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Tripterine alleviates LPS-induced inflammatory injury by up-regulation of miR-146a in HaCaT cells

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ABSTRACT

Background: Corticosteroid-dependent dermatitis (CDD) is an inflammation caused by long-term repeated inappropriate external use of corticosteroids. Tripterine is a natural product from the root of *Tripterygium wilfordii* Hook.f. This study aimed to explore the effect and mechanism of tripterine on LPS-induced inflammatory injury in HaCaT cells.

Methods: In this study, different concentrations (0, 1, 2, 3 and 4 μM) of tripterine and 10 μM of LPS were used to treat HaCaT cells. The expression of miR-146a was altered by transfection. Cell viability, apoptosis, and the release of pro-inflammatory cytokines (IL-6 and TNF-α) were detected by CCK-8, flow cytometry analysis, qRT-PCR and ELISA, respectively. The expression levels of apoptosis-related and JNK/ NF-κB-related proteins were tested by western blotting.

Results: We found that 3 and 4 μM of tripterine significantly decreased cell viability. Tripterine alleviated LPS-induced reduction of cell viability, increase of apoptosis and the release of IL-6 and TNF-α in HaCaT cells. miR-146a was down-regulated by LPS exposure, while tripterine attenuated this impact. Further, inhibition of miR-146a abolished the protective effect of tripterine on cell damage triggered by LPS. Finally, tripterine deactivated JNK and NF-κB pathways through up-regulation of miR-146a.

Conclusion: These results demonstrated that tripterine could attenuate LPS-induced inflammatory injury and deactivate JNK and NF-κB pathways by up-regulation of miR-146a. This study will provide a theoretical basis for further study of tripterine in CDD.

1. Introduction

Corticosteroid-dependent dermatitis (CDD) is the non-purulent inflammation caused by drug dependence due to the long-term external use of hormone preparation in patients [1]. The main etiology of this disease is hormone abuse caused by the lack of understanding of hormone preparations, improper selection of drugs, inappropriate medication site or too long time [1,2]. The clinical manifestations of CDD include epidermis and dermal thinning, pigmentation, blood vessels exposure, local itching and burning, acne-like dermatitis and folliculitis, and even induces fungal and bacterial infection [3–5]. Therefore, it is necessary to completely disable hormone topical preparations, and select other more suitable non-hormonal drugs, such as pimecrolimus combined with photodynamic therapy and tacrolimus ointment [6,7]. Chinese doctors often use traditional Chinese medicine to cure CDD, of which tripterygium glycosides is one of the commonly used drugs in China [8,9]. However, there are few studies on the mechanism of drug therapy for CDD.

Tripterine (CAS: 34157-83-0, $C_{29}H_{38}O_4$), also named as 'celastrol', is a pentacyclic triterpenoid isolated from the root of *Tripterygium wilfordii* Hook.f; it belongs to the family of quinone methides [10,11]. At the meanwhile, it is also one of the effective components of tripterygium wilfordii tablet and tripterygium glycosides [12]. Accounting researches have proved that tripterine exhibits anti-oxidant, anti-inflammatory, anti-cancer and insecticidal activities [10,13–15]. For example, tripterine inhibits cell invasion and induces apoptosis in laryngeal carcinoma and breast cancer [16,17]. It suppresses the inflammatory response of macrophages and protects mice from skin inflammation [10]. Furthermore, tripterine prevents endothelial barrier dysfunction by inhibiting endogenous peroxynitrite formation [18]. However, the mechanism and role of tripterine in the treatment of CDD remains unclear.

microRNAs (miRNAs) are small non-coding RNA molecules found in plants, animals and some viruses. They contain about 22 nucleotides and regulate gene expression in RNA silencing and post-transcription [19]. miRNAs can inhibit translation and degradation from mRNA

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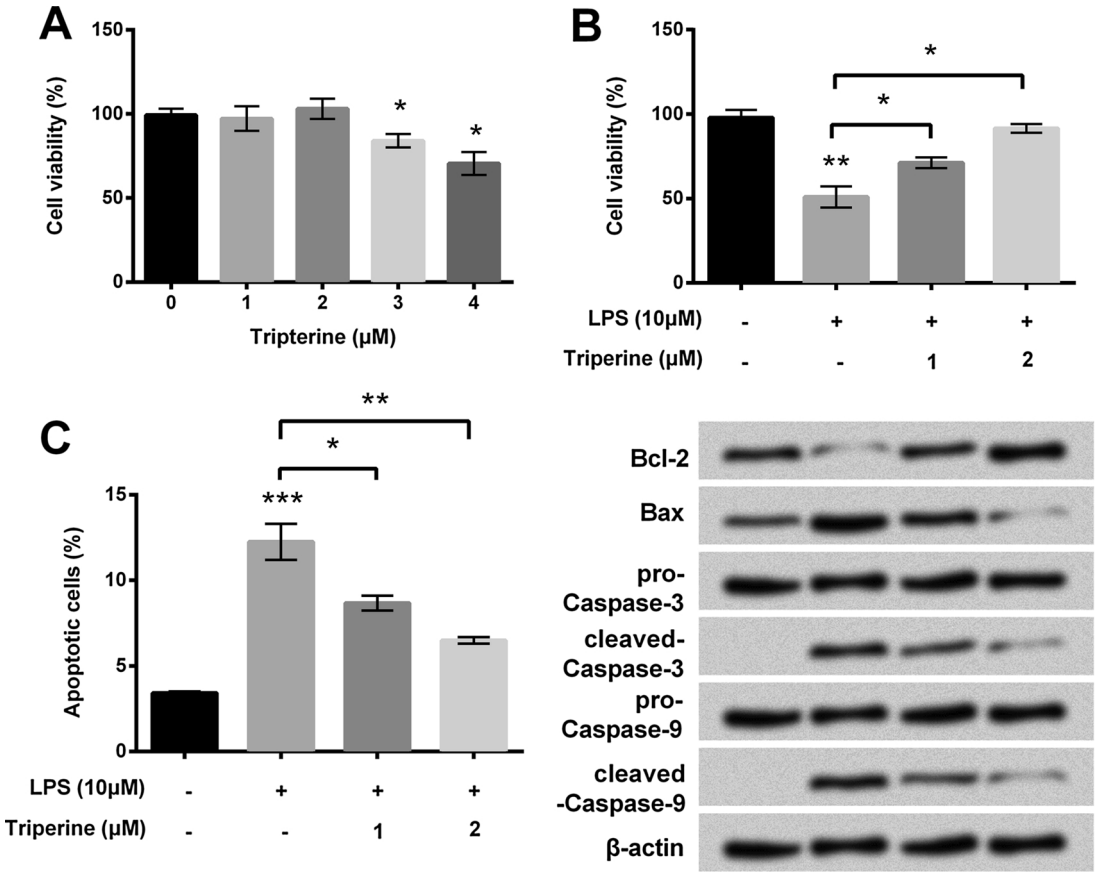

Fig. 1. Tripterine alleviated LPS-induced apoptosis in HaCaT cells. Cells were treated with 10 μM of LPS. (A–B) Cell viability was detected at the different concentrations of tripterine (0, 1, 2, 3 and 4 μM) by CCK-8 assay. (C) Apoptosis was measured by flow cytometry analysis and western blotting. *$p < 0.05$; **$p < 0.01$; *** $p < 0.001$. LPS, lipopolysaccharide; CCK-8, Cell Counting Kit-8.

transcripts by binding the 3′ untranslated regions (3′UTR), leading to negative regulation of the gene expression [20]. According to Ramasamy et al, the regulation of miRNAs with apoptotic regulatory potential is one of the key factors in determining pathology of diseases [21]. Therefore, miRNAs have recently become a hotspot in the study of various disease mechanisms. Many studies have reported that miR-146a is involved in the development, differentiation and regulation of various inflammatory diseases, such as osteoarthritis [22].

Thus, in this study, we aimed to investigate the effect and mechanism of tripterine pretreatment on LPS-induced inflammatory injury in HaCaT cells. Meanwhile, the regulatory relationship of tripterine and miR-146a were detected.

2. Materials and methods

2.1. Cell culture and treatment

The HaCaT cells were purchased from CLS Cell Lines Service GmbH (Eppelheim, Germany) and maintained in Dulbecco's Modified Eagle's Medium/Ham's F12 nutrient medium (DMEM/F12; 1:1; Sigma–Aldrich Co. LLC, St. Louis, USA) supplemented with 10% fetal bovine serum (FBS; Sigma–Aldrich Co. LLC) at 37 °C (5% CO_2). Cells were treated by 10 μM of lipopolysaccharide (LPS; Sigma–Aldrich Co. LLC) for 6 h. Tripterine (C0869) was purchased from Sigma–Aldrich Co. LLC and dissolved in dimethyl sulphoxide (DMSO; Sigma–Aldrich Co. LLC) with a final concentration of less than 0.1% in the culture medium. Cells were treated by tripterine for 4 h in a series of concentrations (0, 1, 2, 3 and 4 μM).

2.2. Cell transfection

In order to detect the effect of miR-146a on HaCaT cells, we synthesized miR-146a inhibitor and its scrambled control (SC) through GenePharma Co. (Shanghai, China). HaCaT cell transfections were conducted by Lipofectamine 3000 Reagent (Invitrogen Technologies, Carlsbad, USA) according to the manufacturer's instruction. The miRNA sequences used in this procedure were listed as follows. miR-146a inhibitor: 5′-AACCCAUGGAAUUCAGUUCUCA-3′. SC: 5′-CAGUACUUUU GUGUAGUACAA-3′.

2.3. Cell viability assay

HaCaT cells (5×10^3 cells/well) were seeded in 96-well plate and cultured at 37 °C (5% CO_2) for 48 h. Cell viability was assessed by Cell Counting Kit-8 (CCK-8, Dojindo Molecular Technologies, Gaithersburg, MD, USA) after LPS and tripterine treated cells. Actually, the culture medium was added CCK-8 solution and incubated at 37 °C (5% CO_2) for 1 h. The absorbance was measured at 450 nm by a Thermo Scientific Microplate Reader (Thermo Fisher Scientific, Waltham, MA, USA).

2.4. Apoptosis assay

HaCaT cells (5×10^3 cells/well) were seeded in 96-well plate and cultured at 37 °C (5% CO_2) for 48 h. Annexin V-FITC/PI Apoptosis Detection Kit (Sigma–Aldrich Co. LLC) was used to analyzed apoptosis. Briefly, cells were washed in phosphate buffered saline (PBS) twice and were stained in Annexin V-FITC/PI in the presence of 50 μg/mL RNase A (Sigma–Aldrich Co. LLC), and then incubated for 1 h at room temperature in the dark. The incubated cells were assessed by an Attune