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Gambogic acid exhibits anti-psoriatic efficacy through inhibition of angiogenesis and inflammation

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SUMMARY

Background: Psoriasis is a chronic T cell-mediated inflammatory skin disease. Studies have shown that angiogenesis plays an important role in the pathogenesis of psoriasis. Studies have also indicated that Gambogic acid (GA) inhibits angiogenesis and may be a viable drug candidate in anti-angiogenesis therapies.
Objective: The aim of this study was to investigate the anti-psoriatic effect of GA and the possible mechanisms.
Methods: MTT test on HaCaT cells and immunofluorescence on HUVEC cells were processed. An O/W cream of GA was prepared and topically applied to the ears of K14-VEGF transgenic mice and psoriasis-like guinea-pigs, and the tail skin of Balb/C mice independently. Furthermore, hematoxylin-eosin staining of tissues from three models and immunohistochemistry staining of ear samples from K14-VEGF mice were performed.
Results: In vitro, GA inhibited proliferation of HaCaTs and TNF-α-induced activation of NF-κB in HUVECs. In vivo, animals treated with GA showed significant morphological and histological improvements. Immunohistochemical analysis of K14-VEGF transgenic mice revealed that hyperplastic and inflamed vessels were suppressed with GA treatment. The expression of adhesion molecules such as ICAM-1 and E-selectin was significantly decreased. GA inhibited angiogenesis and the expression of VEGFR2 and p-VEGFR2. T lymphocyte infiltration and the expression of IL-17 and IL-22 were also reduced by GA treatment.
Conclusion: Our results suggest that GA has anti-psoriatic efficacy through inhibition of angiogenesis and inflammation. Therefore, GA is attractive and offers future potential for application in patients with psoriasis.

1. Introduction

Psoriasis is a chronic T cell-mediated inflammatory skin disease, characterized by thickened and scaly plaques, commonly on the elbows, knees and trunk which is caused by complex, aberrant relationships between the skin and immune system, as well as genetic makeup and environmental factors [1,2]. The histological features of psoriasis include inflammation, epidermal hyperproliferation and disturbed epidermal differentiation, angiogenesis and dilation of dermal blood vessels [3,4]. The pathogenesis of psoriasis contains three major factors: keratinocyte hyperproliferation, abnormal keratinocyte differentiation, and infiltration of inflammatory cells [5]. It is reported that keratinocytes also have an important contribution to the development of optimal cutaneous immune response since it can produce many cytokines [6].

In psoriatic lesions, hyperplastic and hyper-permeable blood vessels with increased expression of E-selectin, ICAM-1 and VCAM-1 have been demonstrated in the dermal papillae. And in the papillary dermal micro-vessels the expression of VEGF and its high affinity receptors VEGFR-1 and VEGFR-2 was increasingly observed [7]. Extensive efforts aimed at transgenically delivering inflammatory mediators or keratinocyte growth factors to the skin have not completely reproduced the psoriatic phenotype [8,9]. Delivery of VEGF to the skin of mice results in epidermal overexpression of VEGF, and thus produces a phenotype with many of the cellular

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and molecular hallmarks of human psoriasis, such as hyperplastic blood vessels, epidermal thickening, and inflammatory infiltrates [10]. The transgenic K14/VEGF mice have been reported to develop a cutaneous inflammatory condition resembling psoriasis [7]. Classical models including psoriasis-like model of guinea pigs and mouse-tail model are also used in anti-psoriatic efficacy studies. Topical application of 5% propranolol to guinea-pig skin causes thickening of the skin with histological changes strikingly resembling psoriasis in man [11]. The mouse tail test has been used to evaluate anti-psoriatic activity on epidermal differentiation, crucially disturbed in psoriasis [12]. Previous studies have shown that the adult mouse tail possesses a granular layer and exhibits orthokeratosis only in perifollicular areas of the epidermis. The areas of epidermis between the follicles in adult mice do not possess a granular layer and exhibit parakeratosis [13].

Traditional treatments for psoriasis contain topical agents (betamethasone, calcipotriol, tazarotene, dithranol, tacrolimus) [13], phototherapy (PUVA) and systemic therapy (methotrexate, acitretin, cyclosporine). Targeted immunobiological therapies including alefacept, efalizumab and etanercept have a great improvement in the treatment of moderate-to-severe psoriasis [2]. Topical treatment is still a fundamental and very current mainstay in the management of mild or moderate psoriasis [14].

Gambogic acid (GA) is the main active compound isolated from the resin of the Garcinia hurburyi tree-in Southeast Asia. It has a long history of medicinal use in Southeast Asia, and it is used as detoxification, homeostasis, anti-inflammatory, and parasiticide medicines for thousands of years. Recent studies showed that GA could inhibit angiogenesis and may be a viable drug candidate in anti-angiogenesis therapies [15].

Until recently, no information of the effectiveness of GA in psoriasis has been reported. In this study, we reported the preparation of GA cream and investigation of anti-psoriatic effects of GA cream in K14-VEGF transgenic model, psoriasis-like model of guinea pigs and mouse-tail model. We found topical administration of GA cream for those three psoriasis-like models could result in an anti-psoriatic efficacy.

2. Materials and methods

2.1. Materials

GA (≥98% in purity) was provided by our laboratory. Stearic acid, glycerin monostearate, white vaseline, liquid paraffin, glycerin, water-soluble azone, SDS and propylparaben were purchased from Changzheng chemical factory (China). DMSO was purchased from Sigma-Aldrich (USA). Primary antibodies including anti-mouse VEGFR2 and p-VEGFR2 were purchased from Cell Signaling Technology (USA). Anti-mouse CD31 was ordered from Sino Biological Inc. Anti-mouse CD54 and CD62E was ordered from Santa Cruz Biotechnology (USA). Anti-human p65 antibody was also purchased from Santa Cruz Biotechnology. Alexa Fluor 488-labeled Goat Anti-mouse IgG and DAPI were ordered from Beyotime. Anti-mouse CD3 was ordered from Bidegen. Anti-mouse IL-17 and IL-22 was purchased from Beijing Biosynthesis Biotechnology.

2.2. Animals

K14-VEGF transgenic mice were purchased from Jackson Laboratories (Bar Harbor, ME, USA). Transgenic animals over-expressing VEGF-A164 in the epidermal basal layer were generated on FVB background by inserting an expression cassette containing the human K14 promoter and the gene encoding murine VEGF-A164 using the pronuclear microinjection technique. Guinea pigs were purchased from Western China Experimental Animal Center. FVB/n mice and BALB/c mice were purchased from Beijing HFK Bioscience Co. Ltd. (HFK). All studies involving mice were approved by the Institutional Animal Care and Use Committee.

2.3. Preparation of GA cream

GA cream can be obtained by preparing oil phase by melting at 90 °C a mixture containing stearic acid, glycerin monostearate, white vaseline, liquid paraffin, addition of GA (0.1%, 0.25%, 0.5%), followed by adding it with stirring to water phase temperature-controlled at 90 °C obtained from an aqueous mixture containing glycerin, distilled water, water-soluble azone, SDS and propylparaben. Wherein homogeneous mixture of oil phase and water phase is provided and the temperature of the mixture is lower to room temperature with constant stirring. The cream base was prepared the same way without GA.

2.4. Cell culture

Human keratinocyte cell line HaCaT cells (American Type Culture Collection) and HUVEC cells were cultured in DMEM supplemented with 10% heat inactivated fetal bovine serum (FBS), 2 mM glutamine, and 100 U/ml penicillin and 100 μg/ml streptomycin, and maintained at 37 °C in a humidified incubator with 5% CO_2.

2.5. Cell growth inhibition assay

In vitro growth inhibition of GA for HaCaT cells was assessed using an MTT assay. Briefly, cells were seeded in a 96-well plate at a density of 5×10^4 ml^{-1} and cultured for 24 h to allow them to adhere to the plate. Cells were exposed in presence or absence with different concentrations of GA (0.0625–1 μmol/l) in DMEM medium. The plates were placed at 37 °C for 24 h. Then a volume of 10 μl of 12 mM MTT (5 mg/ml) was taken for cell incubation performed at 37 °C for 4 h in the darkness. The supernatant were decanted and washed with phosphate-buffer saline solution (PBS). The produced formazan salts were dissolved with DMSO, and the absorbance was measured at 570 nm using a Spectramax M5 Microtiter Plate Luminometer (Molecular Devices) to estimate the formazan concentration.

2.6. Immunofluorescence

To examine the inhibitory activity of GA on TNF-α, NF-κB activity was assessed by immune-localization of p65 in nuclei of HUVEC cells. HUVECs were treated with GA (0.2 μM) for 24 h and then together with 20 ng/ml TNF-α (Prepro Tech) for 1 h. Cells without any treatment were as control. For immunofluorescent staining of p65, the cells were fixed with 4% paraformaldehyde for 10 min, and then the cells were washed with PBS and treated with PBS containing 0.05% of Triton X-100 and 5% serum for 2 h and then washed with PBS. Then, mouse anti-human p65 antibody (diluted 1:100) was incubated with the cells for overnight at 4 °C. After incubation of the primary antibody, the cells were washed with PBS and then incubated with goat anti-mouse FITC antibody (diluted 1:800) for 2 h at 4 °C. For nuclear staining, the cells were incubated with DAPI (diluted 1:5) for 10 min. The pictures were taken by fluorescence microscopy (Olympus, Tokyo, Japan).

2.7. Anti-psoriatic efficacy evaluation of GA cream on K14-VEGF transgenic mice model

The female K14-VEGF transgenic homozygous mice (3 months old) with moderate psoriasis were chosen for the experiment. The