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The synergistic anti-inflammatory effect of the combination of sodium ferulate and oxymatrine and its modulation on inflammation-associated mediators in RAW 264.7 cells

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ABSTRACT

Ethnopharmacological relevance: The combination of Radix *Angelicae sinensis* (Oliv.) Diels and Radix *Sophora flavescens* Ait. was extensively used in traditional Chinese medicine to treat inflammatory diseases, such as acne, heart disease, and hepatitis. Sodium ferulate (SF) and oxymatrine (OMT) were effective component of Radix *Angelicae sinensis* (Oliv.) Diels and Radix *Sophora flavescens* Ait., respectively.
Aim of the study: In this study, we investigated the synergistic anti-inflammatory effect of the combination of SF and OMT, and its modulation on inflammation-associated mediators in RAW 264.7 cells.
Materials and methods: In vivo, the anti-inflammatory effects of the combination of SF and OMT were evaluated with the xylene-induced mouse ear edema model and the carrageenan-induced rat paw edema model. *In vitro*, chemokines and cytokines mRNA expressions in lipopolysaccharide (LPS)-activated RAW 264.7 cells were determined by real-time PCR (RT-PCR) microarray analysis. The levels of interleukin-11 (IL-11), C-reactive protein (CRP) and interferon-γ (INF-γ) in the supernatant of LPS-stimulated RAW 264.7 cells were measured by enzyme-linked immune-sorbent assay (ELISA).
Results: The combination of SF and OMT could significantly inhibit the edema in the xylene-induced mouse ear edema and carrageenan-induced rat paw edema, but no effect was found when each drug was used alone according to above doses. The combination exhibited a better effect in down-regulating mRNA expressions of inflammation-associated mediators in LPS-stimulated RAW 264.7 cells than SF or OMT alone. The ELISA results showed that the combination synergistically inhibited LPS-induced IL-11, CRP and INF-γ production in a dose-dependent manner.
Conclusion: The combination of SF and OMT showed synergistic anti-inflammatory effect, and the activity was probably related to its modulation on inflammation-associated mediators, especially IL-11, CRP and INF-γ.

1. Introduction

Inflammation was the important pathological process of many diseases development, and the inflammatory cellular response and mediators release were the one of crucial part of the defence mechanism against the pathogenic factor (Tao et al., 2008). Also inflammation could produce the harmful effect to the host through the multiple levels of biochemical, pharmacological, and molecular controls (Park et al., 2005). It was well known that the existing non-steroidal anti-inflammatory drugs (NSAID's) were used to treat inflammation diseases, but NSAID's could usually cause undesired and serious side effects, especially gastrointestinal injure. Therefore, development of new and more powerful drugs was still needed.

Angelica sinensis and *Sophora flavescens* was the plant in a family of *Apiaceae* and *Leguminosae*, respectively. The combination of Radix *Angelicae sinensis* (Oliv.) Diels and Radix *Sophora flavescens* Ait. was extensively used in traditional Chinese medicine to treat inflammatory diseases, such as acne, heart disease, and hepatitis.

Abbreviations: OMT, oxymatrine; SF, sodium ferulate; SFDA, State Food and Drugs Administration of China; LPS, lipopolysaccharide; IL-11, interleukin-11; CRP, C-reactive protein; INF-γ, interferon-γ; ELISA, enzyme-linked immune-sorbent assay; SRB, sulforhodamine B; FBS, fetal bovine serum; DEX, dexamethasone sodium phosphate injection.

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For example, Danggui Kushen Wan composed of *Angelica sinensis* and *Sophora flavescens* had been used to treat pimple and acne. In our previous study, we found that the effect of the combination was better than the single herbal medicine, so we presumed that there were some effective compounds in the two herbs, which maybe have a combinative synergistic effect. Sodium ferulate (SF), an effective component of Radix *Angelicae sinensis* (Oliv.) Diels, had been reported with the effects of inhibiting platelet aggregation, increasing coronary blood flow, anti-oxidation, and anti-inflammation (Wang and Ou-Yang, 2005; Wang et al., 2007; Liu et al., 2008, 2010). Oxymatrine (OMT), an active component extracted from traditional Chinese herb Radix *Sophora flavescens* Ait., had been reported to be effective in the clinical treatment of viral hepatitis, bronchial asthma and ischemia reperfusion injury in China, because of its anti-oxidative, anti-inflammatory, and anti-apoptotic effects (Zheng et al., 2005; Fan et al., 2008; Zhao et al., 2008;). In another one of our study (not yet published), we evaluated the anti-inflammatory effects of the OMT combined the different parts extracts of *Angelicae sinensis*, and found that the anti-inflammatory effect of combination of SF and OMT was the best.

In addition, the remarkable analgesic effect of the combination of SF and OMT had been found and reported in our previous study (Liu et al., 2010). The combination was being developed into a new drug to treat acute soft tissue injury, and the clinical trial application had been submitted to the State Food and Drugs Administration of China (SFDA). This study reported the anti-inflammatory effects of the combination and its modulation on inflammation-associated mediators.

In vivo, the xylene-induced mouse ear edema model and carrageenan-induced rat paw edema model were important models commonly used to evaluate anti-inflammatory activity of a new drug. *In vitro*, lipopolysaccharide (LPS)-stimulated RAW 264.7 cells could produce a variety of inflammatory mediators, including interleukin, interferon-γ (INF-γ), tumor necrosis factor-α (TNF-α) and so on (Feng et al., 2010), and the inflammatory mediator levels in the supernatant of LPS-stimulated RAW 264.7 cells were reliable signals reflecting the grade of inflammation. Various chemicals of plant origin showed anti-inflammatory effects by modulating the levels of inflammation-associated gene and inhibiting the releases of inflammation-associated mediators. Hence, LPS-stimulated RAW 264.7 cells were usually used to explore the mechanism of the anti-inflammatory drugs.

In this study, we evaluated the anti-inflammatory effects of the combination of SF and OMT by the xylene-induced mouse ear edema model and carrageenan-induced rat paw edema model, and measured the level of interleukin-11 (IL-11), C-reactive protein (CRP) and interferon-γ (INF-γ) in the paw homogenate. *In vitro*, the mRNA expressions of inflammatory cytokines and chemokines were analyzed by real-time PCR (RT-PCR) microarray analysis in the LPS-induced RAW 264.7 cells, and the production of IL-11, CRP and INF-γ were measured further by enzyme-linked immune-sorbent assay (ELISA).

2. Materials and methods

2.1. Materials

SF (molecular formula: $C_{10}H_9NaO_4{\cdot}2H_2O$; molecular weight: 252.20; CAS: 24276-84-4; HPLC purity > 99%), OMT (molecular formula: $C_{15}H_{24}N_2O_2{\cdot}H_2O$; molecular weight: 282.38; CAS: 16837-52-8; HPLC purity > 98%) were provided by Beijing SL Pharmaceutical Co. Ltd (Beijing, China). Trichloroacetic acid and sulforhodamine (SRB) were obtained from Acros Organics (New Jersey, USA). LPS was purchased from Sigma–Aldrich (St. Louis, MO, USA). RPMI-1640 was purchased from GIBCO Invitrogen Corporation (Grand Island, NY, USA). Fetal bovine serum (FBS) was obtained from NQBB International Biological Corporation (Hong Kong, China). The ELISA kits for determination of CRP, IL-11and INT-γ was purchased from Groundwork Biotechnology Diagnosticate Ltd (San Diego, CA). The murine macrophage cell line (RAW 264.7 cells) was purchased from American Type Culture Collection (Rockvill. Maryland, USA).

2.2. Animals

Kunming mice of both sexes weighing 18–22 g (four weeks old) and Sprague-Dawley rats of both sexes weighing 150–200 g (two months old) were supplied by Shandong Luye Pharmaceutical Co., Ltd (Quality Certificated Number: Lu 20090013). These animals were approved models by the Experimental Animal Management Center of Shandong Province, China. The animals were kept under standard conditions (temperature: $23 \pm 2\,^{\circ}C$, humidity: $55 \pm 5\%$, 12 h light/dark cycle) and acclimatized to the laboratory environment for 3–7 days prior to the experiments. Only water but no food was supplied to the animals within 12 h before the experiment.

All experimental procedures carried out in this study were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals, and approved by the Experimental Animal Management Center of Shandong Province.

2.3. The xylene-induced ear edema of mice

According to the paper (Hosseinzadeh et al., 2003), the xylene was typically applied on the right ear to duplicate the ear edema model. Seventy Kunming mice of both sexes weighing 18–22 g were divided into 7 groups, including model control group (saline), dexamethasone (DEX, 1 mg/kg), SF (15.4 mg/kg), OMT (34.6 mg/kg), SF + OMT (7.7 + 17.3 mg/kg), SF + OMT (15.4 + 34.6 mg/kg) and SF + OMT (30.9 + 69.1 mg/kg). Optimal ratio (molar ratio = 1:2) of SF and OMT was obtained by the pharmaceutical and pharmacological tests. When the molar ratio of SF and OMT was 1:2, the solution system was the most stable with pH vale of 7.0, and the pharmacological activity was also the best. After 30 min of the treatment, xylene (20 μL) was spread on both sides of the right ear of the mouse. After 30 min of xylene application, the animals were killed by cervical dislocation, and a cylindrical plug (diameter, 7 mm) was excised from each of ears. The inflammatory activity (edema index) was evaluated by the weight difference of the right and left ear disk in the same mouse.

2.4. Carrageenan-induced paw edema of rat

To measure the anti-inflammatory activity, the carrageenan-induced paw edema assay was used according to the previously described procedures (Winter et al., 1962) with slight modification. Seventy Sprague-Dawley rats of both sexes weighing 150–200 g were divided into 7 groups, including model control group (saline), DEX (1 mg/kg), SF (15.4 mg/kg), OMT (34.6 mg/kg), SF + OMT (7.7 + 17.3 mg/kg), SF + OMT (15.4 + 34.6 mg/kg) and SF + OMT (30.9 + 69.1 mg/kg). The combination of SF and OMT molar ratio was 1:2. The normal right hind paw volume of each animal was first determined using a plethysmometer before establishing the model. Then, each rat was treated with 0.1 mL of l% carrageenan by subcutaneously injection into the right hind paw. The drugs were diluted with saline and administered intragastrically after 2 h of the carrageenan injection. The paw volume was measured again after 4 h of injecting carrageenan (our previous study showed that the notable edema inhibition was exhibited at 4 h after carrageenan injection, data not showed). The swelling volume was calculated as