Journal of Ethnopharmacology 155 (2014) 1483–1491

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Research Paper

The total alkaloids of *Aconitum tanguticum* protect against lipopolysaccharide-induced acute lung injury in rats

Guotai Wu [a,1], Lidong Du [b,1], Lei Zhao [b], Ruofeng Shang [a], Dongling Liu [b], Qi Jing [b], Jianping Liang [a,*], Yuan Ren [b,**]

[a] Key Laboratory of New Animal Drug Project of Gansu Province, Key Laboratory of Veterinary Pharmaceutics Discovery, Ministry of Agriculture, Lanzhou Institute of Animal Science and Veterinary Pharmaceutics Science, Chinese Academy of Agricultural Sciences, Lanzhou, Gansu 730050, PR China
[b] Key Laboratory of Pharmacology and Toxicology of Traditional Chinese Medicine of Gansu Province, Gansu University of Traditional Chinese Medicine, 35 Dingxi Road, Chengguan District, Lanzhou, Gansu 730000, PR China

ARTICLE INFO

Article history:
Received 4 June 2014
Received in revised form
5 July 2014
Accepted 18 July 2014
Available online 4 August 2014

Chemical compounds studied in this article:
6-benzoylheteratisine (PubChem CID: 5487064)
Heteratisine (PubChem CID: 441735)
Atisine (PubChem CID: 6426913)
Aconitine (PubChem CID: 245005)
Hypaconitine (PubChem CID: 23337)
Benzoylaconitine (PubChem CID: 49868330)
Benzoylhypacoitine (PubChem CID: 3047329)
Benzoylmesaconine (PubChem CID: 3036967)
Hordenine (PubChem CID: 68313)
Heterophylline (PubChem CID: 251575)

Keywords:
Total alkaloids of *Aconitum tanguticum*
Acute lung injury
TNF-α
IL-6
IL-1β
NF-κ B

ABSTRACT

Ethnopharmacological relevance: *Aconitum tanguticum* has been widely used as a remedy for infectious diseases in traditional Tibetan medicine in China. The total alkaloids of *Aconitum tanguticum* (TAA) are the main active components of *Aconitum tanguticum* and have been demonstrated to be effective in suppressing inflammation. Our aim was to investigate the protective effects of TAA on acute lung injury (ALI) induced by lipopolysaccharide (LPS) in rats.

Materials and methods: TAA was extracted in 95% ethanol and purified in chloroform. After vacuum drying, the TAA powder was dissolved in dimethyl sulfoxide. Adult male Sprague-Dawley rats were randomly divided into six groups. Rats were given dexamethasone (DXM, 4 mg/kg) or TAA (60 mg/kg, 30 mg/kg) before LPS injection. The $PaO_2$and PaO_2/FiO_2 values, lung wet/dry (W/D) weight ratio and histological changes in lung tissue were measured. The cell counts, protein concentration, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and interleukin-1β (IL-1β) in bronchoalveolar lavage fluid (BALF), and myeloperoxidase (MPO) activity in lung tissue were determined at 6, 12 or 24 h after LPS treatment. In addition, the NF-κ B activation in lung tissue was analyzed by western blot.

Results: In ALI rats, TAA significantly reduced the lung W/D ratio and increased the value of PaO_2 or PaO_2/FiO_2 at 6, 12 or 24 h after LPS challenge. TAA also reduced the total protein concentration and the number of total cells, neutrophils or lymphocytes in BALF. In addition, TAA decreased MPO activity in the lung and attenuated histological changes in the lung. Furthermore, TAA inhibited the concentration of TNF-α, IL-6 and IL-1β in BALF at 6, 12 or 24 h after LPS treatment. Further study demonstrated that TAA significantly inhibited NF-κ B activation in lung tissue.

Conclusions: The current study proved that TAA exhibited a potent protective effect on LPS-induced ALI in rats through its anti-inflammatory activity.

1. Introduction

Aconitum tanguticum (*Maxim.*) Stapf has long been used for the treatment of infectious fever, pneumonia, enteritis, hepatitis, prevalent influenza and common inflammation. It is a traditional Tibetan medicine in China and is widely distributed in the south-eastern Qinghai-Tibet Plateau, southern Gansu, western Sichuan and northern Yunnan provinces (Zhang et al., 2012a). Phytochemical studies found that *Aconitum tanguticum* contains 22 types of alkaloids, three types of flavonoids, 35 types of essential oils and some polysaccharides (Luo et al., 2012a). The main ingredients of

Abbreviations: TAA, total alkaloids of *Aconitum tanguticum*; AC, aconitine; HDTAA, high dose of TAA; LDTAA, low dose of TAA; ALI, acute lung injury; LPS, lipopolysaccharide; H–E, hematoxylin–eosin; BALF, bronchoalveolar lavage fluid; TNF-α, tumor necrosis factor-alpha; IL-6, interleukin-6; IL-1β, interleukin-1β; W/D, lung wet/dry ratio; MPO, myeloperoxidase; NF-κ B, nuclear factor-kappa B; DMSO, dimethyl sulfoxide; PBS, phosphate buffer solution; DXM, dexamethasone; ELISA, enzyme-linked immunosorbent assay; TCM, traditional Chinese medicine

* Correspondence to: Lanzhou Institute of Husbandry and Pharmaceutical Sciences, CAAS, 335 Jiangouyan Road, Qilihe District, Lanzhou 730050, PR China. Tel./fax: +86 931 2115287.
** Corresponding author. Tel./fax: +86 931 8762653.
E-mail addresses: tcm888gs@163.com (J. Liang), 1018830451@qq.com (Y. Ren).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.jep.2014.07.041
0378-8741/

Aconitum tanguticum were mono- or dimeric diterpenoid alkaloids (Pelletier and Joshi, 1991; Wang et al., 2002, 2005; Li et al., 2004). Pharmacological studies showed that *Aconitum tanguticum* exhibited various biological effects such as antibiosis, antivirus and anti-inflammation (Zhang et al., 2009, 2010). Diterpenoid alkaloids are considered to be the main composition of anti-inflammatory and analgesic (Ha and Li, 2010; Luo et al., 2012b).

Acute lung injury (ALI) is a severe inflammatory disease characterized by neutrophilic infiltration, pulmonary edema, and hypoxemia (Dushianthan et al., 2011; Vadasz and Sznajder, 2011). ALI is associated with the development of multiple organ dysfunction syndromes, which plays a pivotal role in the death of patients with shock, sepsis, and multiple transfusions (Lee and Downey, 2001; Gu and Song, 2012). ALI is characterized by the activation of multiple inflammatory cells and production of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) (Shinbori et al., 2004; Wright et al., 2004), and proteases (Matsuda et al., 2011). The major cytokine is TNF-α, which induces pulmonary endothelial cell activation, migration of leukocytes, neutrophil degranulation and capillary leakage, alveolar cell perfusion and oxygen exchange (Ma et al., 2009). Thus, inhibition of these factors may potentially be a therapeutic target in lung injury. Recently, natural products of plant origin with immense ethnopharmacological importance have been given top priority as treatments for inflammatory diseases (Talhouk et al., 2007; Li et al., 2010). In particular, the understanding of the anti-inflammatory effects of alkaloids has made great progress (Meng et al., 2003). Cyclooxygenase-2, TNF-α, IL-1 and prostaglandin E_2 (PGE_2) can be inhibited by TAA (purity > 75%) in arthritic rats (Zeng et al., 2009). In addition, auricular edema, permeability of celiac blood capillaries in mice and paw swelling in rats were inhibited by the total alkaloids of *Aconitum naviculare*, which is often used instead of *Aconitum tanguticum* in Tibetan pharmaceutical applications due to the similar main chemical compositions (Qu, 2009).

Although *Aconitum tanguticum* is used to treat diseases related to inflammation in the Tibetan folk of China and TAA has exhibited a therapeutic effect on inflammation, research has mainly led to partial findings. Whether TAA has protective effect on ALI and what the underlying mechanisms of TAA action are have never been investigated. In the present study, we studied the effects of TAA on an experimental model of ALI induced by LPS and tried to clarify the mechanism involved. Our results might provide a pharmacological basis for its folkloric use in the treatment of ALI.

2. Materials and methods

2.1. Material and chemicals

2.1.1. Preparation of TAA

Aconitum tanguticum was obtained from Qinghai Jinhe Tibetan Medicine Pharmaceutical Co., Ltd. (Qinghai, China), and identified by Dr. Feng lin Liu, Gansu University of Traditional Chinese Medicine(TCM). A voucher specimen (reference number 130812-01) has been deposited in the herbarium stock room of the College of Pharmacy, Gansu University of TCM, Lanzhou, China. The extraction and purification of TAA were carried out according to previous reports (Wang et al., 2002). Briefly, the whole *Aconitum tanguticum* plant (20 kg) was powdered and immersed into 200 L 95% ethanol for 24 h, then boiled under reflux for 2.0 h twice. The solution was filtered and concentrated. The concentrate was extracted with 2% hydrochloric acid solution several times, and Na_2CO_3 was added to the filter liquor to adjust the pH to 9–10. The solution was allowed to sit for 24 h. After precipitation, the alkaline solution was extracted by chloroform, and 40.8 g of gray powder was obtained.

2.1.2. Quality control of TAA

TAA (50 mg) was precisely weighed and dissolved in 5% potassium hydroxide in methanol (100 ml), then boiled under reflux for 1.0 h until the methanol had evaporated. The residue was adjusted to pH 3–4 with 0.5 mol/L sulfuric acid, then transferred to a separator funnel. The residue was extracted twice with 10 ml ether, then the ether liquid was combined, and the ether was removed. The residual powder was dissolved in 10 ml methanol and allowed to sit for 15 min. The test sample was finally filtered through a 0.45 μm micro-porous membrane before analysis. An Aichrom C18 ($4.6 \times 150\ mm^2$, 5 μm) chromatographic column was employed. The column temperature was kept at 35 °C, the mobile phase was methanol–acetic acid–isopropanol–0.05 mol/L aqueous potassium dihydrogen phosphate (67:4:4:173), the flow rate was l.0 ml/min, the detection of wavelength was 230 nm, and the injection volume was 10 μl. Three replicates of each sample were assessed. Approximately 5 mg of AC was precisely weighed and the general procedure was carried out. Benzoic acid was used as the internal standard (Chen et al., 2009).

2.1.3. Components and toxicity of TAA

A study from another group showed the content for each main alkaloid in TAA was specific, such as AC, hypAC, mesacomhne, atisine, 6-acetylheteratisine, and 6-benzoylheteratisine and so on. The results of these studies will be reported in another paper. The acute toxicity of TAA was known from our previous studies and preliminary experiments when administered to mice via intraperitoneal injection, LD_{50} = 338.80 mg/kg, 95% confidence limit 315.87–363.39 mg/kg.

2.2. Rats

Male Sprague Dawley (SD) rats (6 weeks old, 200–220 g) were purchased from the Center of Experimental Animal of Gansu University of TCM (Lanzhou, Gansu, China). Rats were housed in groups of 5 under standard conditions (12 h light/dark cycle, temperature 25 ± 0.5 °C, and relative humidity 55 ± 5%) with standard food and water ad libitum and allowed to adapt to experiment environment for 7 days. All of the procedures for the animal study were approved by the Institutional Animal Care and Use Committee of Gansu University of TCM (Lanzhou, Gansu, China). All experiments were performed in accordance with the guidelines for the care and use of laboratory animals of the National Institute of Health.

2.3. Experimental design

Male SD rats were assigned to six groups and treated as follows: (1) normal control group (accession to a small amount of dimethyl sulfoxide (DMSO, Sigma, St. Louis, MO) in saline, the final concentration of DMSO was 0.05%, v/v), (2) LPS alone group (LPS 5 mg/kg), (3) TAA alone group (TAA 60 mg/kg, the final concentration of DMSO was 0.05%, v/v), (4) LPS + dexamethasone (LPS + DXM, 4 mg/kg) treatment group, (5) LPS + HDTAA (60 mg/kg, a high dose of TAA) treatment group, and (6) LPS + LDTAA (30 mg/kg, a low dose of TAA) treatment group. The doses of these drugs and LPS were based on our previous studies and preliminary experiments. The dose of 1/20 LD_{50} (LD_{50} = 338.80 mg/kg) could not obviously protect against LPS-induced ALI in rats, so close to 1/10 LD_{50} and 1/5 LD_{50} as low dose and high dose, respectively, were selected in the present study. The TAA powder was dissolved in a small amount of DMSO, and was diluted to the required concentration in normal saline (the final