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ERK inhibitor JSI287 alleviates imiquimod-induced mice skin lesions by ERK/IL-17 signaling pathway

Xiaoling Huang[a], Pengxia Yu[a], Minyu Liu[a], Yifang Deng[a], Yuqiong Dong[c], Quanhai Liu[a], Jintao Zhang[b], Tong Wu[a,*]

[a] State Key Laboratory of Drug Innovation and Pharmaceutical Technology, Shanghai Institute of Pharmaceutical Industry, China state Institute of Pharmaceutical Industry, Shanghai 200437, China
[b] JS InnoPharm (Shanghai) Ltd., Shanghai, China
[c] Shanghai Bioenergy Medicine Science &Technology Co., Ltd., Shanghai, China

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ABSTRACT

Many studies confirmed that the over-activation of RAF-MEK-ERK signaling pathway plays a central role in human cancers. To avoid drug resistance during cancer treatment, many researchers focused on the study of the downstream therapeutic target of RAF-MEK-ERK signaling pathway. Therefore, ERK1/2 became a hot anticancer target. It has been shown that ERK phosphorylation could activate Th17 cells and therefore induce inflammatory diseases. Due to these results, inhibition of ERK, as a potential drug target, could provide a solution for autoimmune diseases, especially T cell mediated diseases. In this study, a small synthetic molecule JSI287 was found with the function of alleviating IMQ-induced mice skin lesions through ERK/IL-17 signaling pathway during the screening of small molecule databases targeting ERK. The results showed that JS1287 small molecule alleviated epidermal thickness, epidermis congestion, edema and inflammatory cell infiltration, decreased release of inflammatory cytokines of IL-6, IL-12 and IL-17A, and further regulated the mRNA expression of ATF1 and protein expression of ERK1/2 in IMQ-induced skin lesions. Our study suggested that ERK inhibitor JSI287 could be a promising candidate for psoriasis treatment.

1. Introduction

Psoriasis is a chronic, recurrent and inflammatory skin disease, and it is characterized by erythema, skin hyperplasia, scales, and hyperproliferation of keratinocyte [1]. It is reported that the psoriasis occurrences are about 3% worldwide [2], but the number varies depending on ethnicity and geography [3].

The pathogenesis of psoriasis is associated with many factors, such as genetic polymorphisms, environmental factors and life style. It's reported that diverse populations have unique clinical presentation, genetic susceptibility, treatment response and drug adverse [4]. Although psoriasis was considered as a T cell mediated disease, recent studies shows its association with other T cell subsets such as Th17, Th22 and Treg cells during development [5]. Excessive activation of the mitogen-activated protein kinases (MAPK) pathway is a common cause of many diseases including autoimmune diseases and sustention of the extracellular signal-related kinases (ERK) phosphorylation activates T helper 17 cells [6,7]. Therefore, ERK signaling pathway is found to be related to psoriasis [8–10].

JSI287 is identified from the screening of small molecule databases targeting ERK kinase. The series of studies on IMQ-induced psoriasis model are conducted to explore the mechanism of JSI287 regrading to psoriasis treatment.

2. Material and methods

2.1. Drug and reagents

JSI-287 was synthesized by JS Innopharm (Shanghai) Ltd. (Fig. 1). IMQ 5% Cream was obtained from Sichuan Med-shine Pharmaceutical Co. Ltd., Chengdu, China.

Abbreviations: ANOVA, analysis of variance; ATF1, Activating Transcription Factor 1; ERK, the extracellular signal-related kinases; IMQ, imiquimod; LCK, lymphocyte-specific protein tyrosine kinase; MAPK, the mitogen-activated protein kinases; PKA, protein kinase A; VEGF, vascular endothelial growth factor

* Corresponding author.
E-mail address: tongwu88@163.com (T. Wu).

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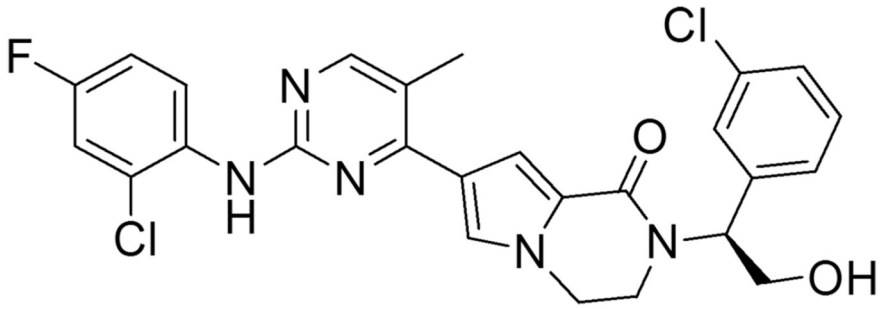

Fig. 1. Structure of JSI287.

2.2. Experiment animals

Adult male BALB/C mice (6–8 weeks, 19–23 g) were obtained from Shanghai Sino-British SIPPR/BK Lab Animal, (Shanghai, China). Prior to experimental study, animals were housed and bred for 5 days for adaptation under a temperature- and humidity-controlled, specific pathogen-free condition with a 12 h light-dark cycle. Food and distilled water ad libitum were provided to the animals simultaneously. All animal experiments were approved by the Animal Ethical Committee at Shanghai Institute of Pharmaceutical Industry, which was conformed to the National Institutes of Health Guidelines on Laboratory Research and Guide for the Care and Use of Laboratory Animals (Eighth Edition, 2011).

2.3. Animal treatment

The mice were randomly divided into 4 groups (n = 6 per group) based on the body weight, the four groups were blank, model, 2% JSI287 (200 mg JSI287 dissolved in 0.5 ml diethylene glycol monoethyl ether, then added 9.5 ml polyoxyethylene castor oil), and 0.5% JSI287 (50 mg JSI287 dissolved in 0.5 ml diethylene glycol monoethyl ether, then added 9.5 ml polyoxyethylene castor oil). For experiment, all groups of animals were shaved using pet hair clipper for a 2 cm × 3 cm area on the back. Except for blank group, all other groups were given a daily dose of 62.5 mg 5% IMQ cream by smearing for 7 continuous days after shaving. After the IMQ cream topical application for 4–5 h, 0.5% JSI287 and 2% JSI287 were smeared onto the shaved skin for 7 continuous days, and the model group was given corresponding solvent. About 1 h after the last administration of JSI287 or corresponding solvent, all mice were sacrificed and experimental back skin samples were collected for additional experiments.

2.4. Scoring severity of skin inflammation

To evaluate the severity of mice back skin lesions, a score system was used based on the clinical Psoriasis Area and Severity Index (PASI) [11]. The scores were collectively calculated in erythema, scaling, and thickening from 0 to 4: 0, none; 1, slight; 2, moderate; 3, marked; and 4, very marked. The cumulative score indicated a measure of the severity of inflammation (scale 0–12) [11]. The scores for each mouse were recorded for 7 continuous experimental days.

2.5. Histology examination and Measurement the thickness of epidermis

The collected skin samples of mice back lesions were fixed in 4% paraformaldehyde for 24 h. Tissue samples were dehydrated and then embedded in paraffin. Paraffin sections (4 μm in thickness) were stained with hematoxylin and eosin (H&E). The pathology of skin was observed under a Nikon eclipse 90i microscope (Nikon, Japan). The epidermis thickness was measured using skin sections by Nikon eclipse 90i microscope and NIS-Elements BR3.2 imaging software. Data collected from each section slide were averaged in 10 fields under 100× view of microscope and used to evaluate epidermis proliferation. Skin pathological sections were examined using 100× view of microscope, the score was conducted as follows: negative (−) 0, suspicious (±) 1, mild (+) 2, moderate (++) 3, severe (+++) 4.

2.6. ELISA

The release of IL-6, IL-12 and IL-17A in back skin supernatants were detected by ELISA Kit using protocols provided by the manufacturer (eBioscience, CA, USA). Briefly, 100 mg skin was washed 3 times with cold phosphate buffer (PH 7.4) to remove blood and residual drugs. The tissue was chopped into about 1 mm pieces quickly using the surgical scissors in ice-cold bathing and placed in homogenized tube. Following that, samples were homogenized using 2 small magnetic beads of 2 mm and 2 ml of cold phosphate buffer (PH 7.4) containing protease inhibitor (MCE, NJ, USA) by homogenizer (Scientz-42, Ningbo, China) at a speed of 60 Hz/min for 70 s. The resultants were centrifuged at 5000 rpm for 10 min at 4 °C. The tissue debris were separated and the supernatants were collected and stored at −20 °C until use.

2.7. Q-PCR

Total RNA was isolated from 100 mg skin tissues using 1 ml Trizol reagent (Invitrogen, USA) and 2 small magnetic beads of 2 mm by Bead Ruptor 12 Homogenizer (Omni, NW Kennesaw, USA) according to the manufacturer's instructions. Reverse transcription of 2000 ng RNA was performed with Revert Aid First Strand cDNA Synthesis Kit (Thermo, NY, USA) based on the attached protocol with 50 ng of random primers (Thermo, NY, USA). ATF1 transcripts levels were detected using FastStart Universal SYBR Green Master (Rox) (Roche, SC, USA) with 0.2 μM primers: ATF1; 5′-CAGCGGACAGTACATTGCCAT-3′ (sense), 5′-TCCCCTGCTGAGTACTGCTTG-3′ (antisense), GAPDH; 5′-CCTCGTC CCGTAGACAAAATG-3′ (sense), 5′-TGAGGTCAATGAAGGGGTCGT-3′ (antisense). Q-PCR was performed on 7300 Fast Real-Time PCR system (Applied Biosystems, CA, USA) using the pre-incubation at 95 °C for 10 min and two steps cycle (40 cycles) of denaturation at 95 °C for 15 s, annealing and extension at 60 °C for 1 min. Relative ATF1 expression level was normalized to the control (GAPDH) using the $2^{-\Delta\Delta Ct}$ method.

2.8. Western blot

One Step Animal Tissue Active Protein Extraction Kit (Sangon Biotech, Shanghai, China) was used for total protein extraction. Briefly, 100 mg of skin tissues were washed 3 times with cold phosphate buffer (pH 7.4) to remove blood and residual drugs. Then 1 ml cold extract reagent (1 ml extraction reagent containing 1 μl of DTT, 10 μl of PMSF, 1 μl of protease inhibitor, added before use) were added after cutting the skin tissue into 1 mm pieces. The tissues were incubated at 4 °C for 30 min and vortexed every 5 min, and then were spun for 10 min at 8000*g* at 4 °C. The primary antibodies were obtained from CST (ERK 1:2000, P-ERK 1:1000) and Servicebio (GAPDH 1:45,000, Wuhan, China). For ERK protein expression analysis, antibody binding was

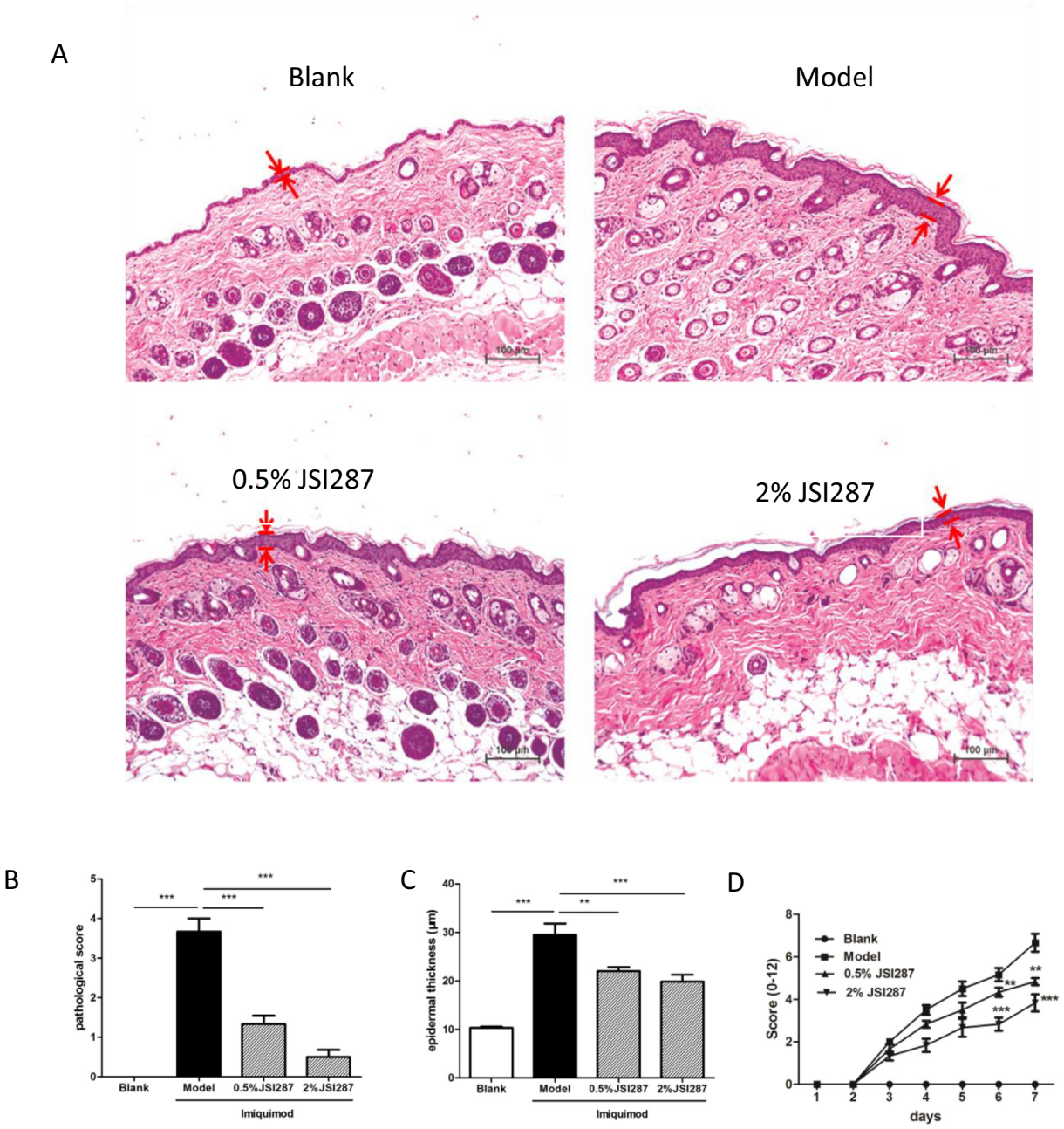

Fig. 2. The histology examination (A), pathological score (B), epidermal thickness (C) and clinical score (D) were decreased in epidermal skin of the IMQ-induced psoriasis like dermatitis model. Red arrows showed the thickness of epidermal skin. Statistical analysis was carried out by ANOVA followed by Dunnett's test to compare all groups to model group ($^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ compared with model group). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

visualized using appropriate horseradish peroxidase-conjugated secondary antibody (Servicebio, Wuhan, China) and DAB detection system. The signals were detected by an image scanning densitometer (Cell Bioscience, CA, USA).

2.9. Statistical analysis

The results were presented as the Mean ± SEM. Statistical analysis was determined using Graphpad Prism 5.0. ANOVA analysis and Dunnett's tests were used. A p value of < 0.05 was assigned significant.

3. Results

3.1. Histological examination and clinical score

For the blank group, histological examination showed no obvious pathological changes. The model group revealed the spine layer of epithelial cell thickening and local inflammatory infiltration when compared with the blank group (Fig. 2A). From evaluation of pharmacological scores conducted from epidermis congestion, edema, and inflammatory cell infiltration, JSI287 group significantly ameliorated pathological situations of skin lesions when compared to the model group (Fig. 2B). The epidermal thickness was measured by NIS-Elements BR3.2 software as described before. The epidermal thickness of JSI287 group was significantly decreased when compared to the model

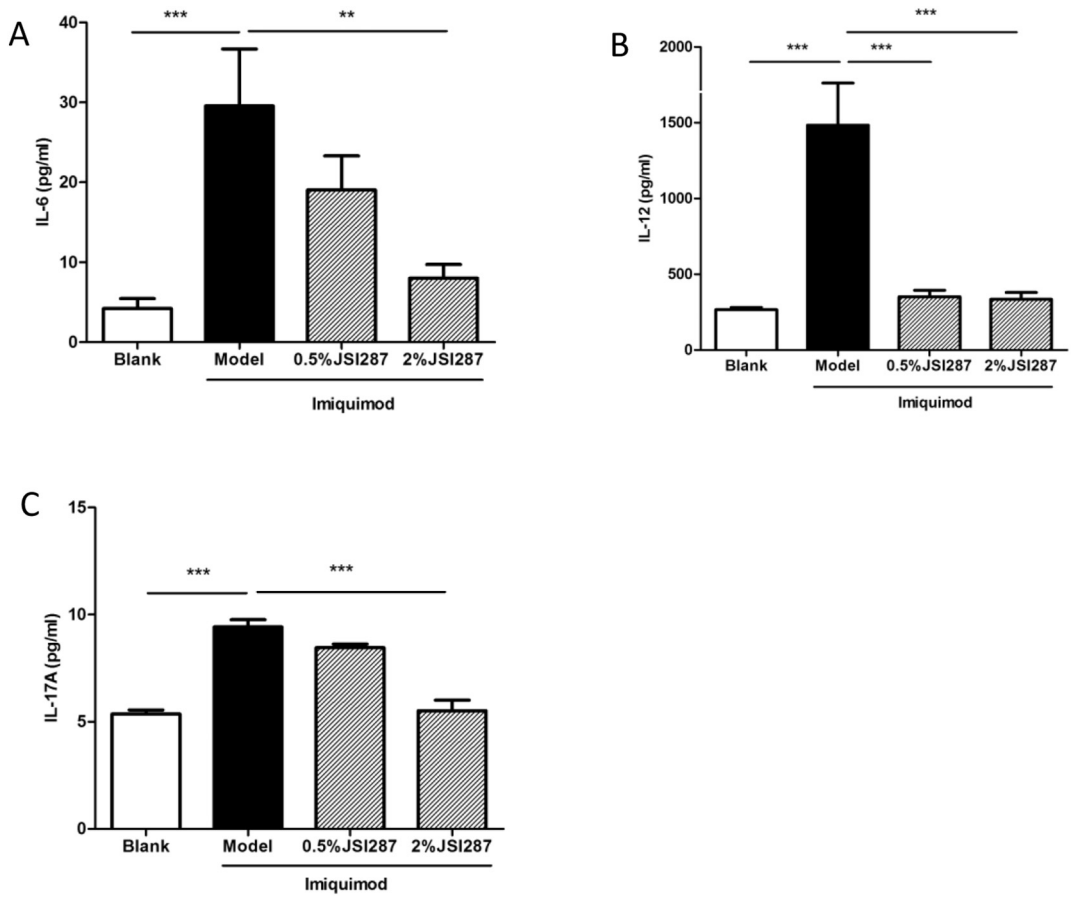

Fig. 3. The release of IL-6 (A), IL-12 (B) and IL-17A (C) was inhibited in skin tissues of the IMQ-induced psoriasis like dermatitis model by ELISA. Statistical analysis was carried out by ANOVA followed by Dunnett's test to compare all groups to model group ($^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ compared with model group).

group (Fig. 2C). These data suggested that JSI287 may inhibit the histological changes and reduce the epidermal thickness of skin caused by IMQ-induced psoriasis-like dermatitis.

Erythema, scaling, and thickness of the back skin were observed and scored daily during the experiment. From day 3, the sign of erythema, scaling, and thickness started appearing. On day 6–7, severity of skin inflammation in JSI287 groups were decreased comparing to the model group. The cumulative scores were depicted in Fig. 2D.

3.2. Inhibition of IL-6, IL-12 and IL-17A by JSI287

The cytokines in the skin supernatants were tested by ELISA kit. The release of IL-6, IL-12 and IL-17A levels were decreased in skin tissues in JSI287-treated group compared with model mice, and among all these markers, IL-12 was significantly reduced. The results demonstrated that JSI287 down-regulated inflammatory cytokines in the IMQ-induced mice psoriasis like dermatitis (Fig. 3).

3.3. Reduction of ATF1 mRNA levels by JSI287

The gene expression levels of ATF1 decreased significantly in JSI287-treated groups compared with model mice, therefore, indicating that the downstream transcription factor of MEK/ERK signal pathway was suppressed (Fig. 4).

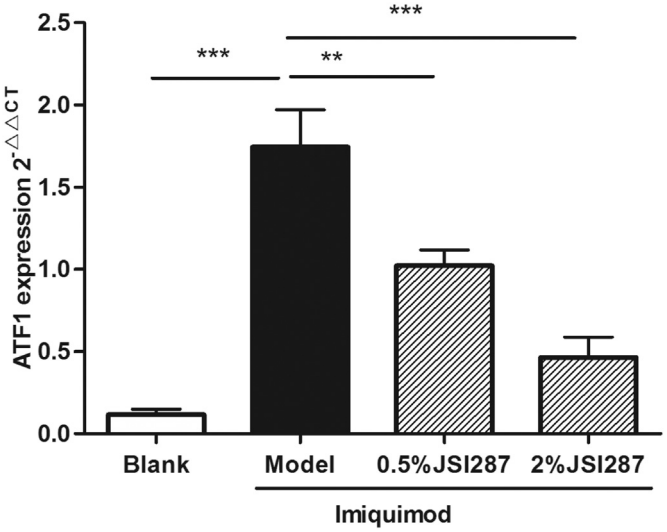

Fig. 4. The expression ATF1 mRNA was downregulated in skin tissues of the IMQ-induced psoriasis like dermatitis model by QPCR. Statistical analysis was carried out by ANOVA followed by Dunnett's test to compare all groups to model group ($^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ compared with model group).

3.4. Effects of JSI287 on ERK1/2 expression

Western blot analysis of ERK1/2 and P-ERK1/2 were performed with skin tissues of the IMQ-induced mice skin dermatitis. The results showed that JSI287 significantly decreased the expression of P-ERK1/2

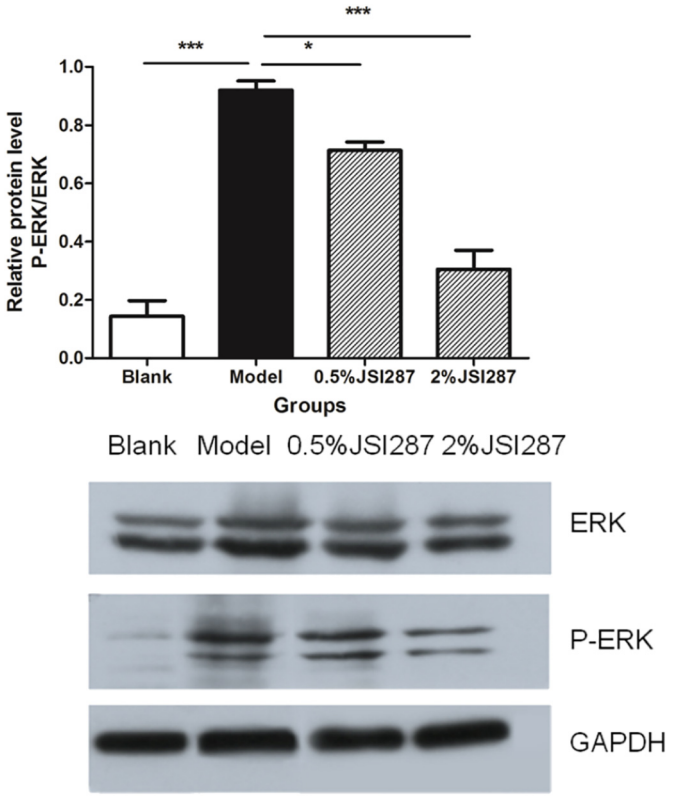

Fig. 5. JSI287 significantly decreased ERK1/2 expression in skin tissues of the IMQ-induced mice psoriasis like dermatitis: (A) protein level changes of ERK and P-ERK; (B) relative protein level of P-ERK/ERK. Statistical analysis was carried out by ANOVA followed by Dunnett's test to compare all groups to model group ($^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ compared with model group).

(Fig. 5A). The relative protein level of P-ERK/ERK also decreased in JSI287 group (Fig. 5B).

4. Discussions

Many studies confirmed that the over-activation of RAF-MEK-ERK signaling pathway plays a central role in human cancers [12–15]. Furthermore, the close relationship between the ERK pathway and autoimmune disease was also identified. Because of these, ERK inhibitors development attracted many more researchers. For example, BVD-523 (Ulixertinib) clinical trials of phase I/II study in patients with acute myelogenous leukemia or myelodysplastic syndromes has been completed. Also, MK8353's safety, tolerability and efficacy of advanced solid tumors treatment in patients was in process [13].

In this study, the compound JSI287 is screened from small molecule databases targeting ERK kinase, and the results showed that JSI287 was specific towards ERK kinase binding. Among all the tested kinases, JSI287 was most specific to ERK kinase and its activation was 50 folds more comparing that on other kinases. Furthermore, JSI287, based on test results, could specifically inhibit ERK kinase with an IC50 of 1.9 nM (The patent international publication number: WO2016/192064A1). ERK1/2 expression in mice skin lesions was detected by WB in this study and the results demonstrated ERK1/2 expression was significantly decreased in JSI287 group comparing to model group.

It has been shown that ERK phosphorylation could activate Th17 cells, and therefore induces inflammatory diseases [7,16]. The inflammatory cytokines TNF-α, IL-2, IL-6, IL-8, IL-18, IL-20 and IL-23 induced or produced by Th17 cell were overexpressed in serum of psoriasis patients and were positively correlated with the psoriasis severity [17–19]. Moreover, the study suggested IL-17 was a psoriasis risk factor for men. The researchers found rapamycin and fingolimod could downregulate pERK level in EAE mice, and further modulate the concentration of IL-17 and TGF-β. Mansouri et al. reported that Th17 cells and its products played an important role in skin diseases process, moreover, clarified IL-17 as a production of Th17 cells was the optimism treatment target for rheumatoid arthritis, psoriatic arthritis, psoriasis and ankylosing spondylitis [20–22]. Drugs targeting IL-17 and IL-12R had been approved for psoriasis therapy.

Cytokines, such as IL-6 and IL-12, play an important role in regulating autoimmune diseases. IL-17 could induce keratinocytes to produce IL-6 and defensins, and following that IL-6 was able to initiate the Th17 cell subsets formation from the primary T cells [23,24]. Other cytokines, such as IL-22, IL-6 and IL-20, contributed to induce keratinocyte proliferation; also, TNF-α could elevate keratinocytes to produce more TNF-α, IL-1, and IL-8 [25,26]. It has been reported that IL-6 mediated ERK signaling pathway played a critical role in epidermal differentiation, and IL-12 affected the development of Th1 cells and activation and function of cytotoxic T cells [27,28]. IL-12 and IL-23 inhibitor ustekinumab phase II, randomized, and controlled studies were completed. Regarding to the importance of IL-17A and correlated cytokines IL-6 and IL-12 in psoriasis, we studied the release of all three cytokines in imiquimod-induced mice skin lesions. The results suggested that JSI287 group significantly alleviated the release of IL-17A, IL-6 and IL-12.

Transcription factor 1 (ATF1) was significantly up-regulated in mice cervical cancer model, and phosphor-CREB/ATF1 binding to IL-6 promoter influenced IL-6 expression [29,30]. Therefore, we studied CREB and AFI1 gene expression, the data showed that JSI287 downregulated mRNA of ATF1. Therefore, the JSI287 alleviated the syndrome severity for cancer treatment through regulation of ATF1.

In this study, we revealed that JSI287 alleviated IMQ-induced psoriasis-like dermatitis by ERK/IL-17 signaling pathway. As a new compound targeting ERK1/2, JSI287 displayed its therapy potential on psoriasis. It has great potential, although further development needed, to treat inflammatory disease. This current study only focused on Th17 pathway, and possible effects of JSI287 on other pathways requires further investigation.

5. Conclusions

In conclusion, JSI287 was shown to be an ERK pathway inhibitor. It reduced epidermal thickness, alleviated epidermis congestion, edema and inflammatory cell infiltration, decreased release of inflammatory cytokines of IL-6, IL-12 and IL-17A, down regulated the mRNA expression of ATF1 and ERK1/2 protein expression through the ERK/IL-17 pathway. Therefore, JSI287 may act as an effective potential drug for psoriasis treatment.

Conflicts of interest

The authors declare that there is no conflict of interest associated with this work.

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References

[1] K.E. Nograles, B. Davidovici, J.G. Krueger, New insights in the immunologic basis of psoriasis, Semin. Cutan. Med. Surg. 29 (1) (2010) 3–9.
[2] R. Parisi, D.P. Symmons, C.E. Griffiths, D.M. Ashcroft, Global epidemiology of psoriasis: a systematic review of incidence and prevalence, J. Invest. Dermatol. 133 (2) (2013) 377–385.
[3] V. Chandran, S.P. Raychaudhuri, Geoepidemiology and environmental factors of psoriasis and psoriatic arthritis, J. Autoimmun. 34 (3) (2010) J314–J321.

[4] B.P. Kaufman, A.F. Alexis, Psoriasis in skin of color: insights into the epidemiology, clinical presentation, genetics, quality-of-life impact, and treatment of psoriasis in non-white racial/ethnic groups, Am. J. Clin. Dermatol. 19 (3) (2018) 405–423.
[5] J. Karczewski, A. Dobrowolska, A. Rychlewska-Hanczewska, Z. Adamski, New insights into the role of T cells in pathogenesis of psoriasis and psoriatic arthritis, Autoimmunity 49 (7) (2016) 435–450.
[6] G. Huang, Y. Wang, H. Chi, Regulation of TH17 cell differentiation by innate immune signals, Cell Mol. Immunol. 9 (4) (2012) 287–295.
[7] F. Mele, C. Basso, C. Leoni, D. Aschenbrenner, S. Becattini, D. Latorre, A. Lanzavecchia, F. Sallusto, S. Monticelli, ERK phosphorylation and miR-181a expression modulate activation of human memory TH17 cells, Nat. Commun. 6 (2015) 6431–6442.
[8] R. Guo, F.M. Zhou, C.J. Su, T.T. Liu, Y. Zhou, L. Fan, Z.H. Wang, X. Liu, Y. Huang, T. Liu, J. Yang, L.H. Chen, Epigallocatechin-3-gallate attenuates acute and chronic psoriatic itch in mice: involvement of antioxidant, anti-inflammatory effects and suppression of ERK and Akt signaling pathways, Biochem. Biophys. Res. Commun. 496 (4) (2018) 1062–1068.
[9] C. Johansen, K. Kragballe, M. Westergaard, J. Henningsen, K. Kristiansen, L. Iversen, The mitogen-activated protein kinases p38 and ERK1/2 are increased in lesional psoriatic skin, Br. J. Dermatol. 152 (1) (2005) 37–42.
[10] X.J. Yu, C.Y. Li, H.Y. Dai, D.X. Cai, K.Y. Wang, Y.H. Xu, L.M. Chen, C.L. Zhou, Expression and localization of the activated mitogen-activated protein kinase in lesional psoriatic skin, Exp. Mol. Pathol. 83 (3) (2007) 413–418.
[11] L. van der Fits, S. Mourits, J.S. Voerman, M. Kant, L. Boon, J.D. Laman, F. Cornelissen, A.M. Mus, E. Florencia, E.P. Prens, E. Lubberts, Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis, J. Immunol. 182 (9) (2009) 5836–5845.
[12] J.M. Buonato, M.J. Lazzara, ERK1/2 blockade prevents epithelial-mesenchymal transition in lung cancer cells and promotes their sensitivity to EGFR inhibition, Cancer Res. 74 (1) (2013) 309–319.
[13] Z. Yu, S. Ye, G. Hu, M. Lv, Z. Tu, K. Zhou, Q. Li, The RAF-MEK-ERK pathway: targeting ERK to overcome obstacles to effective cancer therapy, Future Med. Chem. 7 (3) (2015) 269–289.
[14] C. Neuzillet, A. Tijeras-Raballand, L. de Mestier, J. Cros, S. Faivre, E. Raymond, MEK in cancer and cancer therapy, Pharmacol. Ther. 141 (2) (2013) 160–171.
[15] D.D. Stuart, W.R. Sellers, Targeting RAF-MEK-ERK kinase-scaffold interactions in cancer, Nat. Med. 19 (5) (2013) 538–540.
[16] M. Raman, W. Chen, M.H. Cobb, Differential regulation and properties of MAPKs, Oncogene 26 (22) (2007) 3100–3112.
[17] A. Michalak-Stoma, J. Bartosinska, M. Kowal, M. Juszkiewicz-Borowiec, A. Gerkowicz, G. Chodorowska, Serum levels of selected Th17 and Th22 cytokines in psoriatic patients, Dis. Markers 35 (6) (2013) 625–631.
[18] M.L.Z. Lise, T.S.A. Baptista, L.E. Petersen, M.E. Bauer, C.A.L. Ungaretti, E. Torres, K. Harter, H.L. Staub, Subclinical atherogenesis in patients with mild psoriasis: a role for IL-6? Rev. Assoc. Med. Bras. 63 (9) (2017) 747–752.
[19] F. Bai, W. Zheng, Y. Dong, J. Wang, M.A. Garstka, R. Li, J. An, H. Ma, Serum levels of adipokines and cytokines in psoriasis patients: a systematic review and meta-analysis, Oncotarget 9 (1) (2017) 1266–1278.
[20] J.J. Jaller Char, J.A. Jaller, J.S. Waibel, D.G. Bhanusali, N. Bhanusali, The role of IL-17 in the human immune system and its blockage as a treatment of rheumatoid arthritis, ankylosing spondylitis, and psoriatic arthritis, J. Drugs Dermatol. 17 (5) (2018) 539–542.
[21] E. Lubrano, F.M. Perrotta, The role of IL-17 in the treatment of psoriatic arthritis, Expert. Rev. Clin. Immunol. 13 (8) (2017) 815–821.
[22] N.C. Brembilla, L. Senra, W.H. Boehncke, The IL-17 family of cytokines in psoriasis: IL-17A and beyond, Front. Immunol. 9 (2018) 1682–1694.
[23] C. Thompson, R. Davies, E. Choy, Anti cytokine therapy in chronic inflammatory arthritis, Cytokine 86 (2016) 92–99.
[24] Y.C. Tsai, T.F. Tsai, Anti-interleukin and interleukin therapies for psoriasis: current evidence and clinical usefulness, Ther. Adv. Musculoskelet. Dis. 9 (11) (2018) 277–294.
[25] R.L. Miller, J.F. Gerster, M.L. Owens, H.B. Slade, M.A. Tomai, Imiquimod applied topically: a novel immune response modifier and new class of drug, Int. J. Immunopharmacol. 21 (1) (1999) 1–14.
[26] J.K. Wu, G. Siller, G. Strutton, Psoriasis induced by topical imiquimod, Aust. J. Dermatol. 45 (1) (2004) 47–50.
[27] R.F. van Vollenhoven, B.H. Hahn, G.C. Tsokos, C.L. Wagner, P. Lipsky, Z. Touma, V.P. Werth, R.M. Gordon, B. Zhou, B. Hsu, M. Chevrier, M. Triebel, J.L. Jordan, S. Rose, Efficacy and safety of ustekinumab, an IL-12 and IL-23 inhibitor, in patients with active systemic lupus erythematosus: results of a multicentre, double-blind, phase 2, randomised, controlled study, Lancet 392 (10155) (2018) 1330–1339.
[28] Y. Wu, L. Liu, C. Bian, Q. Diao, M.F. Nisar, X. Jiang, J.W. Bartsch, M. Zhong, X. Hu, J.L. Zhong, MicroRNA let-7b inhibits keratinocyte differentiation by targeting IL-6 mediated ERK signaling in psoriasis, Cell Commun. Signal 16 (1) (2018) 58–69.
[29] Y. Shi, W. Wang, B. Yang, H. Tian, ATF1 and RAS in exosomes are potential clinical diagnostic markers for cervical cancer, Cell Biochem. Funct. 35 (7) (2017) 477–483.
[30] Q. Jia, H.R. Zhou, Y. Shi, J.J. Pestka, Docosahexaenoic acid consumption inhibits deoxynivalenol-induced CREB/ATF1 activation and IL-6 gene transcription in mouse macrophages, J. Nutr. 136 (2) (2006) 366–372.