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Immunopharmacology and inflammation

Uvaol attenuates pleuritis and eosinophilic inflammation in ovalbumin-induced allergy in mice

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ABSTRACT

Uvaol, a triterpene present in olives and virgin olive oil, has been shown to possess anti-inflammatory properties and antioxidant effects. However, until now, no studies have demonstrated its potential effects on allergic inflammation. The aim of this study was to evaluate the anti-inflammatory effects of uvaol in a mouse model of allergy characterized by eosinophil-dominant inflammation in actively sensitized mice. The anti-inflammatory effect of uvaol was analyzed in two murine models of allergic inflammation (pleurisy and asthma). In these models, Swiss mice were sensitized and challenged with ovalbumin (OVA). In the pleurisy model, the pleural eosinophilic inflammation and IL-5 concentrations were examined 24 h after the OVA challenge, while in the asthma model were examined the airway inflammation via bronchoalveolar lavage (BAL) fluid cytology and lung histopathology analyses. Our results showed that uvaol decreased the accumulation of eosinophils and the concentration of IL-5 in pleural effluent. Uvaol also demonstrated important anti-inflammatory activity by inhibiting production of IL-5 and influx of leukocytes, mainly of eosinophils, in BAL fluid, but without interfering with levels of reactive oxygen species in leukocytes. Moreover, the eosinophil infiltration, mucus production, number of alveoli that collapsed, and IL-5 levels in the lung were clearly decreased by uvaol treatment. These findings indicate that uvaol can be a good candidate for the treatment of allergic inflammation by inhibiting eosinophil influx and IL-5 production in ovalbumin-induced allergy.

1. Introduction

Epidemiological studies report that the prevalence of allergic diseases has increased dramatically worldwide (Sole et al., 2014) in both industrialized and developing countries. The World Health Organization estimates that about 700 million people worldwide have some type of allergic disease (Rutkowski et al., 2014), which affects the quality of life of these individuals and their families, thereby negatively influencing the socioeconomic welfare of society.

In allergic reactions that require specific-allergen sensitization, the re-exposure to the antigen causes activation and secretion of a wide spectrum of mediators in target cells that directly damage the surrounding tissue and induce leukocyte infiltration, contributing to exacerbation of the inflammatory response (Barnes, 2011). This leads the antigen-induced accumulation of eosinophils into the tissue, which contributes significantly to tissue damage at sites of allergic inflammation (Nauta et al., 2008; Rose et al., 2010). Indeed, eosinophil-derived inflammatory mediators can be measured in the sputum, in bronchoalveolar lavage, and around areas of damaged epithelium of asthmatics (Brightling et al., 2003). In addition, eosinophilic infiltrate has been correlated clinically with the airway hyperresponsiveness (Siddiqui et al., 2007).

Interleukin-5 (IL-5) has been implicated as a key factor in eosinophil function associated with allergic conditions by promoting recruitment, activation, and survival at inflammatory sites and differentiation and maturation in the bone marrow (Barnes, 2011; Corren, 2012). Therefore, because of the importance of eosinophils in allergy and other associated disorders, IL-5 has been proposed as a potential target in the treatment of these diseases (Corren, 2011; Wechsler, 2008). In fact, the use of monoclonal antibody against IL-5, mepolizumab (Liu et al., 2013) or reslizumab (Kips et al., 2003), reduces the risk of exacerbations and inhibits the

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development of pulmonary eosinophilia, but not improvement the lung function. The first choice therapy to prevent the clinic manifestations associated with allergies is the use of corticosteroids. However, after extended periods of high-dose treatment, corticosteroids can have substantial side effects (Rizzo and Sole, 2006). Thus, the development of efficient alternative agents and therapeutics for allergic conditions is urgently needed.

Several plant-derived secondary metabolites that reduce the production and/or activity of pro-inflammatory mediators have been proposed as alternative therapeutic agents (Calixto et al., 2004). This therapeutic potential has enabled the development of new drugs, such as Acheflan®, from natural products (Calixto, 2005), for the treatment of various inflammatory conditions. Thus, the natural biological compounds continue to contribute to the commercial drugs being manufactured currently.

Pentacyclic triterpenes are widespread in the plant kingdom and are present in the fruits, leaves, and barks of medicinal plants (Hill and Connolly, 2013). These secondary plant metabolites are attracting increasing interest due to their beneficial anti-inflammatory (de Oliveira et al., 2015), anti-diabetic (Sheng and Sun, 2011), and antibacterial (Gilabert et al., 2015) effects. Recently, studies have also reported its effects on immune regulation (Martin et al., 2012a), regulation of blood sugar (de Melo et al., 2010), lowering of blood pressure (Somova et al., 2003), skin inflammation (Passos et al., 2013), and antitumor activity (Zhang et al., 2014). Thus, considering the wide range of biological activities of triterpenes, there has been increased interest in using them for pharmacological studies and for prospective new drug development.

Although several studies have shown the biological effects of a large number of triterpenoids, studies reporting the *in vivo* anti-inflammatory activity of uvaol are still scarce. Uvaol (Urs-12-ene-3,28-diol) is a biologically active molecule present in several foods as well as in plants used in folk medicine for their antioxidant (Allouche et al., 2011) and antibacterial effects (Martins et al., 2011). Despite its known pharmacological effects, the actions of uvaol on allergic inflammatory response are not yet elucidated. Moreover, there have been any studies on the anti-asthmatic or anti-inflammatory effects of uvaol in a murine model of allergy. Here, we evaluated the effect of uvaol on the eosinophilic response triggered by an allergen in two models of allergic inflammation in actively sensitized mice.

2. Materials and methods

2.1. Animals

Male Swiss mice weighing 25–30 g were obtained from the breeding colonies of the Federal University of Alagoas (UFAL). Animals were maintained with free access to food and water and were kept at 22 ± 2 °C with a controlled 12-h light–dark cycle in an animal housing facility at the Institute of Biological and Health Sciences. Experiments were performed during the light phase of the cycle. The animals were allowed to adapt to the laboratory for at least 2 h before testing and were used only once. All experimental procedures were performed in accordance with the guidelines for the ethical use of conscious animals in pain research published by the International Association for the Study of Pain (Zimmermann, 1983).

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the Brazilian Society of Laboratory Animals Science (SBCAL). The protocol was approved by the Committee on the Ethics of Animal Experiments at the Federal University of Alagoas (Comissão de Ética no Uso de Animais – CEUA, License no. 9244/2011-45). All efforts were made to minimize the suffering of the animals.

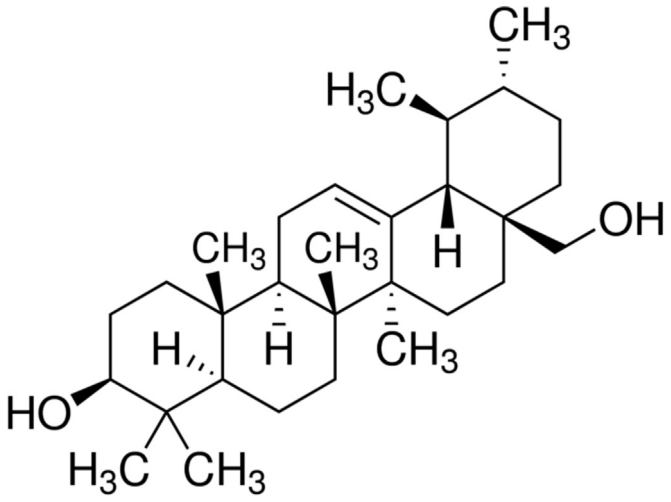

Fig. 1. Chemical structure of uvaol.

2.2. Reagents

The following substances, purchased from Sigma Chemical Co. (St. Louis, MO, USA), were used: uvaol (Urs-12-ene-3,28-diol, ≥ 95% purity) (Fig. 1), ovalbumin (OVA), 2′,7′-dichlorofluorescein diacetate (DCF-DA), protease inhibitor cocktail (MDL number MFCD00677817), Tween-20, eosin, hematoxylin, periodic acid-Schiff (PAS) kit and elastic stain kit, and phosphate-buffered saline (PBS). Aluminum hydroxide was from Alfa Aesar (Ward Hill, MA, USA), dexamethasone (DEXA; Decadron®) from Teuto-Brasileiro (Goiânia, GO, BRA), xylazine (Anasedan®) and ketamine (Dopalen®) from Ceva (Paulínia, SP, BRA), ethylenediaminetetraacetic acid (EDTA) and dimethyl sulfoxide (DMSO) from Synth (Diadema, SP, BRA), and May-Grunwald-Giemsa from Merck (São Paulo, SP, BRA). All drugs were dissolved in sterile 0.9% (w/v) NaCl (saline). Commercially available enzyme-linked immunosorbent assays (ELISA Ready-Set-Go® eBioscience, San Diego, CA, USA) were used to measure IL-5 levels in the bronchoalveolar lavage (BAL) and lung, according to the manufacturer's instructions.

The uvaol was dissolved in 2% DMSO, and the drugs were dissolved to concentrations so as to allow for administration of a constant volume of 10 μl/g in accordance with the average weight of animals. Control animals received similar volumes of the vehicle only. Oral pre-treatments (p.o.) were always administered 60 min before injection of the inflammatory stimuli with OVA.

2.3. Allergic pleurisy

Mice were immunized via subcutaneous (s.c.) injection on days 0 and 7 with 0.2 ml of a solution containing 50 μg of OVA adsorbed to 5 mg of aluminum hydroxide. At day 14, sensitized mice were then challenged intrapleurally with OVA (12.5 μg/cavity) dissolved in a final volume of 50 μl with sterile saline. Groups of mice were treated with vehicle (2% DMSO in sterile saline) or uvaol (100, 200, or 500 μmol/kg) orally 60 min prior to the allergen challenge. Control animals were pre-treated (60 min) with dexamethasone (10 μmol/kg) via intraperitoneal (i.p.) injection. Mice were euthanized 24 h later and the thoracic cavity was washed with 1 ml of PBS containing EDTA (10 mM). The exudate and washing solution were removed by aspiration, and the total volume was measured. Any exudate that was contaminated with blood was discarded. Total cell counts were performed in a Neubauer chamber,