)-1β, IL-6, and high-mobility group box 1 (HMGB-1); the expression of both HMGB-1 messenger RNA (mRNA) and protein in lung tissue was significantly enhanced. EA at ST36 significantly downregulated the levels of inflammatory cytokines and improved lung tissue injury. However, pretreatment with α-bungarotoxin reversed the effects of electrical stimulation of ST36.

Conclusions: EA at ST36 might have a potential protective effect on severe thermal injury-induced remote acute lung injury via limitation of inflammatory responses in rats.

1. Introduction

The pathogenesis of thermal injury is a complex process that may be rooted in the additive effects of inadequate tissue perfusion, hypermetabolic condition, free radical damage, and systemic alterations in the cytokines [1]. Although the pathophysiological basis of thermal injury remains insufficiently understood, based on the current research findings, it is evident that local burn insult produces the release of large

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pro-inflammatory cytokines, including tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β), IL-6, high-mobility group box 1 (HMGB-1), and other inflammatory mediators [2,3]. It might result in unlimited activation of inflammatory responses. Overwhelming release of inflammatory cytokines would develop further into systemic inflammatory response syndrome (SIRS), acute lung injury (ALI), and multiple organ dysfunction syndrome (MODS) [4,5].

The lung is one of the most easily attacked target organs (indirectly and second). ALI is not only a disease of the lungs but also a more important part of the systemic inflammatory response. Severe burns trigger the body's inflammatory response, which induces ALI or acute respiratory distress syndrome (ARDS) occurrence [6,7]. Therefore, the prevention of inflammatory cytokine release from phagocytic cells following thermal injury could be an extremely important approach to treating severe thermal injury-induced remote acute inflammatory lung injury. HMGB1 is known as a mediator of delayed critical disease lethality, severe burns, and systemic inflammatory response. Lutz et al. [8] revealed that HMGB-1 could play a critical role as a late-acting mediator of acute lung inflammation. Abraham et al. [9] found that HMGB-1 given intratracheally produced acute inflammatory lung injury, with neutrophil accumulation, the development of lung edema, and increased pulmonary production of TNF-α and IL-1β.

In recent years, many scholars have conducted massive research on developing the theory of the role of ST36 in traditional Chinese medicine. In 2011, Suo et al. [10] discovered that electroacupuncture (EA) at ST36 points could inhibit the expression of the serum TNF-α, and it produces a protective effect on hemorrhagic shock in dogs. In 2013, Geng et al. [11] found that EA at ST36 could reduce lung injury in a chronic obstructive pulmonary disease (COPD) rat model, and beneficial effects might be related to downregulation of inflammatory cytokines. Furthermore, Hu et al. [12] found that EA at ST36 could prevent intestinal barrier and remote organ dysfunction following gut ischemia via activation of the cholinergic anti-inflammatory-dependent mechanism.

Therefore, we hypothesize that EA at ST36 could attenuate pro-inflammatory cytokine responses by signaling the α7 nicotinic acetylcholine receptor subunit. In the present study, we replicate the model of third-degree thermal injury of 30% of the total body surface area (30% TBSA) in rats, investigating the effects of EA at ST36 and administration of α-bungarotoxin (α-BGT, α7 nicotinic acetylcholine receptor subunit antagonist) on the expression of the pro-inflammatory cytokines TNF-α, IL-1β, and IL-6, and the expression of HMGB-1 mRNA and protein in lung tissue.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley (SD) rats weighing 250–300 g were maintained on sterile, standard laboratory chow and water ad libitum in individual ventilated cages under specific pathogen-free (SPF) conditions in the animal facility of the Experimental Research Centre of Wuhan University. All animal experiments were approved by the Institutional Animal Care Committee of Wuhan University and were in accordance with the guidelines of the National Institutes of Health on the care and use of animals.

2.2. Animal thermal injury model procedure

The models of rats with thermal injury were prepared according to the literature [13]. After animals were anesthetized by an intraperitoneal injection of ketamine (75 mg kg^{-1}) plus midazolam (5 mg kg^{-1}), the dorsum of the rats was shaved (20% sodium sulfide), exposed to a water bath at 99–100 °C for 12 s, which resulted in partial-thickness third-degree skin thermal injury involving 30% TBSA. After 30% TBSA scald injury or the sham operation procedure, all animals were immediately resuscitated with Ringer's solution via the intraperitoneal route (50 mL kg^{-1} body weight), and then the scald wound was treated with 1% silver sulfadiazine to prevent wound infection.

2.3. Experimental protocols

Forty SD rats were randomly assigned into five groups ($n = 8$ each): (1) the sham (S) group, (2) the thermal injury (TEM) group subjected to 30% TBSA scald, (3) the EA at ST36 (ST36) group subjected to EA stimulation at ST36 (3 V, 2 ms, and 3 Hz) after scald, (4) the EA at non-acupoint (NA) group subjected to EA stimulation at non-acupoint after scald, and (5) the α-BGT (α7 nicotinic acetylcholine receptor subunit antagonist) group administered 1.0 μg kg^{-1} α-BGT [14] before EA stimulation. The backs of the sham group animals were only immersed in a water bath at 25 °C for the same period. In the ST36 group, the NA group, and the α-BGT group, electrical stimulation at ST 36 or non-acupoint was performed for 12 min immediately, once every 8 h, and 48 h continuously after the thermal injury model was established, respectively. An intraperitoneal injection of 1.0 μg kg^{-1} α-BGT was administered to rats of the α-BGT group before electrical stimulation. Analgesia was administered with 0.1 mg kg^{-1} buprenorphine every 12 h subcutaneously. At the end of the study, the animals were sacrificed via bleeding from the right carotid artery under ketamine anesthesia.

2.4. EA stimulation at ST36

Animals were anesthetized by an intraperitoneal injection of ketamine (75 mg kg^{-1}) plus midazolam (5 mg kg^{-1}) before EA at ST36. Then dual hind limb skin shearing was carried out, with the ST36 points located between the tibia and fibula, laterally to the distal end of the cranial tuberosity of the tibia, approximately 5 mm lateral to the anterior tubercle of the tibia, and with the non-acupoint located 5 mm next to ST36 outside, using a 7-mm vertical needle. The animals were not required to be fixed. The rats were awake about 30 min after EA. EA at ST 36 or non-acupoint was performed for 12 min immediately, once every 8 h, and 48 h continuously after scald. Constant voltage stimuli (3 V, 2 ms, and 3 Hz) with an electrical stimulation module via the BL-420F Biological Function Experimental System (Tai-Meng Technology Company, Chengdu, China) were applied to the bilateral ST36. The stimulus was shown to be effective by Yim et al. [15]