Moxibustion at Mingmen Reduces Inflammation and Decreases IL-6 in a Collagen-Induced Arthritis Mouse Model

Morihiro Kogure*, Naomi Mimura, Hideshi Ikemoto, Shintaro Ishikawa, Takako Nakanishi-Ueda, Masataka Sunagawa, Tadashi Hisamitsu

Department of Physiology, Showa University, School of Medicine, 1-5-8, Hatanodai, Shinagawa-ku, Tokyo 142-8555 Japan

Available online Dec 13, 2011

Abstract

The purpose of this study was to compare the effectiveness of moxibustion (MOX) treatment at the GV4 and CV12 acupoints, and to determine the correlations between MOX treatment and interleukin (IL)-6 and corticosterone levels in a collagen-induced arthritis (CIA) mouse model. CIA mice were immunized twice intradermally over a 3-week interval with bovine type II collagen. After the second immunization (day 21), MOX was applied to the mouse equivalent of the GV4 and CV12 acupoints with a 1 mg moxa cone five times/day. Clinical symptoms of CIA were observed three times/week until day 35. The concentrations of IL-6 and corticosterone in the blood samples were measured by immunoassay kits. At day 35, the incidence of CIA was significantly decreased in mice treated with MOX at the GV4 acupoint (78%, n = 23, p < 0.05), compared to untreated CIA mice (100%) and mice treated with MOX at the CV12 acupoint (100%). IL-6 levels significantly decreased in mice treated with MOX at the GV4 acupoint. These results suggest that MOX treatment suppressed CIA at the GV4 acupoint, not at the CV12 acupoint, possibly through inhibition of IL-6 production.

1. Introduction

Moxibustion (MOX) has been used as a traditional therapy for the treatment of some immune-related diseases [1,2]. According to traditional Chinese medicine, the Governor Vessel (GV) meridian, located at the dorsal midline, possesses the function of invigorating the body’s resistance and eliminating the cold-damp sensation [3]. MOX treatment at the GV meridian has been shown to suppress arthritis and pain in rheumatoid arthritis patients in China [4,5]. MOX has been used to treat autoimmune diseases such as rheumatoid arthritis, to suppress arthritis, for pain relief and to improve a person’s ability to perform activities associated with daily living. However, the underlying
mechanism of MOX treatment is not fully understood. In rheumatoid arthritis, interleukin-6 (IL-6) increases the generation of T cells, B cells, macrophages, monocytes and endothelial cells, and acts on specific receptors on different target cells [6]. IL-6 induces the migration of lymphocytes, and also induces the proliferation of T cells and the production of antibodies from plasma cells [7]. IL-6 induces vascular endothelial cell growth factor (VEGF) production from localized inflammatory sites and regulates neovascularization in rheumatoid joints [8]. IL-6 also promotes differentiation of osteoclasts [9]. Therefore, IL-6 is considered to be a key pathological pro-inflammatory cytokine and is a target in anti-rheumatoid arthritis drug developments [10].

The GV4 (Mingmen) acupoint, one of the commonly used points from the GV meridian, has been utilized in treating human immunological disorders [3,11] and inducing immune modulation in mice [12]. GV4 is also an effective acupoint for MOX treatment. Fang et al reported that MOX treatment at the GV4 acupoint prevented the incidence of and attenuated collagen-induced arthritis (CIA) in an experimental mouse model [13]. The CIA mouse model, created by immunizing mice with type II collagen in adjuvant, is a widely used model of rheumatoid arthritis [14,15]. CIA in mice is characterized by a severe swelling of the paws associated with a massive infiltration of inflammatory cells into the joints. Progression of the disease results in destruction of joints and severe deformities.

MOX treatment is performed by igniting moxa (Mugwort wool) cones on the skin, and can cause a mild burn injury. After a burn injury, serum glucocorticoid levels increase due to activation of the hypothalamic-pituitary-adrenal (HPA) axis [16]. The HPA axis can be triggered by various factors, such as tumor necrosis factor-alpha (TNF-alpha), IL-1 and IL-6 released mainly from immune cells [17,18].

The purpose of this study was to determine and compare the effectiveness of MOX treatment at the GV4 acupoint, and other acupoints such as Conception Vessel (CV) 12 (Zhongwan), in the CIA mouse model. The CV12 acupoint is located on the abdominal wall, and is associated with the pancreas. It can induce secretion of endogenous beta-endorphin, which reduces the plasma glucose concentration in an insulin-dependent manner in diabetic rats. The CV12 acupoint has also been utilized in the treatment of human abnormal peristaltic movement [19]. Correlations between MOX treatment and IL-6 and corticosterone levels were also investigated.

2. Materials and methods

2.1. Animals

Male DBA/1 J mice, aged 7 weeks, were purchased from Sankyo Labo Service Co., Ltd. (Saitama, Japan). Animals were housed with free access to food and water, and acclimatized to standard laboratory conditions (23 °C ± 3 °C, 55% ± 10% humidity, and 12 hour light/dark cycles) for 7 days. The Showa University Committee of Animal Care and Use approved the study protocol.

2.2. CIA mouse model

Type II collagen (CII) from bovine articular cartilage (Collagen Techno Training Institute Ltd., Tokyo, Japan) was solubilized at a concentration of 200 μg/50 μL in 0.01 M acetic acid and the solution was emulsified in an equal volume of Freund’s complete adjuvant (Difco Laboratories, Detroit, MI, USA) on ice. Mice were injected intradermally at the base of the tail with 0.1 mL of the emulsion. Twenty-one days after the primary immunization, the mice received a second immunization of 200 μg of CII in 0.1 mL Freund’s incomplete adjuvant (Difco Laboratories) [12] (Fig. 1).

2.3. MOX treatment

The mice were divided into five groups: (1) the untreated control group; (2) the MOX(GV4)-treated control group (MOX treatment was administered at the GV4 acupoint to non-immunized mice); (3) the untreated CIA group; (4) the MOX(GV4)-treated CIA group; and (5) the MOX(CV12)-treated CIA group.

MOX was conducted by placing small pieces of a moxa (1 mg Mugwort wool) cone directly onto the skin surface at the relevant acupoint and then ignited. The 1 mg moxa cone burned out in an instant.

After the second immunization, a 1 mg moxa cone was applied to the GV4 or CV12 acupoints. The moxa cone was ignited for 5 seconds on the skin surface, five times/day, three times/week, until day 33 (Fig. 1). The location of the mouse GV4 acupoint was determined according to the corresponding human GV4 acupoint, which is located on the lower middle dorsal midline. The location of the mouse CV12 acupoint was determined according to the corresponding human CV12 acupoint, which is located on the middle ventral midline on the opposite side of the body to GV4 (Fig. 2).

2.4. Assessment of arthritis

The clinical symptoms of arthritis were evaluated with a visual scoring inspection, based on the degree of peri-articular erythema, swelling and joint deformity. Inspections were carried out three times/week, from days 21 to...
Lesions on the four paws of each mouse were assessed using previously described criteria [6], and assigned a score of 0 to 4 as follows: 0 = normal (Fig. 3A); 1 = mild inflammation of a single area (i.e., midfoot, ankle or toes; Fig. 3B); 2 = moderately severe arthritis involving toes and ankle or midfoot; 3 = severe arthritis involving an entire paw (Fig. 3C); 4 = severe arthritis resulting in ankylosis and loss of joint movement. The clinical score given to each mouse was the sum of the scores assigned to each of the four paws.

2.5. Quantification of IL-6 in plasma

On day 35, blood was collected using heparin as the anticoagulant, centrifuged at 2000 rpm for 20 minutes, and the plasma stored at −20°C until analysis. Plasma IL-6 was assayed using the Quantikine Mouse IL-6 immunoassay kit (R&D Systems Inc., Minneapolis, MN, USA).

2.6. Quantification of corticosterone in serum

On day 35, blood was collected, centrifuged at 2000 rpm for 20 minutes, and the serum stored at −20°C until analysis.

Serum corticosterone levels were determined using the AssayMax Corticosterone ELISA Kit (Assaypro, St. Charles, MO, USA).

2.7. Statistics

Statistical analysis was performed using a one-way analysis of variance (ANOVA) followed by Fisher’s PLSD for incidence of CIA and Student t test for clinical score of CIA, the levels of IL-6 and corticosterone. All data are expressed as the mean ± SE and differences were considered significant when p < 0.05.

3. Results

3.1. Incidence of CIA

Arthritis was not observed in the untreated control and MOX(GV4)-treated control groups. Mild inflammation was
observed in 40% of CIA mice at day 26, and the incidence of CIA increased in each group over time. At day 28, the incidence of CIA in the MOX(GV4)-treated CIA group (57%, \( n = 23 \)) was significantly lower than in the untreated CIA group (91%, \( n = 22, p < 0.01 \)). On day 30, the incidence of CIA in the MOX(GV4)-treated CIA group (61%, \( n = 23 \)) was significantly lower than in the MOX(CV12)-treated CIA group (100%, \( n = 8, p < 0.05 \)). Clinical symptoms of arthritis were not observed in 22% of mice in the MOX(GV4)-treated CIA group at day 35 (Fig. 4). Increases in the clinical score of CIA also coincided with increases in the incidence of CIA in each group. However, at day 35, the clinical score of CIA in the MOX(GV4)-treated CIA group (5.7 ± 1.0) was significantly lower than that of the untreated CIA group (8.4 ± 0.7, \( p < 0.05 \)), and the MOX(CV12)-treated CIA group (8.3 ± 1.3, \( p < 0.05 \); Fig. 5). In the MOX(GV4)-treated CIA group, the clinical score of CIA was lower than the other groups at each time point from day 28 to day 35.

### 3.2. Levels of IL-6 in plasma

On day 35, plasma IL-6 levels were 1.5 ± 0.6 pg/mL in the untreated control group (\( n = 18 \)) and 1.4 ± 0.2 pg/mL in the MOX(GV4)-treated control group (\( n = 17 \)), respectively. In the untreated CIA group, plasma IL-6 levels (89.5 ± 17.0 pg/mL) were significantly higher than in the untreated control group (\( n = 18, p < 0.05 \)), and were significantly decreased by MOX treatment at the GV4 acupoint (42.0 ± 10.4 pg/mL, \( n = 22, p < 0.05 \); Table 1).

### 3.3. Levels of corticosterone in serum

The levels of corticosterone on day 35 in the untreated CIA and MOX(GV4)-treated CIA groups were 10.3 ± 2.1 ng/mL and 9.9 ± 2.5 ng/mL, respectively. These levels were significantly higher than in the untreated control group (4.6 ± 0.8 pg/mL) and the MOX(GV4)-treated control group (3.2 ± 0.5 pg/mL; Table 2).

### 4. Discussion

The results from this study clearly demonstrate that MOX treatment at the GV4 acupoint reduced the incidence and clinical score of CIA, and significantly decreased the high levels of IL-6 in CIA mice.

In humans, many acupoints are located along the GV and CV meridians. They have special relationships with the liver, kidney, uterus and brain, and thus influence these structures physiologically and pathologically. In this study, MOX was performed at the GV4 (Mingmen) acupoint, which is an effective acupoint for human immunological disorders [3,11], and the CV12 (Zhongwan) acupoint, which is related to gastrointestinal movement and associated with the pancreas [19]. In this study, MOX treatment at the GV4 acupoint significantly reduced the incidence and development of arthritis in CIA mice. CV12, however, was not an effective acupoint. The effectiveness of MOX is not only due to its thermal effect. To achieve the best therapeutic efficacy, it is important to position MOX at the correct acupoint, where its thermal effect may stimulate immune suppression, which in turn suppresses arthritis.

Blocking IL-6 activity with the anti-IL-6 receptor antibody tocilizumab significantly reduces the disease activity...
of rheumatoid arthritis, indicating that IL-6 is strongly involved in the pathogenesis of rheumatoid arthritis [20–22]. In the current study, plasma IL-6 levels were significantly decreased by MOX(GV4) treatment, and the incidence and development of arthritis in CIA mice was also suppressed. These data suggest that MOX(GV4) treatment may inhibit rheumatoid arthritis pathology through the suppression of IL-6 generation.

MOX treatment caused a small burn injury and inflammation at the acupoint. A previous report has shown that increased circulating levels of glucocorticoids are present in the serum after a burn injury [16]. The current study showed that serum corticosterone levels were increased in the untreated CIA and MOX(GV4)-treated CIA groups, compared to the untreated control group. However, there were no differences in corticosterone levels between the untreated control and the MOX(GV4)-treated control groups. This result implies that MOX treatment does not influence serum corticoid levels.

In conclusion, the results from the current study suggest that MOX treatment at the GV4 acupoint may improve immune-related diseases through inhibition of IL-6, and that MOX treatment at the CV12 acupoint is ineffective.

Acknowledgments

None.

References