Phytomedicine 43 (2018) 46–54

Contents lists available at ScienceDirect

Phytomedicine

journal homepage: www.elsevier.com/locate/phymed

Original article

Dehydrocostus lactone, a sesquiterpene from *Saussurea lappa Clarke*, suppresses allergic airway inflammation by binding to dimerized translationally controlled tumor protein

Haejun Pyun, Unwoo Kang, Eun Kyoung Seo, Kyunglim Lee*

Graduate School of Pharmaceutical Sciences, College of Pharmacy, Ewha Womans University, Seoul 03760, Republic of Korea

ARTICLE INFO

Keywords:
Allergic diseases
Dehydrocostus lactone
Dimerized TCTP
Histamine releasing factor
Saussurea lappa Clarke

ABSTRACT

Background: We previously reported that the biologically active form of histamine releasing factor (HRF) is dimerized translationally controlled tumor protein (dTCTP) which is involved in a number of allergic diseases.
Hypothesis/purpose: Hoping that agents that modulate dTCTP may provide new therapeutic targets to allergic inflammatory diseases, we screened a library of natural products for substances that inhibit dTCTP. One such inhibitor we found was dehydrocostus lactone (DCL), a natural sesquiterpene present in rhizome of *Saussurea lappa Clarke,* the subject of this study.
Methods: We evaluated the therapeutic efficacy of DCL in a mouse model of ovalbumin (OVA)-induced allergic airway inflammation, employing the ELISA system using BEAS-2B cells and splenocytes, and confirmed that DCL interacts with dTCTP using SPR assay.
Results: DCL inhibited dTCTP-induced secretion of IL-8 in BEAS-2B cells. From kinetic analysis of dTCTP and DCL, we found that K_D value was 5.33 ± 0.03 μM between dTCTP and DCL. DCL also significantly reduced inflammatory lung eosinophilia, type 2 cytokines in BALF, as well as OVA specific IgE and mucus production in a mouse model of ovalbumin induced allergy. Moreover, DCL suppressed NF-κB activation.
Conclusion: DCL's therapeutic potential in allergic airway inflammation is based on its anti-inflammatory activity of suppressing the function of dTCTP.

Introduction

Translationally controlled tumor protein (TCTP), also known as histamine releasing factor (HRF), p21, p23, and fortilin, is ubiquitously expressed in eukaryotic groups, and is highly conserved in species ranging from humans to alfafa plants (Bommer and Thiele, 2004). Intracellular TCTP has been variously reported to act as a microtubule-stabilizing protein (Yarm, 2002), an anti-apoptotic protein (Li et al., 2001), and a suppressor of Na, K-ATPase (Jung et al., 2004). Extracellular TCTP has also been reported to act as an histamine-releasing factor hence its name as HRF (MacDonald et al., 1995). The potential clinical roles of HRF in allergic inflammatory responses have been reported in many studies. Increased HRF was observed in skin blister fluids of patients with late phase allergic inflammation (Warner et al., 1986) and in bronchoalveolar lavage fluids of patients with idiopathic eosinophilic pneumonia and asthma (Kazuo et al., 2003). TCTP was also found responsible for spontaneous release of histamine in patients with atopic dermatitis and food hypersensitivity (Sampson et al., 1989). Our group has shown that active form of HRF, participating in allergic inflammation, is dimerized TCTP (dTCTP) (Kim et al., 2009). Our prior studies led us to investigate whether modulation of dTCTP's role in the release of inflammatory or allergic mediators can provide an approach to the therapy of allergic inflammation diseases.

We therefore screened the natural products library for substances that inhibit dTCTP and came upon dehydrocostus lactone (DCL), a natural sesquiterpene lactone present in rhizome of *Saussurea lappa Clarke* (Robles et al., 1995). We found that many studies have already been reported that DCL exhibits a variety of biological activities, including playing roles as an anti-inflammatory (Lee et al., 1999), immunomodulatory (Taniguchi et al., 1995), anti-bacterial, and pro-apoptotic agent, and also in inhibitory action of NF-κB activation (Jin et al., 2000), Nrf2 expression (Park et al., 2014), MAPK activation (Hsu et al., 2009) and STAT3 activation (Kuo et al., 2009) We decided to investigate whether DCL modulates dTCTP's role in the release of

Abbreviations: BALF, bronchoalveolar lavage fluid; DCL, dehydrocostus lactone; dTCTP, dimerized translationally controlled tumor protein; ELISA, enzyme-linked immunosorbent assay; HRF, histamine releasing factor; NF-κB, nuclear factor kappa B; OVA, ovalbumin; SPR, surface plasmon resonance

* Corresponding author.
E-mail address: klyoon@ewha.ac.kr (K. Lee).

https://doi.org/10.1016/j.phymed.2018.03.045
Received 1 October 2017; Received in revised form 16 January 2018; Accepted 18 March 2018

inflammatory or allergic mediators, employing a mouse model of ovalbumin (OVA)-induced allergic airway inflammation.

Methods

Preparation of recombinant dTCTP

We prepared this recombinant protein as previously described (Kim et al., 2009). *Escherichia coli* BL21 (DE3) pLysS cells were transformed with PRSET A/mutated TCTPs (dTCTP), and the overexpressed dTCTP was purified using a Ni^{2+}-charged His-Bind column according to manufacturer's protocol (ELPIS, Daejeon, Korea). The NH_2-terminal fusion proteins of TCTP (dTCTP) were separated by fast protein liquid chromatography on a High trap Q column (GE healthcare, Piscataway, NJ, USA) using a NaCl gradient.

Cell viability studies

Cell viability was measured using a Cell Counting Kit-8 (CCK) assay (Dojindo, Kumamoto, Japan) according to the manufacturer's instructions. BEAS-2B cells (1×10^4 cells/well) were incubated in 96 well plates with various concentrations of acetone extracts of *Saussurea lappa* as well as costunolide and DCL for 24 h. CCK-8 reagent was added to each well, followed by incubation for additional 2 h, and the absorbance was measured at 450 nm using a microplate reader (MOLECULAR DEVICES, Sunnyvale, CA, USA). The percentage of viable cells was calculated using the equation: cell viability (%) = (mean absorbance in test wells/mean absorbance in control wells) × 100.

Measurement of IL-8

Cell culture for measuring IL-8 was carried out in the same manner as in the previous experiment (Kim et al., 2009). The indicated amounts (0, 1, 2, 4 μg/ml) of DCL and 8 μg/ml of dTCTP were pre-incubated for 15 min, added to cells in 1% penicillin-streptomycin/BEBM, and incubated for 20 h. IL-8 secreted into medium was measured by ELISA using a commercial kit (Biolegend, San Diego, CA, USA). The same method was applied to the acetone extract of *Saussurea lappa* (0, 0.25, 0.5, 1, 2 μg/ml) and the costunolide (0, 0.125, 0.25, 0.5, 1 μg/ml).

Surface plasmon resonance (SPR)

Binding of DCL to dTCTP was measured using a Reichert SR7500DC instrument (Reichert Technologies, Depew, NY, USA). dTCTP in 10 mM sodium acetate buffer pH 5.0 was immobilized using the standard amino coupling at 15 μl/min on a 500,000 Da carboxymethyl dextran hydrogel surface sensor chip (Reichert Technologies, Depew, NY, USA). The running buffer used in all experiments was PBS pH 7.4 (2% DMSO). All SPR experiments were performed at 25 °C. DCL (6.25, 12.5, 25, 50, 100, 200 μM) was injected over the dTCTP chip at 30 μl/min for 5 min. Complete dissociation of DCL and dTCTP was achieved after 8 min. The binding of DCL and dTCTP was detected as a change in the refractive index at the surface of the chip as measured by response units (RU). A reference flow cell was used to record the background response, and background was subtracted from each of the measured RU values. K_d values were calculated as ratios of K_a/K_d determined from kinetic experiments. Data model were fits using SCRUBBER-2. This experiment was carried out by WOOJUNG BSC (Suwon, Korea).

Animals

Female BALB/c mice (5–6 weeks and weighing 18–20 g) were purchased from the Central Lab. Animal Inc (Seoul, Korea). Before the experiments, the mice were allowed to become accustomed to their new environments for 1 week and were supplied with standard rodent feed and tap water. The animal room was maintained at 60–80% relative humidity at room temperature under a 12/12 h light/dark cycle. All animal studies were approved by Ewha Womans University's Institutional Animal Care and Use Committee (Approval ID: 16-023).

Measurement Th2 cytokines in murine splenocytes

To obtain splenocytes, the spleen was removed from the mouse (BALB/c, 6 weeks old) aseptically. Homogenized purified splenocytes were collected and treated with red blood cell lysing buffer (Sigma, St. Louis, MO, USA). The splenocytes were diluted to 7×10^6 cells/ml in RPMI 1640 media containing 10% FBS, 100 U/ml penicillin and 100 mg/ml streptomycin. The splenocytes were cultured with DCL (0, 1, 2, 4 μg/ml) after treatment with OVA (100 μg/ml) or cultured without OVA and DCL treatment. The plates were incubated at 37 °C for 72 h in a humidified incubator with 5% (v/v) CO_2 and 95% (v/v) air. IL-4 and IL-5 in the supernatants of splenocytes were measured using mouse IL-4 (Biolegend, San Diego, CA, USA), IL-5 (Thermo, Rockford, IL, USA) ELISA kits.

Mouse model of OVA-induced allergic airway inflammation

Female BALB/c mice, 6–7 weeks of age, were injected intraperitoneally, with 100 μl (0.5 mg/ml) of OVA (Thermo, Rockford, IL, USA) conjugated with same amount of alum (Thermo, Rockford, IL, USA), on days 1, and 14. Two weeks after the second injection, the animals were challenged with 20 μl of saline (sham) or 100 μg of OVA injected into the airway via their nasal cavity on days 28, 30, 32, 34 days. Saline (sham), saline containing 2% DMSO (vehicle), Dexamethasone (1 mg/kg) or DCL (2.5 mg/kg, 5 mg/kg, 10 mg/kg) was injected intraperitoneally into each of 3–6 mice from day 28 to day 35. Dexamethasone and DCL were purchased from Sigma (St. Louis, MO, USA).

Bronchoalveolar lavage fluid (BALF) collection and eosinophil count

Mice were anaesthetized and the trachea was cannulated while the thorax was gently massaged. Lungs were lavaged three times with 0.6 ml of phosphate buffer. The collected lavage fluid was cooled on ice and was centrifuged at 1000*g* for 5 min at 4 °C. The supernatant was stored at −80 °C for ELISA assays. The pellets were resuspended in 0.1 ml in phosphate buffer, and the total inflammatory cell numbers and differential counts were assessed by hemavat (Drew Scientific Inc., Oxford, CT, USA).

Enzyme-linked immunosorbent assay (ELISA)

IL-4, IL-5, IL-13, IFN-γ in BALF were measured using specific mouse IL-4 (Biolegend, San Diego, CA, USA), IL-5 (Thermo, Rockford, IL, USA), IL-13 (Biolegend, San Diego, CA, USA), IFN-γ (Biolegend, San Diego, CA, USA) ELISA kits. Serum OVA specific IgE also was measured using an ELISA kit (Biolegend, San Diego, CA, USA). All ELISAs were performed according to the manufacturer's directions.

Histological analysis

Standard procedures (Lin et al., 2014) were employed for fixation, preparation of tissue sections, deparaffination, HE and PAS stains. The densities of total inflammatory cells in the peribronchial areas of mice from different groups, and mucus occlusion of airway diameter were assessed as described previously (Henderson et al., 2005).

Lung protein extraction and western blot analysis

Lung tissue was homogenized with 500 μl lysis buffer (50 mM Tris–HCl, 150 mM NaCl, 1 mM EDTA, 0.25% deoxycholate, 1% triton X-100, phosphatase inhibitor 2, 3, protease inhibitor), incubated for