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The inhibitory effect of Aconiti Sinomontani Radix extracts on the proliferation and migration of human synovial fibroblast cell line SW982

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Celastrol (PubChem CID: 122724)
DMSO (PubChem CID: 679)
Penicillin (PubChem CID: 5904)
Streptomycin (PubChem CID: 19649)
l-glutamine (PubChem CID: 5961)
acetonitrile (PubChem CID: 6342)
formic acid (PubChem CID: 284)

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ABSTRACT

Ethnopharmacological relevance: Aconiti Sinomontani Radix is frequently used in the treatment of Bi syndrome in traditional Chinese medicine. Several reports indicate that Aconiti Sinomontani Radix has therapeutic effects for rheumatoid arthritis (RA). However, the cellular mode of action is still unclear. To investigate the effect of alkaloid extracts of Aconiti Sinomontani Radix on proliferation and migration of human synovial sarcoma SW982 cells as well as the molecular mechanism underlying.

Materials and methods: SW982 cells were examined for proliferation by a 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) method. Wound scratch assays were performed to assess the migrated rate of SW982 cells. Quantitative real-time PCR was used to measure the mRNA expression levels of Wnt5a, Runx2, MMP3, and Bmp2. Western blotting was used to measure the phosphorylated levels of JNK and NF-κB as well as the expression of MMP3.

Results: The alkaloid extract from Aconiti Sinomontani Radix (MQA) and MQB, which removed lappaconitine from MQA significantly inhibited the proliferation of SW982 in a dose-dependent manner. The proliferation inhibitory effect of MQB was more potent. Incubation with 10 μg/ml MQB for 12, 24, and 36 h inhibited the migration of SW982 cells by 83%, 58%, and 42%, respectively. Treatment with different concentrations of MQB for 24 h inhibited mRNA expression of Wnt5a, Runx2, and MMP3, but Bmp2 mRNA expression was elevated by MQB. Further, MQB inhibited phosphorylation of JNK and NF-κB p65 as well as MMP3 expression by Western blotting analysis.

Conclusion: The results showed that MQB inhibited proliferation and migration of SW982 cells possibly through suppressing Wnt5a-mediated JNK and NF-κB pathways. These results indicated that MQB might be an active extract of Aconiti Sinomontani Radix for targeting fibroblast-like synoviocytes (FLS) and be potential for RA therapy.

1. Introduction

Aconiti Sinomontani Radix, also known as “Ma-Bu-Qi”, is the root of Aconitum sinomontanum Nakai, which has been used as a traditional Chinese medicine as well as a Miao medicine for several thousands of years for treating Bi syndrome (Chen et al., 1980; Yuan and Wang, 2012; Singhuber et al., 2009). In China, Rheumatoid arthritis (RA) is believed as Bi syndrome induced by wind, cold and dampness (Pan et al., 2017). Aconiti Sinomontani Radix has the ability of dispelling cold and removing dampness, and is used to cure arthralgia and lumbocrural pain in RA. The major components include lappaconitine, which is reported to have the anticancer and antiarrhythmic activities (Bryzgalov et al., 2013). Recently, it was reported that Aconiti Sinomontani Radix showed therapeutic effect for RA patients (Li and Liu, 2000; Pan, 2007). However, the cellular action of Aconiti Sinomontani Radix treating RA is still unknown.

RA, a chronic systemic auto-immune disease, is characterized by joint synovitis. Abnormal proliferation and migration of fibroblast-like synoviocytes (FLS) play key roles in RA pathogenesis (Asif et al., 2017; Choe et al., 2016). RA FLS activation and migration increase activation of proinflammatory pathways and secretion of matrix-destructive enzymes such as MMPs, thereafter promoting joint destruction. In addition to proinflammatory pathways, multiple signaling pathways are activated during RA development. Evidence show that Wnt signaling

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pathway is involved in the RA FLS pathological process (Miao et al., 2013; Malysheva et al., 2016). Wnt is a secreted protein crucial in cell proliferation, differentiation, cell morphology, adhesion and migration. Intracellular signaling of the Wnt pathway diversifies into canonical β-catenin and noncanonical Wnt pathway that includes jun N-terminal kinase (JNK) and NF-κB pathways (Liu et al., 2017; Katoh and Katoh, 2007).

In the present study, we aimed to investigate the inhibitory effect of Aconiti Sinomontani Radix extracts on FLS proliferation and migration by using a human synovial fibroblast cell linage, SW982 cells. Simultaneously, the mechanism of active Aconiti Sinomontani Radix extract underlying was investigated.

2. Materials and methods

2.1. Preparation of Aconiti Sinomontani Radix extracts

Aconiti Sinomontani Radix (also known as tall monkshood root) was the root of Aconitum sinomontanum Nakai. Aconiti Sinomontani Radix was identified and a voucher (no. NZZ20150507) was lodged in a specific voucher specimen laboratory. 4.3 kg of Aconiti Sinomontani Radix was cut into inch segments and divided into two batches for decocting with 20 L water for 3 times, 2 h once, then the decoction were combined and concentrated to about 4 L on reduced pressure. The concentrated solution was added with 95% ethanol 12 L for precipitating overnight, then the alcohol solution was separated and concentrated on reduced pressure to no alcohol flavor (about 1.6 L). The extract in concentration was calculated to be 530 g (12.3%). The extract concentration was adjusted pH to 9 ~ 10 with sodium hydroxide, followed by partition with equal volume of dichloromethane for 5 times to get the alkaloids (MQA) 79.4 g (1.9%).

500 mg alkaloid extract (MQA) was dissolved into 10 ml methanol and separated on pre-HPLC with 75% methanol/water (0.01% ammonia) after filtration. The peak of lappaconitine at 11.1 min was collected. The baseline was the residual alkaloids (MQB) that removed most lappaconitine.

2.2. HPLC analysis

High-performance liquid chromatography (HPLC) was performed with Waters e2695 HPLC with UV detector, cooling autosampler, and column oven enabling control of the temperature of the analytical column. Data were collected and processed by Qualitative software. Injections (10 μl) of lappaconitine, MQA and MQB were made using a 10-ml loop operated in partial-loop mode. HPLC separation was achieved on a Xtimate-C18 column (4.6 * 150 mm, 5 μm) (Waters). The mobile phase was an isocratic elution prepared from 75% methanol (component A) and water (0.01% ammonia) (component B). The total run time was held for 20 min. The mobile phase flow rate was 1 ml/min. UV detection of the samples was performed at 254 nm.

2.3. SW982 cell culture

Human synovial sarcoma SW982 cells were obtained from the Type Culture Collection of Chinese Academy of Sciences (Shanghai, China) and cultured routinely with Dulbecco's Modified Eagle's Medium (DMEM) (Gibco, USA)) supplemented with 10% fetal bovine serum (FBS) (Gibco, USA), penicillin (100 U/ml) and streptomycin (100 μg/ml) and maintained at 37 °C with 5% CO_2 in a humidified incubator. Cells were seeded into plates 24 h prior to treatments at approximately 80% confluence and exposed to different doses of the extracts from Aconiti Sinomontani Radix.

2.4. MTT assay

The SW982 cells were seeded into 96-well plates at 5 × 10^3 /ml density. After overnight incubation, the extracts from Aconiti Sinomontani Radix were added to the plates. Following incubation for 24 h, cell growth was measured by 3-(4, 5-dimethylthiazol2-yl)-2,5-diphenyltetrazolium bromide (MTT) with a plate reader (Tecan, Switzerland) as previously described (van Meerloo et al., 2011). The percentage of inhibition was calculated as follows:

$$\text{Inhibitory rate}(\%) = [\text{A492 (control)} - \text{A492 (sample)}]/\text{A492 (control)} \times 100\%$$

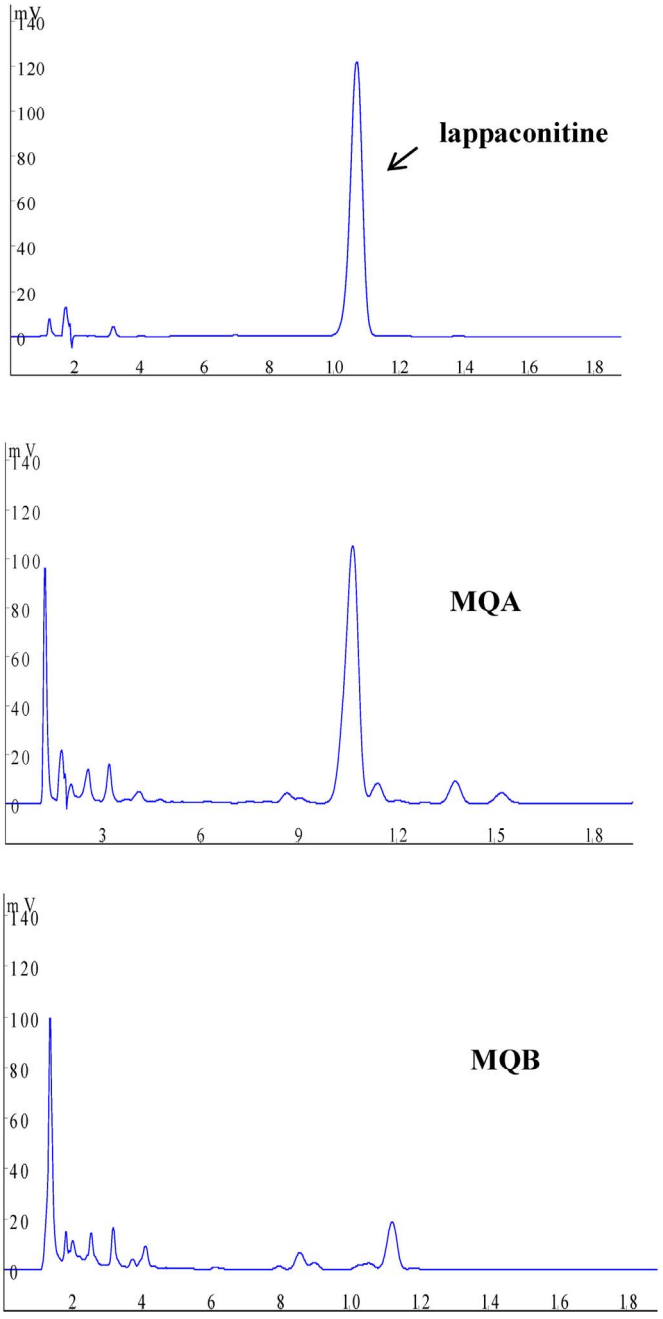

Fig. 1. HPLC analysis of MQA and MQB. HPLC conditions: column 4.6*150 mm, 5 μm, 1.8 μm particle size; mobile phase: component A (75% methanol) and component B (0.01% ammonia). Flow rate: 1 ml/min.

2.5. Wound scratch assays

Cells were seeded at 2 × 10^5 cells/well in 24-well plates and grown until 80% confluence. After serum starvation for 24 h, monolayers were