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Anti-proliferative and anti-inflammatory effects of 3β,6β,16β-Trihydroxylup-20(29)-ene on cutaneous inflammation

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ABSTRACT

Ethnopharmacological relevance: 3β,6β,16β-Trihydroxylup-20(29)-ene (TTHL) is a triterpene isolated from the flowers of *Combretum leprosum,* a plant used in folk medicine in the north of Brazil for the treatment of skin disorders.
Aim of the study: In the present study, TTHL was evaluated as a potential topical anti-inflammatory and anti-proliferative agent through *in vivo* and *in vitro* models.
Material and methods: Anti-inflammmatory and anti-proliferative effects of TTHL were assessed using Swiss mice in acute and chronic models of skin inflammation induced by 12-*O*-tetradecanoylphorbol-acetate (TPA) application. Anti-proliferative activity was proved through *in vitro* experiments with the HaCaT human keratinocyte cell line.
Results: Treatment with TTHL inhibited inflammatory parameters such as oedema formation and cellular infiltration in acute and chronic models. In the chronic model, TTHL also inhibited epidermal hyperproliferation, as evidenced by reduction of epidermis thickness and proliferating cell nuclear antigen expression. The anti-proliferative effect was confirmed by the capability of TTHL in reducing the proliferation and inducing cell apoptosis of HaCaT cells. Suggesting a mechanism of action, TTHL showed activation of corticosteroid receptors, but without the induction of corticosteroid-related cutaneous side effects.
Conclusion: Our results demonstrate consistent anti-inflammatory and anti-proliferative activity and assign TTHL as a valuable tool in the development of a new treatment for skin inflammatory and proliferative diseases, such as psoriasis.

1. Introduction

Psoriasis is a chronic inflammatory skin disease which affects approximately 2% of the world's population (Jacobson et al., 2011). Although raised, erythematous skin plaques with adherent silvery scales are the typical manifestation of the disease, psoriasis also shows an important psychological component which greatly impairs the patient's quality of life (Chalmers, 2015). The scales arise from hyperproliferation of the epidermis which shows precipitate maturation and incomplete cornification of keratinocytes. A defect in apoptosis seems to be one of the factors responsible for the hyperproliferative epidermis (Boehm, 2006). In addition, psoriatic skin shows intense dermal inflammatory infiltrate which contributes to the overall thickness of lesions (Christensen et al., 2006). Facing the complex nature of the disease, most of treatments often do not show satisfactory outcomes. Drug tolerance, side effects, toxicity and inconvenience are the main drawbacks of the available therapies (Gottlieb, 2005; Linden and Weinstein, 1999). In light of the difficulties, there is growing interest in the development of new therapies and drugs to treat inflammatory skin disorders in a safe and effective way (Stern et al., 2004).

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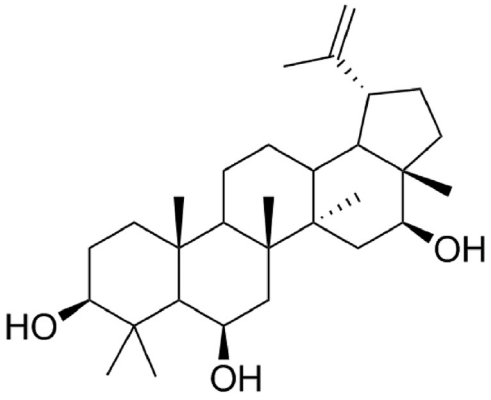

Fig. 1. 3β,6β,16β-Trihydroxylup-20(29)-ene (TTHL).

In the search for new therapeutic agents, natural products represent an abundant source of potentially effective molecules. Molecular richness of natural compounds facilitates target achievement, and the existing biological activity favours bioavailability. Besides, ethnopharmacological clues shorten the distance from crude resources to a successful safe and effective compound (Harvey et al., 2015).

3β,6β,16β-Trihydroxylup-20(29)-ene (TTHL) is a promising pentacyclic triterpene isolated from *Combretum leprosum* Mart. (Combretaceae), a native tree commonly applied in folk medicine in the north of Brazil, especially for the treatment of skin conditions (Facundo et al., 1993) (Fig. 1). Diverse studies have shown the pharmacological activities of this triterpene. The oral administration of TTHL is effective at reducing pain signalling in different models of nociception involving glutamatergic, opioid and serotonergic systems and also Gi/o protein activation and opening of specific K^+ channels (Longhi-Balbinot et al., 2011, 2009; Pietrovski et al., 2006). An anti-inflammatory effect seems to be another valuable pharmacological ability of TTHL, which was able to reduce leukocyte infiltration and cytokine release in an animal model of peritonitis induced by carrageenan (Longhi-Balbinot et al., 2012). Nascimento-Neto et al. (2015) showed reduced inflammatory response and an improvement of the wound healing process when applying TTHL in a mice skin wound healing model. Triterpene is also effective against Gram positive bacterial infections and demonstrated leishmanicidal activity (Evaristo et al., 2014; Teles et al., 2015, 2011). Furthermore, TTHL showed an anti-proliferative effect in several tumorigenic cell lines by inducing apoptosis and increasing intracellular reactive oxygen species levels (Viau et al., 2014).

In a previous study, we showed that the ethanolic extract from flowers of *C. leprosum* reduced skin inflammation and epidermal hyperproliferation in animal models (Horinouchi et al., 2013). In the present study, the potential of TTHL as a topical anti-inflammatory and anti-proliferative agent was evaluated in *in vivo* and *in vitro* skin inflammation models.

2. Material and methods

2.1. General experimental procedure

All procedures were carried out on female Swiss mice (25–35 g). Animals were randomly allocated into groups. Food and water were supplied *ad libitum* and the animals were kept in a 12 h light/dark cycle and in a temperature-controlled room (22 ± 2 °C). Animals were allowed to adapt to the laboratory for at least 1 h before testing and were used only once. All animal procedures were performed after approval of the Institutional Ethics Committee of Federal University of Paraná (protocol numbers: 296 and 567) and were carried out in accordance with the current guidelines for the care of laboratory animals.

2.2. Experimental biological material

Plant material was collected in May 2007 in Viçosa, Ceará State, Brazil, and was classified by Dr Afrânio Fernandes (Universidade Federal do Ceará, Fortaleza) as *Combretum leprosum* Mart. A voucher specimen of this plant was deposited in the Herbarium Prisco Bezerra of the Biology Department, Universidade Federal do Ceará, Brazil, under number 12446 (May 2007). The triterpene 3β,6β,16β-trihydroxylup-20(29)-ene (TTHL) was isolated from the flowers of *C. leprosum* at the Department of Organic Chemistry, Universidade Federal do Ceará, Brazil as previously described; its degree of purity was > 98% (Facundo et al., 1993).

2.3. Chemicals

The following chemicals and substances were used in experimental procedures: 12-*O*-tetradecanoylphorbol-acetate (TPA), dexamethasone, acid fuchsine, Biebrich scarlet, gelatine, aniline blue, tetramethylbenzidine (TMB), 4-Nitrophenyl N-acetyl-β-D-glucosaminide (NAG), Sodium citrate dehydrate, Glycine anhydride, hexadecyltrimethylammonium bromide (HTAB), mifepristone, 3-(4,5-dimetylyhiazol-2-yl)−2,5-diphenyltetrazolium bromide (MTT), Triton-X, propidium iodide (PI), and bovine serum albumin (BSA) were purchased from Sigma Chemical Co (St Louis MO); DAB substrate kit was purchased from BD Biosciences (San Diego, CA, USA); acetone, formaldehyde, glacial acetic acid, paraffin, polyethylene glycol (PEG) 400, dimethylformamide and phosphate-buffered saline (PBS) were purchased from Merck Biosciences (Germany); sodium acetate anhydrous, hydrogen peroxide, absolute ethanol, ferric chloride, hydrochloric acid, methanol, phosphomolybdic acid, phosphotungstic acid, eosin, hematoxylin and xylene were purchased from Vetec (Rio de Janeiro, Brazil); Dulbecco's modified Eagle's medium (DMEM), neutral red, penicillin-streptomycin and foetal bovine serum (FBS) were obtained from Cultilab (Campinas, Brazil); cell proliferation assay kit, Cyquant (Invitrogen, Molecular Probes, São Paulo, Brazil) and ribonuclease (RNase) (Amresco, Solon, EUA); and the anti-PCNA (C-20) goat polyclonal IgG antibody and donkey anti-goat IgG HRP antibody were purchased from Santa Cruz Biotechnology, Inc. (CA, USA).

2.4. Ear oedema measurement

Oedema was quantified as the increase in mice ear thickness upon inflammatory challenge. Ear thickness was measured before and after induction of the inflammatory response using a digital micrometer (MT-045B - Shangai Metal Great Tools Co., Ltd., Shangai, China). The micrometer was applied close to the medial edge of the ear, and the thickness was recorded in micrometres. To minimise technique variations, a single investigator performed the measurements throughout each experiment (Otuki et al., 2005). The TPA and dexamethasone were dissolved in 20 μL of acetone, TTHL was dissolved in 20 μL of ethanol-acetone (1:1).

2.5. TPA-Induced irritative contact dermatitis

Acute oedema was induced in the right ear of the animals by single topical application of TPA (2.5 μg/ear). Treatment with TTHL (0.002–1.313 μmol/ear) or dexamethasone (0.255 μmol/ear) (reference drug) was topically applied immediately after induction with TPA on the right ears. Control animals were challenged with TPA without receiving any treatment. Left ears were not handled during the experiments. To verify the possible involvement of glucocorticoid receptors, animals