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Methylallyl sulfone attenuates inflammation, oxidative stress and lung injury induced by cigarette smoke extract in mice and RAW264.7 cells

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ABSTRACT

In this study, we revealed that methylallyl sulfone (AMSO2), the metabolite of active organosulfur compounds, had anti-inflammatory and antioxidant effect in a cigarette smoke extract (CSE)-induced lung injury model. Firstly, histological analysis showed that the CSE group exhibited lung injury compared with the control, which was alleviated by AMSO2. Secondly, we estimated its anti-inflammatory capacity. The results indicated that pretreatment with AMSO2 significantly decreased CSE-elevated tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) in serum. Thirdly, AMSO2 also showed antioxidant properties through enhancing activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) as well as reducing the level of malondialdehyde (MDA) and myeloperoxidase (MPO). Finally, we elucidated that AMSO2 alleviated inflammation and oxidative stress probably via suppressing ERK/p38 MAPK and inhibiting NF-κB expressions. In conclusion, we proposed that AMSO2 protected against the development of CSE-induced lung injury by reducing inflammatory cytokine levels and augmenting antioxidant activity via ERK/p38 MAPK and NF-κB pathways.

1. Introduction

Chronic obstructive pulmonary disease (COPD) is one of major causes of chronic morbidity and mortality in the world. Until 2015, 2.4% (about 174.5 million) of the global population has been suffered by COPD [1]. Emphysema, characterized by irreversible alveolar wall destruction, is older term used for different types of COPD. As the main pathological feature, it is the hotspot for researchers to study the mechanism of COPD.

Cigarette smoke (CS) contains > 4000 different chemical compounds including free radicals, toxins, and electrophiles [2]. It has been proposed that CS induced an abnormal inflammatory response in the small airways and alveoli. In order to against CS, bronchial epithelium overproduces various cytokines, chemokines and adhesion molecules to modulate other elements of the airway wall land immune cells [3]. Therefore, more and more scientists studied the molecular mechanisms for CS-induced alterations in bronchial and lung cells, which offer many clues into COPD pathogenesis and treatment [4,5]. In this study, we established animal model of lung injury by intraperitoneal injection of CSE.

Garlic (*Allium sativum*) has been used in medicines and cooking by many ancient countries for thousands of years. After years of research, scientists suggest that garlic derivatives, mainly water-soluble and oil-soluble organosulfur compounds, have many biological properties, including anti-inflammatory, antifungal, antimicrobial, antitumor potential and stimulating immune system [6]. It has been reported that the compounds, such as *S*-allylmercaptocysteine (SAMC), *S*-allylcysteine (SAC), diallyl disulfide (DADS) and diallyl trisulfide (DATS), showed antioxidant and anti-inflammatory activity which is considered as capability against aggravation of COPD [7–10]. However, because of their rapid disappearance in vitro and in vivo, we supposed that long-circulating and quick-formed active metabolite methylallyl sulfone (AMSO2) behave similar activity [11] (Fig. 1).

As a type of corticosteroid, dexamethasone (Dex) is used in the treatment of many conditions, including COPD. Although it has good anti-inflammatory and immunosuppressant effects, the long-term use of Dex may result in many problems, like thrush, bone loss, cataracts or muscle weakness [12]. Therefore, it is urgent for scientists to design and discover new candidates which are more safe with less side-effect. In this article, we are the first to research the protective effect of AMSO2

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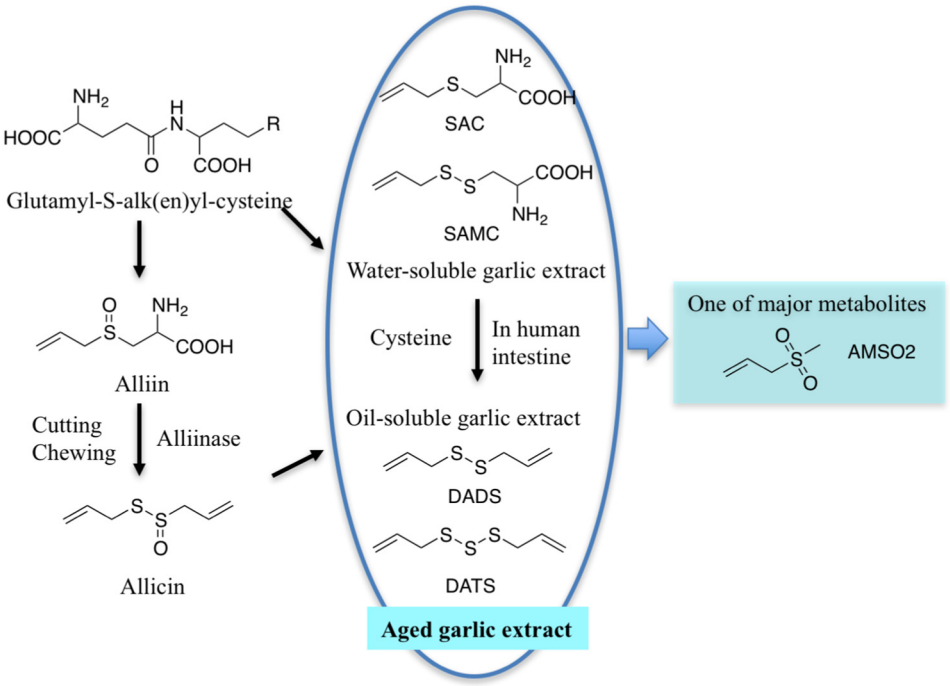

Fig. 1. Glutamyl-*S*-alk(en)yl-L-cysteines are the primary sulfur compounds in the intact garlic, which can be hydrolyzed and oxidized to yield Alliin. Then Alliin are transformed to allicin when chewing or cutting with activating the enzyme alliinase. Allicin is highly unstable and instantly decompose to form various oil-soluble compounds (DADS, DATS et al.). At the same time, glutamyl-*S*-alk(en)yl-L-cysteines are also converted to water-soluble organosulfur compounds (SAMC, SAC et al.). And finally, AMSO2 is one of the major metabolites of garlic extracts, which is quick-formed with a long half-life.

on lung injury in C57BL/6 and discuss underlying mechanism as Dex control.

2. Materials and methods

2.1. Reagents

AMSO2 with a purity of 95% was obtained from Sigma (St Louis, MO, USA) and was diluted with DMEM. Dexamethasone Sodium Phosphate injection (5 mg/ml, Hebei Tiancheng Pharmaceutical Co., LTD) was diluted with DMEM to 0.1 mg/ml. Primary antibodies against ERK, p38, phospho-ERK, phospho-p38, β-actin and horseradish peroxidase-conjugated second antibodies were all purchased from Cell Signaling Technology. The SuperSignal West Pico Chemiluminescent Substrate for horseradish peroxidase enzyme was obtained from Pierce (Rockford, IL, USA).

2.2. Cell culture

RAW264.7 macrophages were maintained in DMEM supplemented with 10% (v/v) fetal bovine serum, at 37 °C (5% CO_2). Exponentially growing RAW264.7 cells were suspended in DMEM to a concentration of 2×10^5 cells/ml, and the total number of cells was determined with a hemocytometer. Subsequently, the cells were plated in 6-well flat-bottomed microculture plates (2 ml/well) and cultured at 37 °C in a 5% CO_2 atmosphere for 4 h. The cultures were washed to remove non-adherent cells and then incubated with 2 ml of DMEM supplemented with 10% (v/v) fetal bovine serum for an additional 20 h.

2.3. Preparation of CSE

CSE was prepared using a modified method [13]. Taishan cigarettes were purchased. The smoke generated by cigarettes contains 11.0 mg tar, and 1.10 mg nicotine/cigarette. Briefly, three cigarette smoke extracts were collected into a centrifuge of 10 ml PBS and which were attached to a vacuum pump machine. Next, the PBS containing smoke was filtered through a 0.22 μm filter to remove large particles and considered a 100% CSE solution. CSE was prepared within 30 min and stored at −80 °C for additional experiments. The CSE was diluted and added to cells in submerged culture conditions.

2.4. Cell viability assay

Cell viability was measured by SRB method. Cells (5×10^3 per well) seeded in 96-well plates were exposed to increasing concentrations of SAMC for the indicated time. The treated cells were then fixed with 10% TCA for 1 h at 4 °C; the 96-well plates were washed three times with distilled water and allowed to dry in the air. Each well was added with 100 μl of sulforhodamine (SRB) solution and the staining was completed at room temperature for 15 min. The SRB stain solution was removed by washing the plates quickly with 1% (v/v) acetic acid three times, and the plates were dried in the air. The dried materials in each well were solubilized by adding 200 μl of 10 mM unbuffered Tris Base (pH 10.5). The cell viability was detected by measuring the absorbance at 540 nm on a plate reader (Safire2, TECAN, France). All experiments were repeated at least three times.

2.5. Treatment of animals

Male C57BL/6 mice (16 g, aged 5–6 weeks) were purchased from Institute of Laboratory Animal Sciences, Cams&Pumc (Beijing, China, SCXK 2014-0004), and housed under standard conditions (12:12 h light/dark cycle at 25 ± 2 °C and 40–70% humidity) in a specific pathogen free (SPF) environment. The mice had free access to standard commercial diet and water. All experimental procedures involving animals were performed in accordance with the institutional guidelines of the Animal Care and Use Committee of Shandong University. As seen in Table 1, the mice were randomly divided into five groups (n = 6 per group): 1) control group, 2) CSE group, 3) CSE + 1 mg/kg/day of Dex, 4) CSE + 50 mg/kg/day of AMSO2 (P-L group) and 5) CSE + 100 mg/kg/day of AMSO2 (P-H group). The mice from negative control were injected intraperitoneally with 500 μl of vehicle (normal saline) while the others were injected intraperitoneally with 500 μl of CSE-PBS solution on days 7, 14 and 21. AMSO2 and DEX were administered daily for 21 days. All the mice were fed under the same conditions for 3 weeks. On the 21st day, after starting the experiment, the rats were killed, and the blood samples and lung tissues were obtained as described below.