Pharmacological Research xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Pharmacological Research

journal homepage: www.elsevier.com/locate/yphrs

Invited Perspective

Topical administration of reversible SAHH inhibitor ameliorates imiquimod-induced psoriasis-like skin lesions in mice via suppression of TNF-α/IFN-γ-induced inflammatory response in keratinocytes and T cell-derived IL-17

Ze-Min Lin[a,1], Meng Ma[a,1], Heng Li[b,c,1], Qing Qi[a], Yu-Ting Liu[b,c], Yu-Xi Yan[b,c], Yun-Fu Shen[a], Xiao-Qian Yang[b], Feng-Hua Zhu[b], Shi-Jun He[b,c,*], Wei Tang[b,c,*], Jian-Ping Zuo[a,b,c,*]

[a] *Laboratory of Immunology and Virology, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China*
[b] *Laboratory of Immunopharmacology, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China*
[c] *University of Chinese Academy of Sciences, No.19A Yuquan Road, Beijing 100049, China*

ARTICLE INFO

Article history:
Received 10 April 2017
Received in revised form 2 November 2017
Accepted 11 November 2017
Available online xxx

Keywords:
Reversible *S*-adenosyl-L-homocysteine hydrolase inhibitor
Keratinocyte
Inflammation
Interleukin-17
Imiquimod
Psoriasis

ABSTRACT

DZ2002, a reversible *S*-adenosyl-L-homocysteine hydrolase (SAHH) inhibitor with immunosuppressive properties and potent therapeutic activity against various autoimmune diseases in mice. The present study was designed to characterize the potential therapeutic effects of DZ2002 on murine model of psoriasis and reveal the correlated mechanisms. In this report, we demonstrated that *in vitro*, DZ2002 significantly decreased the expression of pro-inflammatory cytokines and adhesion molecule including IL-1α, IL-1β, IL-6, IL-8, TNF-α and ICAM-1 by inhibiting the phosphorylation of p38 MAPK, ERK and JNK in TNF-α/IFN-γ-stimulated HaCaT human keratinocytes. Topical administration of DZ2002 alleviated the imiquimod (IMQ)-induced psoriasis-like skin lesions and inflammation in mice, the therapeutic effect was comparable with the Calcipotriol. Moreover, the inflammatory skin disorder was restored by DZ2002 treatment characterized by reducing both of the $CD3^+$ T cell accumulation and the psoriasis-specific cytokines expression. Further, we found that DZ2002 improved IMQ-induced splenomegaly and decreased the frequency of splenic IL-17-producing T cells. Our finding offered the convincing evidence that SAHH inhibitor DZ2002 might attenuate psoriasis by simultaneously interfering the abnormal activation and differentiation of keratinocytes and accumulation of IL-17-producing T cells in skin lesions.

1. Introduction

Psoriasis is a multi-factorial skin disease with a complex pathogenesis. The disease is characterized by focal formation of inflamed, raised plaques that constantly shed scales derived from excessive growth of skin epithelial cells [1]. A consensus now emerges that the cross-talk between epithelial cells and immune cells shapes and maintains the inflammatory milieu. The psoriatic lesions involve hyperproliferation and abnormal differentiation of epidermal keratinocytes, lymphocyte infiltration consisting mostly of T lymphocytes, and various endothelial vascular changes in the dermal layer [2,3].

In the skin, cytokines and chemokines are produced both by resident cells such as keratinocytes (KCs), Langerhans cells (LCs), mast cells, as well as by infiltrated cells like lymphocytes and

Abbreviations: SAHH, *S*-adenosyl-L-homocysteine hydrolase; IMQ, imiquimod; KC, keratinocytes; IL, interleukin; ICAM-1, intercellular adhesion molecule-1; EGFR, epidermal growth factor receptor; MAPKs, mitogen activated protein kinases; ERK, extracellular signal-regulated kinase; JNK, c-Jun NH2-terminal kinase; APC, antigen-presenting cell; T17, IL-17-producing $CD3^+$ T cells; Th17, $CD4^+$ IL-17^+ T cell; Tc17, $CD8^+$ IL-17^+ T cell; CCL, chemokine CC ligand; CCR, chemokine CC receptor.

* Corresponding authors at: Laboratory of Immunopharmacology, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zuchongzhi Raod, Zhangjiang, Shanghai, 201203, China.
E-mail addresses: heshijun@simm.ac.cn (S.-J. He), tangwei@simm.ac.cn (W. Tang), jpzuo@simm.ac.cn (J.-P. Zuo).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.phrs.2017.11.012
1043-6618/

neutrophils [4,5]. It is now well accepted that KCs may facilitate or promote an amplification of inflammatory response with the additional production of tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ), in the initiation of various skin diseases [6]. TNF-α and IFN-γ are called the primary inflammatory factors, as they induce the synthesis of secondary inflammatory cytokines and chemokines [4]. Stimulation of KCs with TNF-α and IFN-γ induces the expression of pro-inflammatory cytokines and chemokines such as interleukin (IL)-6, IL-8, IL-1β, TNF-α, as well as adhesion molecules intercellular adhesion molecule-1 (ICAM-1). These mediators contribute to the subsequent recruitment of immune cells into inflammatory lesions in the skin [7,8].

A common feature of hyperproliferative skin disorders, including psoriasis, is epidermal hyperplasia and thickening. By sustained release of inflammatory mediators from activated T cells and ongoing traffic of T cells in the epidermis, there would be a continuous set of signals for chronic epidermal hyperplasia in psoriatic lesions [9,10]. Moreover, TNF-α and IFN-γ are potent inducers of epidermal growth factor (EGF) family factors and EGF receptors (EGFR), and the consequence activation of EGFR signal transduction cascade leading to epidermal proliferation through the persistent induction of mitogen activated protein kinases (MAPKs) pathway such as extracellular signal-regulated kinase (ERK), p38 MAPK and c-Jun NH2-terminal kinase (JNK) [11].

The pathophysiology of psoriasis is complex and dynamic, involving particular epidermal KCs and residing or recruited immune cells in the skin. It was previously assumed that KC hyperproliferation associated with abnormal epidermal differentiation was the primary cause of the disease. Nevertheless, recent studies have defined a role for the interactive network of immune cells and cytokines in disease pathogenesis. The responses of KCs upon stimulation with cytokines produced by immune cells, actively maintain inflammatory microenvironments and sustain plaque development [9,12]. One of the chief cell types in the dermal infiltrates is $CD3^+$ T cells, in psoriatic lesions. Among the pro-inflammatory cytokines produced by T cells, both IL-17 and IFN-γ are up-regulated in disease skin, yet they regulate distinct psoriasis-related gene sets from KCs, and playing unique roles in disease pathogenesis [13]. Although this is the case, the inhibition of IL-17 alone showed desired clinical results in patients with psoriasis, suggesting a critical character of IL-17 in psoriasis pathogenesis [12,14].

S-adenosyl-L-homocysteine hydrolase (SAHH) and its substrate S-adenosyl-L-homocysteine (SAH) are deeply involved in the process of transmethylation mediated by S-adenosylmethionine (SAM) [15,16]. Immune cells are especially subject to transmethylation [17], and modifications of both DNA and protein by methylation are key factors in T and B cell immune responses in the development of inflammatory disease[18]. Since blockade of SAHH results in accumulation of SAH and global inhibition of methyltransferases activity, the immunosuppressive properties of SAHH inhibitors have been well known for years. The irreversible type I SAHH inhibitors have been reported to be effective in T-cell mediated immune responses *in vivo* and *in vitro* [19–21]. Previous studies from our team and other researchers showed that the reversible type III SAHH inhibitor DZ2002 [methyl-(adenin-9-yl)-2-hydroxybutanoate] has an immunomodulatory activity and to alleviate disease in several inflammatory and autoimmune animal models, by reducing pro-inflammatory cytokine production from macrophage [22], inhibiting antigen-induced specific immune responses [23], suppressing T cell activation [24] and regulating Toll-like receptor (TLR)-triggered antigen-presenting cells (APCs) functions [25].

In the present study, we reported that DZ2002 suppressed the TNF-α/IFN-γ-elicited inflammatory response in human keratinocytes through inhibition of the ERK/p38 MAPK signaling pathways activation. In addition, we provided evidence to demonstrate that topical administration of DZ2002 improved the clinical and histological features of imiquimod (IMQ)-induced psoriasis-like inflammation in mice, a murine model highly resembles human psoriatic lesions phenotypically and histologically. These therapeutic effects may resulted from the efficient prevention of the inflammatory responses in keratinocyte and the restraint of IL-17-producing T cells by DZ2002.

Table 1
Sequences of primers used for quantitative real-time PCR.

Target gene	Primer sequence
h-IL-1α	F:5′-TGGTAGTAGCAACCAACGGGA-3′ R: 5′-ACTTTGATTGAGGGCGTCATTC-3′
h-IL-1β	F:5′-TTCGACACATGGGATAACGAGG-3′ R: 5′-TTTTTGCTGTGAGTCCCGGAG-3′
h-IL-6	F:5′-ACTCACCTCTTCAGAACGAATTG-3′ R: 5′-CCATCTTTGGAAGGTTCAGGTTG-3′
h-IL-8	F:5′-TTTTGCCAAGGAGTGCTAAAGA-3′ R: 5′-AACCCTCTGCACCCAGTTTTC-3′
h-TNF-α	F:5′-CCTCTCTCTAATCAGCCCTCTG-3′ R: 5′-GAGGACCTGGGAGTAGATGAG3′
h-ICAM-1	F: 5′-ATGCCCAGACATCTGTGTCC-3′ R: 5′-GGGGTCTCTATGCCCAACAA-3′
h-GAPDH	F: 5′-GGAGCGAGATCCCTCCAAAAT-3′ R: 5′-GGCTGTTGTCATACTTCTCATGG-3′
m-TNF-α	F: 5′-CAGGCGGTGCCTATGTCTC-3′ R: 5′-CGATCACCCCGAAGTTCAGTAG-3′
m-IFN-γ	F: 5′-GCCACGGCACAGTCATTGA-3′ R: 5′-TGCTGATGGCCTGATTGTCTT-3′
m-IL-17	F: 5′-TTTAACTCCCTTGGCGCAAAA-3′ R: 5′-CTTTCCCTCCGCATTGACAC-3′
m-IL-6	F:5′-CTGCAAGAGACTTCCATCCAG-3′ R: 5′-AGTGGTATAGACAGGTCTGTTGG-3′
m-IL-1α	F:5′-CGAAGACTACAGTTCTGCCATT-3′ R: 5′-GACGTTTCAGAGGTTCTCAGAG-3′
m-IL-1β	F:5′-GAAATGCCACCTTTTGACAGTG-3′ R: 5′-TGGATGCTCTCATCAGGACAG-3′
m-IL-8	F:5′-TCGAGACCATTTACTGCAACAG-3′ F:5′-CATTGCCGGTGGAAATTCCTT-3′
m-β-actin	F: 5′-GGCTGTATTCCCCTCCATCG-3′ R: 5′-CCAGTTGGTAACAATGCCATGT-3′

IL, Interleukin; TNF, tumor necrosis factor; IFN-γ, interferon gamma; ICAM, intercellular cell adhesion molecule; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; h-: human; m-: mouse; F: forward; R: reverse.

2. Material and methods

2.1. Cell culture

Immortalized human keratinocyte cell line HaCaT (ATCC, Rockville, MD, USA) was maintained in Dulbecco's modified Eagle's medium (DMEM, Hyclone Laboratories, Logan, Utah, USA) supplemented with 10% fetal bovine serum (FBS, Hyclone Laboratories, Logan, Utah, USA) and antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin, purchased from North China Pharmaceutical, Hebei, China). The cells were incubated in a humidified incubator with 5% CO_2 at 37 °C and cells were subcultured twice weekly.

Primary normal human epidermal keratinocytes (NHEK, single donor) were purchased from PromoCell (Heidelberg, Germany) and maintained in Keratinocyte Growth Medium 2 (PromoCell, Heidelberg, Germany) supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin. Cells were cultured at 37 °C in a humidified incubator of 5% CO2 and were subcultured weekly using the Accutase solution (PromoCell, Heidelberg, Germany).

Please cite this article in press as: Z.-M. Lin, et al., Topical administration of reversible SAHH inhibitor ameliorates imiquimod-induced psoriasis-like skin lesions in mice via suppression of TNF-α/IFN-γ-induced inflammatory response in keratinocytes and T cell-derived IL-17, Pharmacol Res (2017), https://doi.org/10.1016/j.phrs.2017.11.012