International Immunopharmacology 64 (2018) 319–325

Contents lists available at ScienceDirect

International Immunopharmacology

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

Aromatic-turmerone ameliorates imiquimod-induced psoriasis-like inflammation of BALB/c mice

Yong-Liang Li[a], Zhi-Yun Du[a,*], Peng-Hui Li[a], Longjia Yan[a], Wei Zhou[a], Ya-Dong Tang[a], Guang-Rong Liu[c], Yan-Xiong Fang[a], Kun Zhang[a], Chang-Zhi Dong[a,d], Hui-Xiong Chen[a,b,**]

[a] Faculty of Light Industry and Chemical Engineering, Guangdong University of Technology, Guangdong 510006, China
[b] CNRS, UMR8601, Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, CBNIT, Université Paris Descartes, PRES Sorbonne Paris Cité, UFR Biomédicale, 75006 Paris, France
[c] Infinitus Company Ltd., 11 Sicheng Road, Tianhe District, Guangzhou 510000, China
[d] Université Paris Diderot, Sorbonne Paris Cité, ITODYS, UMR 7086 CNRS, 75013 Paris, France

ARTICLE INFO

Keywords:
Aromatic-turmerone
Psoriasis
Anti-inflammation
Proinflammatory cytokines
Imiquimod

ABSTRACT

Psoriasis is a usual immune-mediated inflammatory skin disease with undefined pathogenesis. Aromatic-turmerone (ATM) is a mainly constituent of essential oil from *Curcuma longa* L. It has been shown to exhibit strong anti-oxidant, anti-tumor activities and anti-inflammatory effects. In this study, we investigated the effects of ATM on imiquimod (IMQ)-induced psoriasis-like BALB/c mice and its molecular mechanisms for anti-inflammatory effect. ATM showed inhibition of the transfer of $CD8^+$ T cells in epidermis, and reduced expression of NF-κB and COX-2 as well as phosphorylation of p38 MAPK. It also decreased the level of TNF-α and IL-6, and down-regulates IL-17 IL-22 and IL-23 mRNA synthesis. Notably, we demonstrated that topically applied ATM alleviated skin inflammation in IMQ-induced mice. These results indicate that ATM, a natural active compound exhibits anti-inflammatory activity and is a promising candidate molecule to treat inflammatory skin diseases, such as psoriasis.

1. Introduction

Psoriasis is an immune-mediated inflammatory skin disease, one of the most common chronic diseases effected more than 125 million people but onset mainly arises in the 18 to 39 years of age or 50–69 years of age in worldwide [1,2]. The primary characteristic of psoriasis manifests on the skin and psoriasis lesions are characterized by hyperproliferation of epidermal keratinocytes associated with inflammatory cellular infiltrate in both dermis and epidermis. The major histological traits of psoriasis encompass acanthosis and hyperkeratosis of the epidermis, elongation of dermal capillary vessels and a lymphohistiocytic inflammation which are clinically reflected by erythema, infiltration and scaling. Psoriasis might also induced various comorbidities such as psoriatic arthritis, non-melanoma skin cancer, melanoma and lymphoma [3]. Current research suggested that immunological dysfunction plays a crucial role in the process of this disease. In particular, immune response mediated by T cells and keratinocytes has participated in the initiation and maintenance of psoriasis [4]. Numerous inflammatory cytokines produced by immune cells, including IL-6, IL-17, IL-22, IL-23, TNF-α and CCL20, contribute to the proliferation of keratinocytes [5]. However, the exact etiological and pathogenic mechanisms are still not fully clear [6]. The treatment of psoriasis has been developed by the introduction of biologic and small molecule inhibitor targeted therapy. Several of these therapies have been released and are available for general use, such as ixekizumab, brodalumab, guselkumab, secukinumab and methotreaxate, but they caused different degrees of side-effects [7–10]. Thus, it is urgent to develop another drug that is effective and does not induce side effects.

Aromatic-turmerone (ATM), as the basis in essential oil contented 61.79% from *Curcuma longa* L., which has been used in Southeast Asia both as medicinal purposes and food [11]. ATM was reported to exert positive modulation on murine dendritic cells [12], and to possess immunomodulatory, antinociceptive and anti-tumor activities [13–16], as well as anti-inflammatory effects by reducing the IFN-γ and IL-2 expression without affecting IL-4 production in $CD4^+$ T cell [17,18]. ATM could also reduce Aβ-induced neuroinflammatory molecules through blocking of NF-κB, JNK, and p38 MAPK signaling pathways [19]. However, there is no research about the effects and the

* Correspondence to: Z.-Y. Du, Guangdong University of Technology, China.
** Correspondence to: H.-X Chen, UFR Biomédicale, Université Paris Descartes, France.
E-mail addresses: zhiyundu@gdut.edu.cn (Z.-Y. Du), huixiong.chen@parisdescartes.fr (H.-X. Chen).

https://doi.org/10.1016/j.intimp.2018.09.015
Received 11 July 2018; Received in revised form 12 September 2018; Accepted 12 September 2018

mechanisms of ATM used in the treatment of psoriasis.

Imiquimod (IMQ) is an agonist of toll-like receptor 7/8 (TLR7/8), and a potent immune activator. Tropical treatment with IMQ in mice can induce psoriasis-like skin, which resembles human psoriatic lesions phenotypically and histologically [20]. In this study, we investigated the effects of ATM on IMQ-induced psoriasis-like BALB/c mice. We focused on the change of the infiltration of $CD8^+$ T, the expression of NF-κB, COX-2, and the phosphorylation of p38 MAPK as well as key cytokines including TNF-α, IL-6, IL-17, IL-22 and IL-23 induced by ATM. Together, these findings might provide novel insights into the anti-psoriasis mechanism of ATM.

2. Materials and methods

2.1. Materials

BALB/c mice (six to eight weeks, 20–25 g, female) were bought from Guangdong University of Chinese Medicine (Guangdong, China). Imiquimod cream was purchased from Sichuan Med-shine Pharmaceutical co., LTD (Sichuan, China). ATM (98%) was separated from curcuma oil, provided by LvYuan Natural spice oil refinery (Ji'an, China). Antibodies against phosphorylated p38 (p-p38), NF-κB (p65), CD8 alpha, COX2, TNF-α and IL-6 were purchased from ABcam (Cambridge, MA, USA), and β-actin was purchased from Beijing Biosynthesis Biotechnology CO., LTD (Beijing, China). Trizol Reagent and RevertAid First Strand cDNA Synthesis Kit were purchased from Thermo Fisher Scientific (Shanghai, China).

2.2. Mice treatments

BALB/c mice were randomly divided into four groups ($n = 4$), including the control group (C), the imiquimod-induced group (IMQ), the high dose of the intervention group (H) and the low dose of the intervention group (L). All mice were fed with formal forage and water for 2 days, and then shaved for an area of 5 cm × 4 cm from the backs of mice. All experiments were conducted and approved according to the principles of Guide for the Care and Use of Laboratory Animals in China.

The control group were applied with 60 mg/d Vaseline and the IMQ group were only applied with 60 mg/d 5% imiquimod cream topically once a day for 6 consecutive days. The high dose (H) group and the low dose (L) group were firstly applied imiquimod cream, and then, after 4 h applied topically with ATM dissolved in butanediol in a dosage of 40 and 0.4 mg/kg/d, respectively for 6 consecutive days. All groups' mice were killed on the sixth day, and the skins were collected for next experiments.

2.3. Evaluating the severity of skin inflammation

To evaluating the severity of inflammation of back skin, a clinical Psoriasis Area and Severity Index (PASI) as scoring system was used [21]. Scales, erythema and thickness were marked independently on a range from 0 to 4, the score of 0, 1, 2, 3 and 4 was meant the severity of none, slight, moderate, striking and very striking, respectively. A total score was applied to measure the severity of inflammation (score range: 0–12).

2.4. Histopathological and immunohistochemical examination

The back skin samples from each mouse were soaked in 4% paraformaldehyde for 24 h and embedded in paraffin. The tissues were stained by hematoxylin and eosin (H&E) after sectioned and observed under a microscope (BDS-DM500, Chongqing OP Tec Instrument CO. LTD, China). For immunohistochemistry examinations, the paraffin sections were treated with primary antibody: anti-NF-κB p65 (ab16502, Abcam), anti-CD8 alpha (ab17147, Abcam) and anti-COX2 (ab15191, Abcam), and then horseradish peroxidase (HRP) labeled anti-rabbit secondary antibodies (SH-0031, Ding Guo Changsheng Biotechnology Co. Ltd., China) were used. After incubating secondary antibodies, the DAB Kit (DA1010, Beijing Soledad Symbol Technologies LTD., China) was used. The image was collected with a microscope (Olympus IX71, Japan) and optical density was calculated by Image J software.

2.5. Western blotting

Back skins collected from BALB/c were kept in −80 °C and incubated in RIPA buffer on ice-bath for 30 min. The skin extractions were centrifuged at 12,000 rpm for 10 min at 4 °C. The supernatant was collected and protein concentration was measured by BCA kit for protein quantification. Equivalent protein (40 μg) from each sample was load to SDS-PAGE and transferred to PVDF membrane. The PVDF membranes were blocked with 5% (w/v) BSA in TBST and incubated with anti-NF-κB (p65), anti-TNF-α, anti-p-p38, anti-IL-6 and β-actin over night at 4 °C, subsequently washed using TBST, probed with specific secondary antibodies coupled to HRP. An enhanced chemiluminescence method (ECL) was used to detect the immunoreactive protein by ECL reagents and the signal was recorded by FuJi medical X-ray film.

2.6. Real-time polymerase chain reaction (RT-PCR)

Total RNA was extracted from collected back skin on BALB/c by using Trizol Reagent. The synthesis of cDNA on 2 μg RNA was using the RevertAid First Strand cDNA Synthesis Kit, and the expression of relative genes were measured with ABI Stepone plus Fast Real-Time PCR system by using a FastStart Universal SYBR Green Master (Rox) (Roche Diagnostics, China). Cycle parameters were as follows: 95 °C for 10 min; 40 cycles of 95 °C for 15 s, and 60 °C for 60 s. The GAPDH gene was used as a reference to normalize the data that was quantitatively analyzed by using the $2^{-\Delta\Delta Cq}$ method. The PCR primer sequences are listed in supplementary materials (Table S1).

2.7. Statistical method

The means ± standard error of mean (SEM) and one-way ANOVA were applied to statistical analysis, the data of RT-PCR was a mean value on three time repeated. Graph Pad Prism 5 software was used to analyze the experiments data and $P < 0.05$ was considered to be significance and produce the diagrams. Pictures were dealt in Photoshop CS5 software.

3. Result

3.1. ATM improves psoriatic lesions in a mouse model induced by IMQ

Topical application of imiquimod (IMQ) can induce and exacerbate psoriasis at both the local treated areas as well as distant sites which has led to the development of pre-clinical models of psoriasis [20]. Based on the results obtained from Tapinarof, a natural product that resolves skin inflammation in mice and humans [22], we initially investigated the effect of different doses of ATM (Fig. 1) on IMQ-induced psoriasis-like BALB/c mice for 6 consecutive days through structural features characteristic for psoriasis. Typical erythema, scaling and thickening were observed in the IMQ-induced skin lesions as compared to the control group (C) which did not show any sign of inflammation, while ATM significantly inhibited these pathological changes in a dose-dependent manner. Indeed, as shown in Fig. 1a, two or 3 days after the start of IMQ application, the back skin of the mice started to display signs of erythema, scaling, and thickening. From days 2–3 onward, inflammation was visible, which continually increased in severity up to the end of the experiment and was similar to human plaque psoriasis. However, in the intervention group treated with different dose of ATM (H and L), only a few thin scales, thickness and erythema were