Moxibustion treatment increases the survival rate of lung infection of bed-ridden patients from osteoporotic fracture of spine via regulating the inflammatory responses

Keywords
mice, pulmonary infection, moxibustion, bed-ridden senile, osteoporotic fracture

Abstract
Introduction
This study aimed to investigate the potential role of moxibustion (MOX) in the treatment of lung infection in bed-ridden patients from osteoporotic fracture of spine.

Material and methods
96 bed-ridden senile patients with pulmonary infection due to osteoporotic fracture of spine were enrolled.

Results
We found that the survival rate was higher for patients who received MOX. TNF-α, IL-1β, IL-6 and IL-18 were down-regulated while IL-10 was up-regulated by MOX. And MOX time-dependently increased the survival while reducing the bacteria left in infected mice.

Conclusions
Moxibustion significantly alleviated the inflammatory responses, thus leading to better survival rate of bed-ridden patients from osteoporotic fracture of spine.
Moxibustion treatment increases the survival rate of lung infection of bed-ridden patients from osteoporotic fracture of spine via regulating the inflammatory responses

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Abstract

This study aimed to investigate the potential role of moxibustion (MOX) in the treatment of lung infection in bed-ridden patients from osteoporotic fracture of spine. A total of 96 bed-ridden senile patients with pulmonary infection due to osteoporotic fracture of spine were grouped into a MOX (-) group and a MOX (+) group. Animal model was established as a SHAM group, a PRIMED group, a MOX 15’ group and a MOX 30’ group. For the patients study, we found that the survival rate was higher for patients who received MOX. Moreover, TNF-α, IL-1β, IL-6 and IL-18 were down-regulated while IL-10 was up-regulated by MOX. And MOX time-dependently increased the survival while reducing the bacteria left in infected mice. In conclusion, moxibustion significantly alleviated the inflammatory responses, thus leading to better survival rate of bed-ridden patients from osteoporotic fracture of spine.
Introduction

Respiratory infections induced by postoperative bed-riding, such as pneumonia and empyema could lead to mortality of lung cancer patients (1), and the mortality rate of pneumonia is high regardless of the fact that the postoperative mortality of lung resection is reduced (2).

Risk factors such as the age, smoking history, weak respiratory functions, invasive operations, intraoperative complications, or pathologic stages are known to be more likely to lead to postoperative respiratory infections (1, 3-5), or even prolonged postoperative hospital stay (6).

Moxa, a traditional Chinese herb which is minced before being burned in moxibustion, has been found to affect neuroendocrine immune functions and induce the production of heat shock proteins to activate the process of pain reduction and self-healing and (7, 8). Moreover, several reports have demonstrated that moxibustion could influence gene expression, thus being beneficial to autoimmune and inflammatory diseases such as ulcerative colitis (9, 10), Crohn's Disease (11), arthritis (12) and intestinal mucositis (13). Also, a previous study upon the recurrent respiratory tract infection in children suffering from cerebral palsy also indicated that moxibustion could exhibit a long-term clinical effect (14). And moxibustion at the acupoint of ST36 (Zusanli) was proved to activate the protective responses against HSV-1 infection in male BALB/c mice by activating production of cytokines including interleukin and TNF-α (15).

Therefore, the aim of this study is to further investigate the potential role of moxibustion treatment in the management of respiratory infection in bed-ridden patients who are suffering from osteoporotic fracture of spine.

Materials and Methods

The human study was approved by the institutional ethics committee and all experiments were performed in strict accordance with the 2013 version of the Declaration of Helsinki. Written informed consents have been obtained from all participants or their first-degree relatives before
the initiation of human study. We recruited a total of 96 bed ridden senile patients with pulmonary infection due to osteoporotic fracture of spine in this study. The patients were randomly distributed into a MOX (-) group and a MOX (+) group in which the patients received moxibustion treatment at points of BL13 (Feishu), RN8 (Shenque) and RN4 (Guanyuan). For the MOX treatment performed once each day for 12 weeks, 1.8 g moxa cones (Hanyi, Nanyang, Henan, CN) were placed burning on a 3.3 cm * 0.7 cm herbal cake (Hanyi, Nanyang, Henan, CN). The animal study was approved by the institutional animal ethics committee and all experiments were performed in strict accordance line with the 8th edition of Guide for the Care and Use of Laboratory Animal by National Research Council (US) Committee. 32 specific-pathogen-free (SPF) male 6-8 weeks of age C57BL/6 mice were randomly divided into 4 groups: a SHAM group a PRIMED group, a MOX 15’ group and a MOX 30’ group. The mice were established as acute infected animal by i.p. injection of 10 LD50 Staphylococcus aureus after 2 days of i.p. injection of 6% starch broth (Sigma-Aldrich, St. Louis, MI, US). At 48h after the moxibustion treatment, peripheral blood were collected from all mice, followed by were euthanasia by i.p. injection of 100mg/kg sodium pentobarbital. And the peritoneal fluids were immediately collected for subsequent bacterial colony formation analysis to evaluate the bacterial clearance, i.e., the number of Staphylococcus aureus colonies as colony-forming units (CFU).

Peripheral blood samples collected from patients and animal models were prepared to study the level of CRP, MPO, IL-18, IL-1β, IL-10, IL-6 and TNF-α using according assay kits following the instructions provided by the manufacture. The p-value no more than 0.05 was deemed as the level of statistical significance. All statistical analyses were carried out with GraphPad Prism software (GraphPad, Santa Barbara, CA, US).

Results

Human study

As indicated in Table 1, demographic and baseline data of all bed-ridden senile patients were collected and compared in the Table, and no significant differences were spotted in respect to the parameters. However, the survival curve up to 6 months showed that patients who received
Moxibustion treatment had a higher survival rate compared with patients in the MOX (-) group (Fig. 1A). Moreover, MOX (+) group had lower levels of TNF-α (Fig. 1B), IL-6 (Fig. 1C), IL-1β (Fig. 1E) and IL-18 (Fig. 1F) compared with the MOX (-) group, and the level of IL-10 (Fig. 1D) was higher in the MOX (+) group, indicating the potential association between MOX treatment and reduced inflammation responses in the patients.

**Animal study**

The survival analysis showed the lowest survival rate in the PRIMED group, while both moxibustion treatment for 15 min or 30 min elevated the suppressed survival rate (Fig. 1G). Meanwhile, moxibustion was found to substantially reduced the high bacteria left in the infected mice (Fig. 1H). Moreover, the production of TNF-α (Fig. 1I), IL-6 (Fig. 1J), IL-10 (Fig. 1K), IL-1β (Fig. 1L) and IL-18 (Fig. 1M) in the PRIMED group was evidently higher compared with those of the SHAM group, whereas treatment of moxibustion restored the expression of these factors in a time-dependent manner. Also, ELISA assay upon the serum level of CPR (Fig. 1N) and MPO (Fig. 1O) also presented evidently promoted CRP and MPO production in the PRIMED group, while moxibustion significantly reduced the CRP and MPO production in the MOX 15’ group and MOX 30’ group. Altogether, the above observations validated the time-dependent inhibitory effect of moxibustion treatment upon inflammatory responses in the bacterial infected mice.

**Discussion**

The over-activation of inflammasome NOD-like receptor protein 3 (NLRP3) induces the maturation and release of proinflammatory cytokines interleukin-1 beta (IL-1β) and IL-18 (16). And since IL-1β participates in the process of inflammatory injuries and damaging colonic barrier, the modulation of NLRP3 could inhibit inflammatory responses and reconstruct colonic mucosal immune homeostasis (17). Meanwhile, NLRP3 is also found to be regulated by the signaling pathway of nuclear factor kappa B (NF-κB), which participates in the inflammatory responses of inflammatory bowel disease (IBD) (18). The high concentration of extracellular adenosine triphosphate (ATP) produced at inflammatory sites could activate P2X7 receptor (P2X7R) (19), and P2X7R and ATP could collaboratively activate the Pannexin-1 channel to promote the transcription of NLRP3 mRNA (20). According to Tourkochristou et al., upon the activation of
NLRP3, apoptosis-associated speck-like protein (ASC) could trigger the activation of caspase-1, which helps to produce mature IL-1β and IL-18 and induce chronic intestinal inflammation (21-23). Also, it was found that the continuous between the harmful gut bacteria and the pathogen-associated molecular patterns will accelerate the activation of NLRP3 inflammasome, which results in severer inflammatory reactions by the subsequent upregulation of the downstream pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), interleukin-1β (IL-1β) and interleukin-18 (IL-18), thereby further aggravating the inflammatory reaction (24, 25). Accordingly, compared with the MOX (-) patient group, we found that moxibustion in the MOX (+) patient group not only suppressed the expression of NLRP3, but also inhibited expression of pro-inflammatory factors such as TNF-α, IL-1β, IL-6 and IL-18 while promoting the level of IL-10. And similar results were obtained from the animal models primed with bacteria and subsequently received moxibustion treatment.

In this study, we not only studied the effect of moxibustion against lung infection, but also observed whether the moxibustion duration potentially influences the effect of moxibustion. Therefore, we treated the bacteria infected mice models for 15 min and 30 min respectively, and we accordingly found that the 30-min treatment of moxibustion exerted more beneficial effect against bacterial infection compared with the 15-min treatment of moxibustion, while both the 15-min moxibustion treatment and 30-min moxibustion treatment exhibited significant beneficial effect compared with the untreated mice.

Conclusions

By observing the lung-infected patients and mice models, we found that the MOX treatment could significantly alleviate the inflammatory responses, thus leading to better survival rate of bed-ridden patients from osteoporotic fracture of spine.

Declarations

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.
Competing interests

The authors declare that they have no competing interests.

Figure legend

Figure 1

A: The survival rate of the MOX (+) patient group was higher than that of the MOX (+) patient group;

B: The level of TNF-α was reduced in the MOX (+) patient group compared with that in the MOX (-) patient group;

C: The production of IL-6 was suppressed in the MOX (+) patient group compared with that in the MOX (-) patient group;

D: Compared with the MOX (-) patient group, the IL-10 level was higher in the MOX (+) patient group;

E: Compared with the MOX (-) patient group, the IL-1β level in the MOX (+) patient group was significantly lower;

F: Compared with the MOX (-) patient group, the IL-18 level in the MOX (+) patient group was evidently reduced;

G: The survival curves of difference mice groups;

H: The bacterial left of difference mice groups.

I: ELISA analysis of TNF-α production in different mice groups;

J: ELISA analysis of IL-6 production in different mice groups;

K: ELISA analysis of IL-10 level in different mice groups;

L: ELISA analysis of IL-1β level in different mice groups;

M: ELISA analysis of IL-18 level in different mice groups;
N: ELISA analysis of CPR production in different mice groups;

O: ELISA analysis of MPO production in different mice groups.

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Table 1. Basic characteristics of the recruited bed ridden senile patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>MOX(-)(N=48)</th>
<th>MOX(+)(N=48)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>56.8 ± 3.2</td>
<td>60.3 ± 6.2</td>
<td>0.243</td>
</tr>
<tr>
<td>Sex, male (%)</td>
<td>32 (66.7)</td>
<td>28 (62.5)</td>
<td>0.298</td>
</tr>
<tr>
<td>Baseline date</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocyte (/μl)</td>
<td>1425 ± 415</td>
<td>1374 ± 447</td>
<td>0.726</td>
</tr>
<tr>
<td>LDH (U/l)</td>
<td>482 ± 112</td>
<td>502 ± 89</td>
<td>0.296</td>
</tr>
<tr>
<td>KL-6 (U/l)</td>
<td>712 ± 154</td>
<td>692 ± 174</td>
<td>0.403</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>0.68 ± 0.23</td>
<td>0.63 ± 0.18</td>
<td>0.657</td>
</tr>
<tr>
<td>CK (U/l)</td>
<td>1835 ± 884</td>
<td>1953 ± 665</td>
<td>0.338</td>
</tr>
<tr>
<td>Ferritin (ng/ml)</td>
<td>235 ± 35</td>
<td>264 ± 41</td>
<td>0.708</td>
</tr>
</tbody>
</table>
Figure 1

A: The survival rate of the MOX (+) patient group was higher than that of the MOX (+) patient group;

B: The level of TNF-α was reduced in the MOX (+) patient group compared with that in the MOX (-) patient group;

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