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Resolvin D1 attenuates imiquimod-induced mice psoriasiform dermatitis through MAPKs and NF-κB pathways

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ABSTRACT

Background: Resolvin D1 (RvD1), a pro-resolution lipid mediator derived from docosahexaenoic acid (DHA), has been described to promote several kinds of inflammatory resolution. However, the effects and anti-inflammatory mechanisms of RvD1 on psoriasis have not been previously reported.
Objective: The present study aimed to determine the protective effects and the underlying mechanisms of RvD1 on imiquimod (IMQ)-induced psoriasiform dermatitis.
Methods: Mice were topically treated with IMQ to develop psoriasiform dermatitis on their shaved back, pretreated intraperitoneally (i.p.) with or without RvD1 or *tert*-butoxycarbonyl Met-Leu-Phe peptide (Boc), a lipoxin A4 (ALX) receptor antagonist. The severity was monitored and graded using a modified human scoring system, the Psoriasis Area and Severity Index (PASI), histopathology, and the signature cytokines of psoriasis (IL-23, IL-17, IL-22 and TNF-α). The mRNA and protein levels of inflammatory cytokines were quantified by quantitative real-time PCR (QRT-PCR) and ELISA. The expressions of signaling proteins MAPKs and NF-κB p65 were analyzed using western blotting. Electrophoretic mobility shift assay (EMSA) was used to check NF-κB p65 DNA binding activity.
Results: Our study showed that RvD1 alleviated IMQ-induced psoriasiform dermatitis and improved skin pathological changes. RvD1 markedly inhibited IMQ-induced activation of ERK1/2, p38, JNK (c-Jun N-terminal protein kinase, a subfamily of MAPKs), and NF-κB. Furthermore, pretreatment with Boc, would not exacerbate skin inflammation of IMQ-induced mice, but significantly reversed the beneficial effects of RvD1 on IMQ-induced psoriasiform inflammation.
Conclusion: RvD1 can obviously improve skin inflammation in IMQ-induced mice psoriasiform dermatitis. The protective mechanisms might be related to its selective reaction with lipoxin A4 receptor/Formyl-peptide receptor 2 (ALX/FPR2), by downregulating relevant cytokines of the IL-23/IL-17 axis expression, the inhibition of MAPKs and NF-κB signaling transduction pathways. Thus, these results show that RvD1 could be a possible candidate for psoriasis therapy.

1. Introduction

Psoriasis is a common, immune-mediated, chronic inflammatory skin disease of man. The prevalence ranges from 0% to 8.5%. The occurrence of psoriasis varies according to age and geographic region [1–3]. Psoriasis is characterized by hyperproliferative keratinocytes, parakeratosis, hyperkeratosis, and massive infiltration of inflammatory leukocytes into the dermis and epidermis. The disease severely affects quality of life. The typical histological traits encompass hyperkeratosis of the epidermis, acanthosis,

Abbreviations: Boc, tert-butoxycarbonyl Met-Leu-Phe peptide; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IL-17, interleukin-17; IL-22, interleukin-22; IL-23, interleukin-23; IMQ, imiquimod; MAPKs, mitogen-activated protein kinases; NF-κB p65, nuclear factor kappa B p65; PASI, Psoriasis Area and Severity Index; QRT-PCR, quantitative real-time PCR; RvD1, resolvin D1; TNF-α, tumor necrosis factor alpha.

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elongation of dermal capillary vessels, and infiltration of inflammatory leukocytes into the dermis and epidermis, which are clinically reflected by erythema, scaling, and thickening. Although much progress has been made in elucidating the pathological mechanisms of psoriasis, the underlying mechanisms are still not fully understood, and thus, so far there are no perfect treatment methods.

Traditional treatments include psoralen plus UVA irradiation (PUVA), acitretin, methotrexate, and cyclosporine, but they have limited therapeutic effects. Therefore, investigating the mechanisms of pathogenesis and developing effective treatments for psoriasis are still necessary. Resolvin D1 (RvD1, 7S, 8R, 17S-trihydroxy-4Z, 9E, 11E, 13-Z, 15E, 19Z-docosahexaenoic acid), a novel lipid mediator derived from docosahexaenoic acid (DHA), was initially discovered in resolving exudates of mice [4,5]. ALX/FPR2 and G protein-coupled receptor-32 (GPR32) are two specific receptors for RvD1. RvD1 specifically interacts with both ALX and GPR32 on phagocytes and this suggests that each plays a role in resolving acute inflammation through relative signal transduction pathways [6]. The effects of RvD1 on inflammation are mostly mediated by these two receptors. Aryl hydrocarbon receptor (AhR) has a direct role in IL-22 production by Th17 cells in mouse ear skin [7].

Previous studies have shown that RvD1 has potent anti-inflammatory pro-resolution actions in several disease models including peritonitis, inflammatory corneal hemangiogenesis, ischemia-reperfusion-induced lung injury, and kidney injury [4,5,8–11]. Recent studies have demonstrated that DHA (which may be converted in vivo via lipoxygenase-initiated mechanisms to 17S hydroxy-containing RvD1) could modulate the balance between pro- and anti-inflammatory cytokines, alter the response of the host to pulmonary bacterial infection, and affect the early outcome of infection [12,13]. Resolvins induce the hallmark functions of resolution of inflammation including prevention of neutrophil penetration and phagocytosis of apoptotic neutrophils, thus enhancing clearance of inflammation within psoriatic lesions and promoting tissue regeneration [14]. An ALX receptor antagonist *tert*-butoxycarbonyl Met-Leu-Phe peptide (Boc) blocks receptor activation by RvD1 [6].

To observe the effects of RvD1 in psoriasis, we took advantage of the recently described IMQ-induced psoriasis mouse model [15]. IMQ is a TLR-7/8 agonist and a potent immune activator widely used for the treatment of certain malignant skin diseases and virus-associated skin diseases. As a side effect, it can induce and exacerbate psoriasis in predisposed humans [16,17]. IMQ-induced psoriasiform dermatitis in mouse is a well-recognized model of psoriasis, closely resembling human psoriatic lesions phenotypically and in many important pathological aspects [15]. Importantly, studies have suggested that the development of IMQ-induced psoriasiform dermatitis is critically dependent on the IL-23/IL-17 cytokine axis [15,18]. Activation of MAPKs (p38, JNK and ERK1/2) signaling pathway plays key roles in regulating the production of multiple important inflammatory mediators in psoriasis, such as TNF-α and IL-23 [19]. While certain interleukins can activate intracellular signaling pathways (such as NF-κB), thus promoting downstream effector expression (such as ICAM) [20]. ICAM-1 can obviously be induced in a variety of cell types by various proinflammatory cytokines such as TNF-α. It plays a central role in tissue recruitment of leukocytes [21] and likely contributes to psoriasis. Keratinocytes can also express ICAM-1, which increases leukocyte infiltration at affected areas [22,23]. This infiltration is one of the most critical steps involved in the development of psoriasis. TNF-α could upregulate the expression of ICAM-1. Clinical manifestations of psoriasis can be improved by downregulating ICAM-1 via the inhibition of TNF-α. TNF-α induces the upregulation of ICAM-1 expression in HaCaT cells [24]. A. Mitsui et al. have reported that the clinical outcome of ICAM-$1^{-/-}$ mice (mice lacking ICAM-1), after topical treatment with IMQ for six consecutive days, was relieved as compared to wild type mice [25]. Therefore, ICAM-1 provides a potentially new strategy against psoriasis [24]. However, few data are available on the role of RvD1 in IMQ-induced skin inflammation. In this study, based on above-mentioned effects of RvD1, we investigated whether RvD1 could exert protective effects on IMQ-induced psoriasiform dermatitis and further revealed its underlying mechanisms.

The goal of this study was to assess the effects of RvD1, a potent anti-inflammatory agent, on IMQ-induced psoriasiform skin inflammation and consequently to evaluate its potential as a new agent in the development of novel therapeutic options.

2. Materials and methods

2.1. Animals

Male BALB/c-Mice at 6–8 weeks of age (weight range 18–20 g) were purchased from Huafukang (Beijing, China). The animals were bred and housed under specific pathogens-free conditions of 12-h light-dark rhythm, 24–26 °C ambient temperature, and 55% humidity. They were fed with a standard laboratory diet and water. They were acclimatized for at least 1 week before use. All experimental animals used in the present study were under a protocol approved by the animal care and use committee of Tongji Medical College of Huazhong University of Science and Technology.

Table 1
Primer sequences for real-time quantitative PCR.

Targeted gene	5′-3′ primer sequence	Product size(bp)	reference
IL-17A	F: TTTAACTCCCTTGGCGCAAAA R: CTTTCCCTCCGCATTGACAC	165	primer bank
IL-17F	F: TGCTACTGTTGATGTTGGGAC R: AATGCCCTGGTTTTGGTTGAA	161	primer bank
IL-23p19	F: ATGCTGGATTGCAGAGCAGTA R: ACGGGGCACATTATTTTTAGTCT	213	primer bank
IL-22	F: ATGAGTTTTTCCCTTATGGGGAC R: GCTGGAAGTTGGACACCTCAA	124	primer bank
TNF-α	F: GACGTGGAACTGGCAGAAGAG R: TTGGTGGTTTGTGAGTGTGAG	228	primer bank
ICAM-1	F: GTGATGCTCAGGTATCCATCCA R: CACAGTTCTCAAAGCACAGCG	213	primer bank
GAPDH	F: AGGTCGGTGTGAACGGATTTG R: TGTAGACCATGTAGTTGAGGTCA	123	primer bank

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