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Costunolide and Dehydrocostuslactone, two natural sesquiterpene lactones, ameliorate the inflammatory process associated to experimental pleurisy in mice

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ABSTRACT

The aim of this study was to investigate the effect of costunolide (CS) and dehydrocostuslactone (DCE) a well-known sesquiterpene lactones contained in many plants, in a model of lung injury induced by carrageenan administration in the mice. Injection of carrageenan into the pleural cavity of mice elicited an acute inflammatory response characterized by fluid accumulation in the pleural cavity which contained a large number of polymorphonuclear cells (PMNs) as well as an infiltration of PMNs in lung tissues and increased production of tumour necrosis factor α (TNF-α). All parameters of inflammation were attenuated by CS and DCE (15 mg/kg 10% DMSO i.p.) administered 1 h before carrageenan. Carrageenan induced an up regulation of the intracellular adhesion molecules-1 (ICAM-1) and P-selectin, as well as nitrotyrosine and poly (ADP-ribose) (PAR) as determined by immunohistochemical analysis of lung tissues. The degree of staining for the ICAM-1, P-selectin, nitrotyrosine and PAR was reduced by CS and DCE. Additionally we show that this inflammatory events were associated with NF-κB and STAT3 activation and these sesquiterpenes down-regulated it. Taken together, ours results clearly shown that CS and DCE may offer a novel therapeutic approach for the management of inflammatory diseases.

1. Introduction

Injection of carrageenan (CAR) into the pleural space leads to local inflammation, infiltration by polymorphonuclear leukocytes (PMNs) and lung injury (Cuzzocrea et al., 1999c). This experimental model of pleurisy has been widely used to investigate the pathophysiology of acute inflammation and also to evaluate the efficacy of drugs in inflammation (Nantel et al., 1999). During the inflammatory response, the high production of reactive oxygen and nitrogen species modulate gene expression as well as apoptosis contributing to the amplification of the inflammatory tissue injury (Fialkow et al., 2007). Recently we and others authors have clearly demonstrated that one mechanism through which reactive oxygen species influences the inflammatory response in the experimental model of lung inflammation is through the activation of the redox-sensitive transcription factor NF-κB (Cuzzocrea et al., 2004), that translocates to the nucleus, where it orchestrates the transcription of a number of pro-inflammatory genes (Peters et al., 2000). Consensus-binding sequences for NF-κB have been identified in the promoter regions of several genes implicated in the pathogenesis of acute and chronic inflammation (Baldwin, 2001).

Another hallmarks of the inflammatory response is the activation of the members of transcription factors, Signal Transducers and Activators of transcription family (STATs), which are induced by cytokines at the early phase of the inflammatory process (Pfitzner et al., 2004). The phosphorylated STATs dimers translocate into the nucleus to regulate expression of target genes (Darnell et al., 1994). Among a number of cytokines released during inflammatory response, interferon (IFN)-γ and interleukin (IL-6) play a pivotal role in regulating the expression of inflammatory genes. IFN-γ exclusively transduces its message by activating cytoplasmic STAT1 whereas the pleiotropic cytokine IL-6 predominantly activates STAT3 and to less extent STAT1. Our previous report (Cuzzocrea et al., 1999b) showed a reduction of carrageenan-elicited lung injury in IL-6-KO mice and a significant attenuation of lung inflammation in the IL-6-WT mice treated with anti-IL-6 antibodies. Furthermore, we have demonstrate

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that the administration of *Hypericum perforatum* extract as well as natural molecule like glycyrrhizin protected lung tissues in CAR-treated mice in association with reduced STAT3 activation (Menegazzi et al., 2008a). Recently, we have reported that costunolide (CS) and dehydrocostuslactone (DCE) two sesquiterpene lactones present in a number of plants such as *Laurus nobilis*, strongly and efficiently inhibit IL-6-induced tyrosine phosphorylation of STAT3 in human leukemic cell line THP-1 (Butturini et al., 2011). Furthermore, these compounds exhibit variety of pharmacological activities including anti-inflammatory and anti-bacterial ones (Robles et al., 1995); pro-apoptotic effect in different human cancer cell line (Hung et al., 2010; Park et al., 2001); inhibitory action on NF-κB activation (Koo et al., 2001); Nfr2 expression (Pae et al., 2007) and MAPK activation (Hsu et al., 2009). Moreover, DCE inhibits the constitutive STAT3 activation (Kuo et al., 2009).

Given that CS and DCE have emerged as a potential anti-inflammatory agent, the present study was designed to explore their possible effect in an experimental model of CAR-induced pleurisy as well as to examine the underlying mechanisms responsible for its effects on this model of acute lung injury.

2. Materials and methods

2.1. Animals

Male CD mice (weight 20–25 g; Harlan Nossan, Milan, Italy) were used in these studies. The animals were housed in a controlled environment and provided with standard rodent chow and water. Animal care was in compliance with Italian regulations on the protection of animals used for experimental and other scientific purposes (D.M. 116192) as well as with EEC regulations (O.J. of E.C. L358/1 12/18/1986).

2.2. Carrageenan-induced pleurisy

Pleurisy by CAR was induced as previously described (Cuzzocrea et al., 2000). Mice were anaesthetized with isoflurane and subjected to a skin incision at the level of the left sixth intercostals space. The underlying muscle was dissected and saline (0.1 mL) or saline containing 2% λ-CAR (0.1 mL) was injected into the pleural cavity. The skin incision was closed with a suture and the animals were allowed to recover. At 4 h after the injection of CAR, the animals were killed by inhalation of CO_2.

The chest was carefully opened and the pleural cavity rinsed with 2 mL of saline solution containing heparin (5 U/mL) and indomethacin (10 μg/mL). The exudate and washing solution were removed by aspiration and the total volume measured. Any exudate that was contaminated with blood was discarded. The amount of exudate was calculated by subtracting the volume injected (2 mL) from the total volume recovered. The leukocytes in the exudate were suspended in phosphate-buffer saline (PBS) and counted with an optical microscope in a Burker's chamber after vital Trypan Blue staining.

2.3. Experimental design

In a separate set of experiment, mice were treated with different dose (5–10–15 mg/kg) of CS and DCE in order to evaluate the efficacy of treatments in dose dependent manner. The different doses of the CS and the DCE were solubilized at a concentration of 10% DMSO. The volume injected to each mouse was the same for each different dose.

Mice were randomly allocated into the following groups:

(i) *CAR+saline group*. Mice were subjected to CAR-induced pleurisy ($N=10$).

(ii) *CAR+costunolide (CS) group*. Same as the CAR+saline group but CS (15 mg/kg 10% DMSO i.p.) was administered 1 h before CAR ($N=10$).

(iii) *CAR+dehydrocostuslactone (DCE) group*. Same as the CAR+saline group but *DCE* (15 mg/kg 10% DMSO i.p.) was administered 1 h before CAR ($N=10$).

(iv) *Sham+saline group*. Sham-operated group in which identical surgical procedures to the CAR group was performed, except that the saline was administered instead of CAR ($N=10$).

(v) *Sham+costunolide (CS) group*. Same as the Sham+saline group but CS (15 mg/kg 10% DMSO i.p.) was administered 1 h before saline solution ($N=10$).

(vi) *Sham+dehydrocostuslactone (DCE) group*. Same as the Sham+saline group but *DCE* (15 mg/kg 10% DMSO i.p.) was administered 1 h before saline solution ($N=10$).

In a separate set of experiment, mice were treated with different dose of CS and DCE in order to evaluate the efficacy of treatments in dose dependent manner.

2.4. Histological examination

Lung tissues samples were taken 4 h after injection of CAR. Lung tissues samples were fixed for 1 week in 10% (w/v) PBS-buffered formaldehyde solution at room temperature, dehydrated using graded ethanol and embedded in Paraplast (Sherwood Medical, Mahwah, NJ, USA). Sections were then deparaffinized with xylene, stained with hematoxylin and eosin. All sections were studied using Axiovision Zeiss (Milan, Italy) microscope. The following morphological criteria were used for scoring: 0, normal lung; grade 1, minimal edema or infiltration of alveolar or bronchiolar walls; grade 3, moderate edema and inflammatory cell infiltration without obvious damage to lung architecture; grade 4, severe inflammatory cell infiltration with obvious damage to lung architecture. All the histological studies were performed in a blinded fashion.

2.5. Measurement of cytokine

TNF-α levels were evaluated in the exudates 4 h after the induction of pleurisy by CAR injection as previously described (Cuzzocrea et al., 1999a). The assay was carried out using a colorimetric commercial ELISA kit (Calbiochem-Novabiochem Corporation, Milan, Italy). The ELISA has a lower detection limit of 5 pg/ml.

2.6. Myeloperoxidase (MPO) activity

MPO activity, an indicator of PMN accumulation, was determined as previously described (Mullane et al., 1985). At the specified time following injection of CAR, lung tissues were obtained and weighed, each piece homogenized in a solution containing 0.5% (w/v) hexadecyltrimethyl-ammonium bromide dissolved in 10 mM potassium phosphate buffer (pH 7) and centrifuged for 30 min at 20,000 × *g* at 4 °C. An aliquot of the supernatant was then allowed to react with a solution of tetramethylbenzidine (1.6 mM) and 0.1 mM hydrogen peroxide. The rate of change in absorbance was measured spectrophotometrically at 650 nm. MPO activity was defined as the quantity of enzyme degrading 1 μmol of peroxide min^{-1} at 37 °C and was expressed in units per 100 mg of wet tissue.

2.7. Immunohistochemical localization of intercellular cell adhesion molecule (ICAM-1), P-selectin, nitrotyrosine and poly(ADP-ribose) polymerase (PARP)

At the end of the experiment, the tissues were fixed in 10% (w/v) PBS-buffered formaldehyde and 8 μm sections were prepared from