Available online at www.sciencedirect.com
ScienceDirect
European Journal of Pharmacology 562 (2007) 155–163
www.elsevier.com/locate/ejphar

Anti-inflammatory and analgesic effects of the sesquiterpene lactone budlein A in mice: Inhibition of cytokine production-dependent mechanism

Daniel A.R. Valério [a,1], Thiago M. Cunha [a,1], Nilton S. Arakawa [b,1], Henrique P. Lemos [a], Fernando B. Da Costa [b], Carlos A. Parada [a], Sergio H. Ferreira [a], Fernando Q. Cunha [a], Waldiceu A. Verri Jr. [a,*]

[a] *Department of Pharmacology, School of Medicine of Ribeirão Preto, University of São Paulo, São Paulo, Avenida Bandeirantes, 3900, 14049-900-Ribeirão Preto, São Paulo, Brazil*

[b] *Department of Pharmaceutical Sciences, Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, São Paulo, Avenida do Café, s/n, 14040-903-Ribeirão Preto, São Paulo, Brazil*

Received 4 September 2006; received in revised form 14 December 2006; accepted 16 January 2007
Available online 1 February 2007

Abstract

The anti-inflammatory activities of some medicinal plants are attributed to their contents of sesquiterpene lactones. In the present study, the anti-inflammatory and anti-nociceptive activity of a sesquiterpene lactone isolated from *Viguiera robusta*, budlein A in mice was investigated. The treatment with budlein A dose—(1.0–10.0 mg/kg, p.o., respectively) dependently inhibited the carrageenan-induced: i. neutrophil migration to the peritoneal cavity (2–52%), ii. neutrophil migration to the paw skin tissue (32–74%), iii. paw oedema (13–74%) and iv. mechanical hypernociception (2–58%) as well as the acetic acid-induced writhings (0–66%). Additionally, budlein A (10.0 mg/kg) treatment inhibited the mechanical hypernociception-induced by tumour necrosis factor (TNF-α, 36%), Keratinocyte-derived chemokine (KC, 37%) and Interleukin-1β (IL-1β, 28%), but not of prostaglandin E_2 or dopamine. Budlein A also inhibited the carrageenan-induced release of TNF-α (52%), KC (70%) and IL-1β (59%). Furthermore, an 8 days treatment with budlein A inhibited Complete Freund's adjuvant (10 μl/paw)-induced hypernociception, paw oedema and paw skin myeloperoxidase activity increase while not affecting the motor performance or myeloperoxidase activity in the stomach. Concluding, the present data suggest that budlein A presents anti-inflammatory and antinociceptive property in mice by a mechanism dependent on inhibition of cytokines production. It supports the potential beneficial effect of orally administered budlein A in inflammatory diseases involving cytokine-mediated nociception, oedema and neutrophil migration.

Keywords: Budlein A; *Viguiera robusta*; Asteraceae; Sesquiterpene lactones; Inflammation; Oedema; Nociception; Hypernociception; Hyperalgesia; Neutrophil migration; Pain; Cytokine; Chemokine

1. Introduction

The pharmacological activities of some medicinal plants, specially those from the sunflower family Asteraceae, are attributed to their contents of sesquiterpene lactones such as mikanolide, helenalin, parthenolide, artemisinin, bis(isoalantodiol-B)glutarate. In fact, sesquiterpene lactones may present a wide variety of activities including *in vitro* antimicrobial (Pickman, 1984), antiviral (Meshnick, 2002), and antitumor activities (Chen et al., 1994). Sesquiterpene lactones also seem promising anti-inflammatory drugs. They inhibit inflammatory oedema induced by cotton pellet granuloma, complete Freund's adjuvant, 4-beta-phorbol 12-myristate 13-acetate, formalin, and carrageenan (Damre et al., 2003; Guardia et al., 2003; Abil'daeva et al., 2004; Silvan et al., 1996; Feltenstein et al., 2004). Additionally, using the acetic acid-induced writhings model it was demonstrated the antinociceptive effect of sesquiterpene lactones (*e.g.* parthenolide, costunolide, dehydrocostus lactone) (Jain and Kulkarni, 1999; Okugawa et al., 2000; Ahmed et al., 2001). Furthermore, Recio et al. (2000), demonstrated the concomitant inhibition by different sesquiterpene

* Corresponding author. Tel.: +55 16 3602 3227; fax: +55 16 3633 0021.
E-mail addresses: waverrij@usp.br, waldiceujr@yahoo.com.br (W.A. Verri).
[1] These authors contributed equally.

0014-2999/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2007.01.029

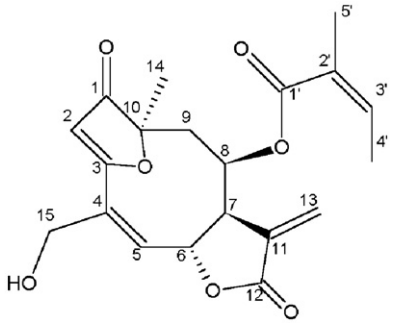

Fig. 1. Chemical structure of budlein A.

lactones (*e.g.* confertdiolide) of inflammatory oedema and leukocyte migration to ear skin challenged with 12-*O*-tetradecanoylphorbol 13-acetate.

Budlein A (Fig. 1) is a sesquiterpene lactone that has been previously isolated from *Viguiera buddleiaeformis* (De Vivar et al., 1976). However, despite its first isolation in the seventies, there are only two reports regarding the budlein A effect on mammalian cells, which demonstrate *in vitro* inhibition of sperm motility (Huacuja et al., 1993) and NF–kB activation (Siedle et al., 2004). Inhibition of NF–kB activity by preventing I–kB degradation has been described for other sesquiterpene lactones including isogoyazensolide, centratherin, atripliciolide tiglate, among others (Hehner et al., 1998; Siedle et al., 2004). The activation of this transcription factor is involved in the production of many inflammatory mediators. After its activation, NF–kB migrates to the cell nucleus and induces the expression of cytokines, such as tumour necrosis factor (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), cyclooxigenase-2 and adhesion molecules (L-selectins, ICAM-1) (Baeuerle and Henkel, 1994; Baeuerle and Baltimore, 1996; May and Ghosh, 1998; for review see Barnes, 2006), which are important for the genesis of inflammatory signals.

Thus, in the present study we isolated budlein A from the diclorometanic extract of *Viguiera robusta*, and demonstrated the anti-inflammatory and anti-nociceptive effects of budlein A in models of carrageenan-induced oedema, leukocyte migration and nociception. Furthermore, the mechanisms involved in budlein A effect were also addressed.

2. Materials and methods

2.1. Isolation of budlein A

2.1.1. Plant material

Leaves of *V. robusta* were collected by F.B.C. in April 2001 in Batatais (35 km, Batatais–Altinopolis highway), state of São Paulo, Brazil. E. E. Schilling (Department of Botany, University of Tennessee, Knoxville, TN, USA) and J. N. Nakajima (Biology Institute, University of Uberlândia, Uberlândia, MG, Brazil) identified the material. A voucher specimen (FBC # 105) is deposited at the Herbarium SPFR of the Department of Biology, FFCLRP, University of São Paulo, Ribeirão Preto, SP, Brazil, with the code SPFR 07155.

2.1.2. Extraction and isolation

Air-dried and entire leaves (2.5 kg) of *V. robusta* were placed in an Erlenmeyer and extracted with dichloromethane in sonicator, at room temperature (28 °C), for 10 min. The residue was filtered through common filter paper and the solvent was removed under vacuum, affording 14 g of dried crude extract, which was analyzed by infrared (IR) spectroscopy. A strong band at 1.760 cm^{-1} in the spectrum corresponded to the carbonyl stretching of γ-lactones, an indication of sesquiterpene lactones in the extract. In order to remove pigments and fats, the extract was dissolved in methanol-water (4:1) and successive partition was made with *n*-hexane, dichloromethane and methanol, affording, respectively 3.1, 4.0, and 6.5 g of organic soluble residues after solvent evaporation under vacuum. After IR spectral analysis, a strong band of γ-lactones was observed in the spectrum of the dichloromethane residue. This residue was fractioned through vacuum liquid chromatography (silica gel, Merck, *n*-hexane: ethyl acetate, increasing polarity) to give nine fractions after thin-layer chromatography (TLC) analysis. Fraction 6 (1042 mg) and 7 (725 mg) were found to contain γ-lactones monitored via IR spectral analysis. Due to the formation of a solid mass, fraction 6 was exhaustively washed with cold ethanol until pure budlein A (500 mg) was obtained as white crystals. Its chemical structure was determined by means of spectrometric analysis, *i.e.* IR and nuclear magnetic resonance (NMR) spectrometry (^{1}H and ^{13}C), as well as comparison with authentic sample and data reported in the literature (Da Costa et al., 2001). The purity of budlein A was determined by chromatographic and spectrometric methods. TLC was carried out using several eluent systems and two spray reagents (1% vanillin–sulphuric acid or concentrated sulphuric acid). A high performance liquid chromatography (HPLC) run was made using methanol–water 55:45 or acetonitrile–water 65:35 as mobile phase, a reversed phase (ODS) analytical column, flow rate 1.0 or 1.3 mL/min, and UV detection at λ_{max} 225 and 265 nm, as described elsewhere (Da Costa et al., 2001). Only one compound was detected in the chromatographic analyses. The ^{13}C NMR spectrum of budlein A showed 20 carbon atoms corresponding to its structure. By means of chromatographic and spectrometric methods, we estimated that the purity of budlein A is between 95–98%, therefore suitable for these biological assays. HPLC chromatograms and NMR spectral data are available upon request.

2.2. Animals

Adult male Swiss mice (22–28 g) obtained from the University of Sao Paulo, campus of Ribeirao Preto, were housed in a temperature-controlled room, with access to water and food *ad libitum* until use. All experiments were double blind and conducted in accordance with the National Institute of Health guidelines on the welfare of experimental animals and with the approval of Ethics Committee of the Faculty of Medicine of Ribeirao Preto (University of Sao Paulo).

2.3. Paw oedema test

The volume of the mice paw was measured with a plesthismometer (Ugo Basil, Italy) before (V_o) the intraplantar stimulus