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The plant extract *Isatis tinctoria* L. extract (ITE) inhibits allergen-induced airway inflammation and hyperreactivity in mice

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ABSTRACT

Background: The herbal *Isatis tinctoria* extract (ITE) inhibits the inducible isoform of cyclooxygenase (COX-2) as well as lipoxygenase (5-LOX) and therefore possesses anti-inflammatory properties. The extract might also be useful in allergic airway diseases which are characterized by chronic inflammation.

Methods: ITE obtained from leaves by supercritical carbon dioxide extraction was investigated in ovalbumin (OVA) immunised BALB/c mice given intranasally together with antigen challenge in the murine model of allergic airway disease (asthma) with the analysis of the inflammatory and immune parameters in the lung.

Results: ITE given with the antigen challenge inhibited in a dose related manner the allergic response. ITE diminished airway hyperresponsiveness (AHR) and eosinophil recruitment into the bronchoalveolar lavage (BAL) fluid upon allergen challenge, but had no effect in the saline control mice. Eosinophil recruitment was further assessed in the lung by eosinophil peroxidase (EPO) activity at a dose of 30 μg ITE per mouse. Microscopic investigations revealed less inflammation, eosinophil recruitment and mucus hyperproduction in the lung in a dose related manner. Diminution of AHR and inflammation was associated with reduced IL-4, IL-5, and RANTES production in the BAL fluid at the 30 μg ITE dose, while OVA specific IgE and eotaxin serum levels remained unchanged.

Conclusion: ITE, which has been reported inhibiting COX-2 and 5-LOX, reduced allergic airway inflammation and AHR by inhibiting the production of the Th2 cytokines IL-4 and IL-5, and RANTES.

Introduction

Isatis tinctoria L. (woad, Brassicaceae) is an ancient European dye and medicinal plant which was used as astringent and for treating skin inflammation and ulcers (*Isatis tinctoria*, monograph 2002). Lipophilic extracts from *Isatis tinctoria* (ITE) significantly inhibit cyclooxygenase 2 (COX-2), 5-lipoxygenase (5-LOX), inducible NO synthase (iNOS), leucocytic elastase, and histamine and serotonine release from stimulated mast cells (Hamburger 2002). ITE showed anti-inflammatory activity in carageenan-induced paw oedema and in tetradecanoylphorbol acetate (TPA)-induced mouse ear oedema (Recio et al. 2006a, b). In a clinical pilot study the anti-inflammatory activity of topical administered ITE was confirmed in skin irritation models (Heinemann et al. 2004). Based on this data it can be suggested that ITE may also be useful in allergic airway inflammations.

There is general consensus that allergic inflammation is driven by the activation of T-helper (Th) type 2 cells. They are known to produce IL-4 and IL-13 contributing to IgE production, mucus hypersecretion, airway hyperresponsiveness (AHR), and IL-5 promoted eosinophilic inflammation (Ahern and Robinson 2005; Robinson 2005). Therefore, several attempts are being made to reduce the inappropriate Th2 response to reduce allergic airway diseases. Therapeutic concepts include Th2 cytokine inhibitors, neutralising antibodies directed towards B-cell IgE, histamine and leukotriene blockers, as well as other targets (Barnes 2008; Holgate and Polosa 2008; Wills-Karp 1999) The aim of the present investigation was to test the ability of the ITE to inhibit Th2 response.

Murine models can be used for such investigations, since systemic responses as well as airway responses are well defined (Kips et al. 2003) It was previously demonstrated that intranasally applied allergens are equally distributed within the upper and

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lower airway systems, and that repeated allergen challenges induced airway hyperresponsiveness (AHR) (Wang and McCusker 2005).

Therefore, Th2 activation, allergic tissue reactions, inflammatory cell recruitment in the bronchoalveolar lavage (BAL), and airway hyperresponsiveness to methacholine were studied in the OVA antigen-induced experimental model in mice, and then compared with negative and positive controls. We report that orally administered ITE, which inhibits COX-2 and 5-LOX, effectively inhibits an established Th2 response in the lung.

Methods

Animals and immunization

Balb/c mice, aged 6–8 weeks, were immunised twice subcutaneously in groups of 6 mice at weekly intervals with a 0.4 ml saline solution containing 1 μg OVA and 1.6 mg alums. One week after the second immunisation, on day 14, intranasal challenge was performed under light i.v. ketamine anaesthesia by applying 50 μl OVA in alum-free saline solution (10 μg) or saline alone as control. The intranasal challenges were given on three consecutive days (Couillin et al. 2004). All experimental studies were approved by the local ethical animal research committee.

Plant extraction and administration

The ITE extract was manufactured using supercritical carbon dioxide with leaves of cultivated *Isatis tinctoria*. Fresh leaves were dried on a band drier operating at 60 °C and coarsely powdered with an SK 100 cross-beater mill (Retsch; Haan, Germany) equipped with a 2 mm sieve. The supercritical CO_2 (SFE) extract was prepared at the Adalbert-Raps-Zentrum, Technical University München-Weihenstephan, in a pilot-plant extractor by extraction with CO_2 at 800 bar and 50 °C for 3 h. The extraction yield was 0.85%. The extract corresponded to the SFE extract in tryptanthrin, indolin-2-one, indirubin, indigo and alpha-linolenic acids at 0.23%, 0.012%, 1.51%, 0.21% and 9.09%, respectively (Hamburger 2002, Recio et al. 2006a, b).

The ITEs extract was given intranasally at a dose of 10, 30, and 100 μg (in 40 μl) per mouse before saline or OVA challenge in OVA sensitised Balb/c mice under light ketamine anaesthesia (Fig. 1).

Airway hyperresponsiveness (AHR)

Airway resistance was evaluated by whole-body plethysmography as described before (Couillin et al. 2004). Bronchial hyperreactivity to aerosolised methacholine was investigated 24 h after the last saline or OVA challenge in OVA sensitized mice as described before (Couillin et al. 2004). Unrestrained conscious mice were placed in whole-body plethysmography chambers (Buxco Electronic, Sharon, CO, USA). Methacholine at 100 mM is aerosolized for 1 min and mean airway bronchoconstriction readings, as assessed by Enhanced Respiratory Pause (PenH), were obtained over 15 min periods. PenH can be conceptualised as the phase shift of the thoracic flow and the nasal flow curves; where an increased phase shift correlates with increased respiratory system resistance. PenH is calculated by the formula $PenH=(Te/RT-1)\times PEF/PIF$, where Te is the expiratory time, RT the relaxation time, PEF the peak expiratory flow, and PIF the peak inspiratory flow.

Bronchoalveolar lavage (BAL)

BAL was performed 24 h after the last antigen challenge by cannulating the trachea under ketamine anaesthesia and washing with 0.5 ml of ice-cold phosphate-buffered saline (PBS) (Couillin et al. 2004). The first 500 μl of the lavage fluid was centrifuged and the supernatant frozen for subsequent cytokine determinations and the trachea was further lavaged with 3×0.5 ml of PBS and centrifuged. The cell pellet was resuspended in PBS, counted by a haemocytometer chamber and cytospin preparations were made using a Shandon cytocentrifuge. The cells were analysed after differential staining with May–Gruenwald–Giemsa.

Lung histology

After bronchoalveolar lavage, the mice were sacrificed 24 h after the last saline or OVA challenge. The whole lung was removed and fixed in 4% buffered formaldehyde for standard microscopic analysis with haematoxylin and eosin (H&E) and periodic acid Schiff reagent (PAS) staining. Peribronchial inflammation, eosinophilic recruitment, and mucus hypersecretion was assessed using a semi-quantitative score (a scale from 0 to 5, absent; mild, slight, moderate, distinct, severe) by two independent observers as described before (Couillin et al. 2004).

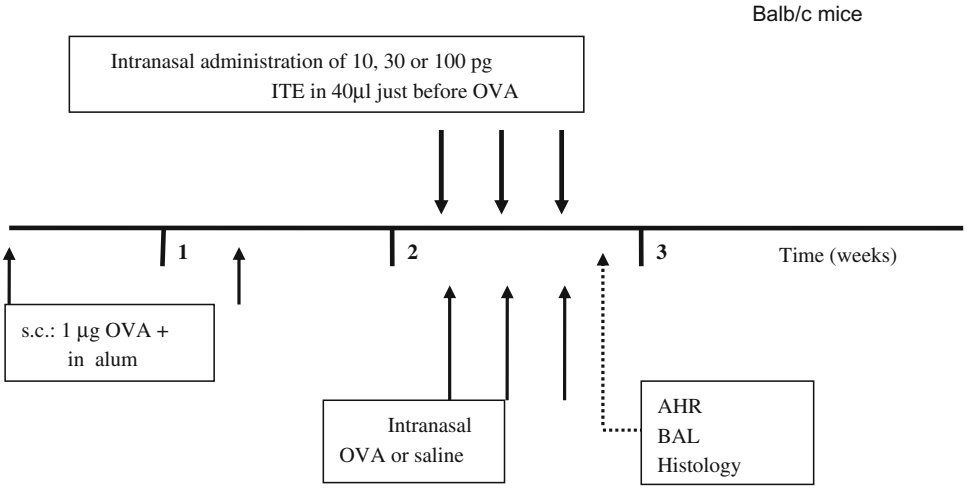

Fig. 1. Scheme of allergen OVA sensitization (days 0 and 7) and intra-nasal instillation of ITE followed by OVA challenge (days 14, 15, 16) and analysis on day 17 (AHR, BAL, cytokines, IgE and histology).