Received: 20 February 2023 | Revised: 6 June 2023 | Accepted: 9 June 2023

DOI: 10.1111/exd.14862

CONCISE COMMUNICATION

Experimental Dermatology WILEY

The opposite effect of tapinarof between IMQ and IL-23 induced psoriasis mouse models

Xingyu Zhu[1,2] | Ruomei Han[1] | Xiaoxue Tian[3] | Mathias Hochgerner[3] | Hui Li[4] | Jiucun Wang[1,3,5] | Jingjing Xia[1,3]

[1]State Key Laboratory of Genetic Engineering, Collaborative Innovation Center for Genetics and Development, School of Life Sciences, Fudan University, Shanghai, China

[2]Institute for Six-Sector Economy, Fudan University, Shanghai, China

[3]Greater Bay Area Institute of Precision Medicine (Guangzhou), School of Life Sciences, Fudan University, Shanghai, China

[4]MOE Key Laboratory of Contemporary Anthropology, School of Life Sciences, Fudan University, Shanghai, China

[5]Research Unit of Dissecting the Population Genetics and Developing New Technologies for Treatment and Prevention of Dermatological Diseases (2019RU058), Chinese Academy of Medical Sciences, Shanghai, China

Correspondence
Jingjing Xia, Greater Bay Area Institute of Precision Medicine (Guangzhou), School of Life Sciences, Fudan University, Shanghai, China.
Email: xiajingjing@fudan.edu.cn

Jiucun Wang, State Key Laboratory of Genetic Engineering, Collaborative Innovation Center for Genetics and Development, School of Life Sciences, Fudan University, Shanghai, China.
Email: jcwang@fudan.edu.cn

Funding information
CAMS Innovation Fund for Medical Science, Grant/Award Number: 2019-I2M-5-066; National Natural Science Foundation of China, Grant/Award Number: 81703097

Abstract

Tapinarof is an aryl hydrocarbon receptor (AHR) ligand which is used to treat plaque psoriasis in adults. However, the underlying mechanism is not yet fully understood. In this study, we applied two of the most studied psoriasis mouse models: topical application of imiquimod (IMQ) and subcutaneous injection of IL-23. Although both models successfully induced psoriasis-like lesions in mice, tapinarof had a completely opposite effect on the two models. Tapinarof decreased the expression of multiple essential cytokines involved in the pathological IL-23/IL-17/IL-22 axis and ameliorated IMQ-induced psoriatic dermatitis, inhibiting keratinocyte proliferation and abnormal differentiation. However, in the IL-23-injection-model, tapinarof instead aggravated the disease. Here, tapinarof increased epidermal thickness and differentiated epidermal dysplasia in mice. Our data suggest that tapinarof may have different effects on varied types of psoriasis.

KEYWORDS
imiquimod, interleukin-23, psoriasis, tapinarof

Xingyu Zhu and Ruomei Han contributed equally to this work.

1 | BACKGROUND

Psoriasis is a common, chronic, inflammatory, proliferative even disfiguring condition of the skin. Both genetic and environmental risk factors contribute to the disease.[1] T lymphocytes are key players in the development of psoriasis, specifically interleukin (IL)-17-producing T helper (Th17) cells drive the disease.[2] Immunological and genetic studies identified the IL-23/IL-17-axis as the main mechanism of psoriasis pathogenesis.[3,4] Psoriasis is currently incurable, but can be treated with retinoids, methotrexate, corticosteroids, cyclosporine, TNF-alpha inhibitors, topical therapy and phototherapy.[5] Tapinarof is a small-molecule aryl hydrocarbon receptor (AHR) agonist.[6] It has been shown to ameliorate psoriasis,[7] and in 2022 was approved by the FDA as treatment for adult plaque psoriasis. The clinical score of tapinarof for plaque psoriasis in phase III, PASI 75, is significantly higher than calcipotriol (50.4% vs. 38.5%).[8] Nevertheless, the exact therapeutic mechanisms of tapinarof are still unclear. To further investigate the mechanism of tapinarof, we used the most studied animal models of psoriasis research, IL-23 and imiquimod (IMQ) induction.[9] Both models induce psoriasis-like lesions in mice, including acanthosis (thickening of the epidermis), hyperkeratosis (thickening of the stratum corneum) and hypokeratosis (retention of nucleated keratinocytes in the stratum corneum).[9]

2 | QUESTIONS ADDRESSED

Here, we demonstrate that in the IMQ-induced psoriasis mouse model tapinarof can significantly inhibit the proliferation and regulate the abnormal differentiation of keratinocytes, as well as reduce expression of pro-inflammatory cytokines but does not ameliorate psoriasis in the IL-23-induced mouse model.

3 | EXPERIMENTAL DESIGN

3.1 | Psoriasis mouse model

C57BL/6J mice (age 6 weeks, female) were purchased from Shanghai SLAC Laboratory Animal Co. Ltd and housed under SPF conditions with light–dark cycle. All mice were fed for 2 weeks to adapt to the new environment before any treatment. Mice were treated with Tapinarof (#HY-109044, MedChemExpress, USA) or vehicle 3 days before induction of psoriaform dermatitis. Dermatitis was induced by topical application of 62.5 mg IMQ/mouse (Sichuan Med-shine Pharmaceuticals) or subcutaneous injection of 500 ng IL-23/mouse (#ab259423, Abcam) into shaved back skin for 5 consecutive days[10] (Figure 1A). Mouse skin was harvested at Day 5. Animal experiments were performed following the general guidelines, and the protocol was approved by the Animal Care and Use Committee of the School of Life sciences at Fudan University, China.

3.2 | Real-time PCR

RNA was isolated from whole mouse skin by Trizol (#15596018, Thermo Fisher Scientific), and cDNA was obtained by reverse transcription (RR047, TAKARA). Real-time PCR was employed to measure the expression of *Il-17f*, *Cxcl1* and *Il-22*. The following primer sequences were used: *Il-17f* Forward 5′–3′ AACCAGGGCATTTCTGT CCCAC, Reverse 5′–3′ GGCATTGA.

TGCAGCCTGAGTGT. *Cxcl1* Forward 5′–3′ TCCAGAGCTTGAAG GTGTTGCC, Reverse 5′–3′ AACCAAGGGAGCTTCAGGGTCA. *Il-22* Forward 5′–3′ GCTTGAGGTGTCCAACTTCCAG, Reverse 5′–3′ ACTCC TCGGAACAGTTTCTCCC. *Il-23* Forward 5′–3′ TCTCGGAATCTCTG CATGCT, Reverse 5′–3′ ACTGGCTGTTGTCCTTGAGT. *Il-1α* Forward 5′–3′ CGAAGACTACA GTTCTGCCATT, Reverse 5′–3′ GACGTTTC AGAGGTTCTCAGAG. *Il-36α* Forward 5′–3′ GCAGCATCACCTTC GCTTAGA, Reverse 5′–3′ CAGATATTGGCATGGGAGCAAG. *Il-36β* Forward 5′–3′ AGAGTATTCAAATGTGGGAACCG, Reverse 5′–3′ GACCCATACCATCTGTTG TGAG. *Il-36γ* Forward 5′–3′ TCCTGACT TTGGGGAGGTTTT, Reverse 5′–3′ TCACGCTGACT GGGGTTACT. *Gapdh* Forward 5′–3′ AACTCCCACTCTTCCACCTTCG, Reverse 5′–3′ TCCAC CACCCTGTTGCTGTAG.

3.3 | Immunohistochemical staining

Mouse skin was fixed in 4% paraformaldehyde overnight and embedded in paraffin. Sections were placed in PH 6.0 citrate buffer for repair, blocked with 5% BSA and incubated at 4°C with primary antibody overnight. On the next day, samples were incubated with secondary antibody for 50 min at room temperature, washed three times in PBS and developed with DAB. Commercially purchased antibodies include Ki67 (#ab15580, Abcam), KRT10 (#905404, Biolegend) and LOR (#905104, Biolegend).

4 | RESULTS

IMQ and IL-23 both significantly induced the expression of psoriasis-associated inflammatory cytokines/chemokines such as IL-23, IL-17f, IL-22, IL-1α and chemokine (C–X–C motif) ligand 1 (CXCL1) (Figure 1B–F) and increased the psoriasis-like skin thickening in mice (Figure 2A,B). As expected, tapinarof ameliorated IMQ-induced psoriatic-like dermatitis (Figure 2A) and significantly decreased expression of IL-23/IL-17/IL-22 (Figure 1B–D). These results are consistent with previous studies which showed that tapinarof can decrease IL-23 and IL-17.[11] However, tapinarof failed to alleviate these symptoms in the IL-23-induced model. Unexpectedly, tapinarof further increased the expression of *Il-23*, *Il-17f*, *Il-22*, *Il-1α* and *Cxcl1*. Since IL-36 signalling was found to be important in regulating the pathological IL-23/IL-17/IL-22 axis and disease development, we also investigated this cytokine family.[12] Analysis of the expression levels of *Il-36α/β/γ* showed the same pattern as before: In IMQ,

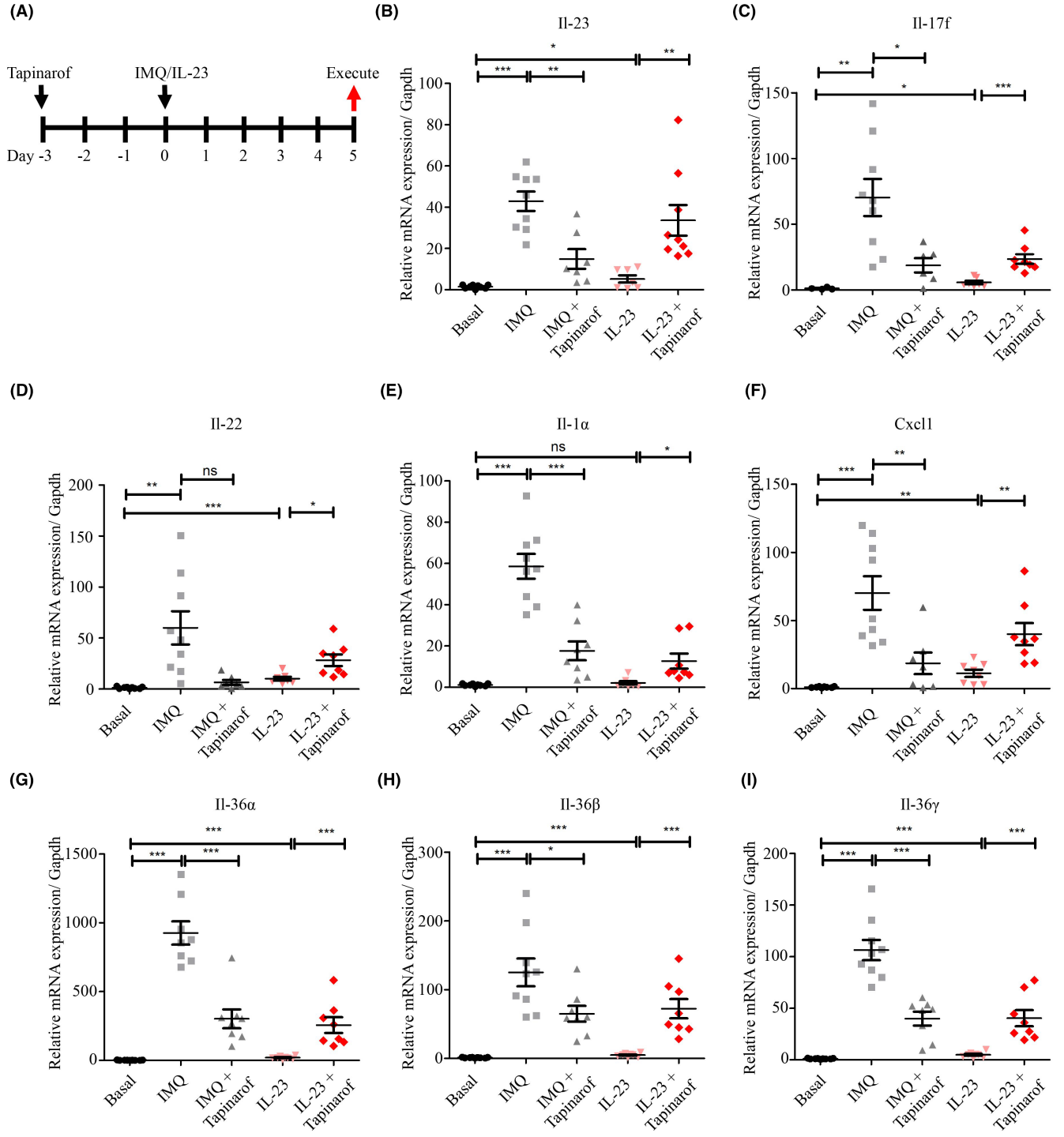

FIGURE 1 Expression of inflammatory genes in psoriasis mouse models. (A) Schematic illustrating experimental design, tapinarof was applied for 3 days before IMQ/IL-23 treatment for five consecutive days. IMQ (62.5 mg/mouse), IL-23 (500 ng/mouse, dissolved in 0.9% NaCl), tapinarof (1%, dissolved in 60% ethanol). mRNA expression of *Il-23* (B), *Il-17f* (C), *Il-22* (D), *Il-1α* (E), *Cxcl1* (F), *IL-36α* (G), *IL-36β* (H) and *IL-36γ* (I) was measured by qRT-PCR in psoriasis mouse skin ($n > 6$). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.00.1$, the differences between groups were assessed by *t*-test.

tapinarof decreases their expression, in IL-23 tapinarof increases the expression (Figure 1G–I). Our data show that tapinarof reduces IMQ-induced upregulation of pro-inflammatory mediators but it clearly increases the same mediators in the IL-23-model.

Psoriasis is characterized by abnormal differentiation and proliferation of keratinocytes due to hyperproliferation in the basal and suprabasal levels of the epidermis, manifesting as epidermal thickening.[13] Our results showed that tapinarof could significantly inhibit the proliferation and abnormal differentiation of

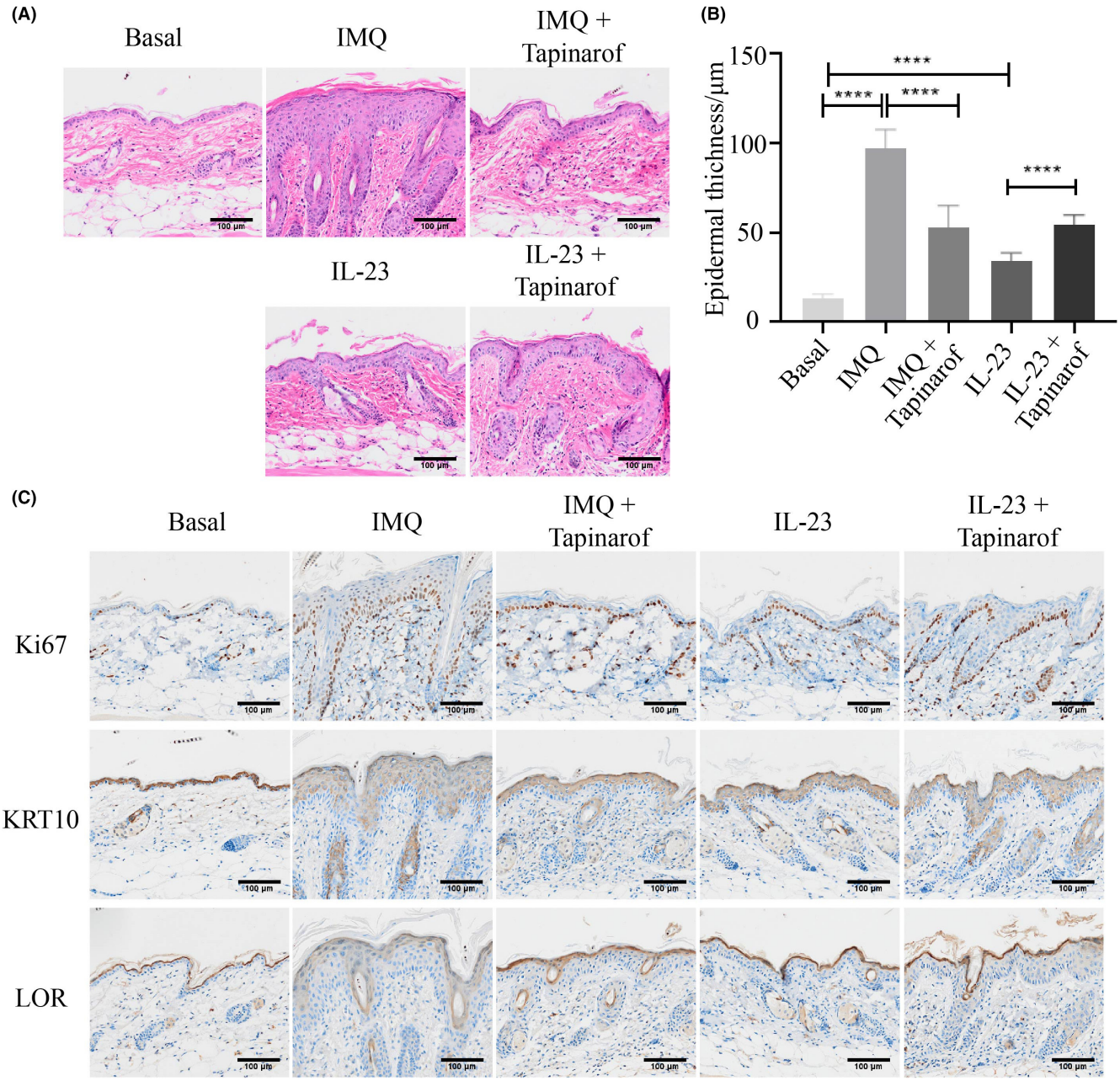

FIGURE 2 Immunohistochemical staining in psoriasis mouse models. (A) HE staining in IMQ- and IL-23-induced mouse models of psoriasis. (B) Quantitative analysis of epidermal thickness. (C) Immunohistochemical staining of Ki67/KRT10 and LOR. $^{****}p < 0.0001$, the differences between groups were assessed by *t*-test.

keratinocytes in the IMQ-model (Figure 2C). In the IL-23-model, tapinarof did not only not ameliorate the epidermal thickening, it even increased the abnormal proliferation and differentiation of the mouse epidermis.

5 | CONCLUSIONS AND PERSPECTIVES

Topical application of IMQ daily for 5–7 days on mouse depilated back or ears can induce significant psoriasis-like skin damage, including parakeratosis and hyperproliferation.[14] Due to its practicality, IMQ has been used as the most common mouse model for psoriasis. IMQ is a Toll-like receptor (TLR) 7/8 agonist which acts on skin dendritic cells (DCs), mediating the secretion of IL-23. This polarizes T cells towards Th17 cells, which then secrete IL-17 and IL-22.[15,16] Psoriatic pathogenesis is almost completely blocked in mice deficient for IL-23 or the IL-17 receptor, demonstrating the significance of the IL-23/IL-17 axis.[15] Abnormal production of pro-inflammatory cytokines is the driver of many inflammatory skin diseases,[17–19] which allows their use as direct disease models. Injection of the pro-inflammatory cytokine IL-23 directly into mouse dermis leads to the activation of Th17 cells and the development of psoriasis[20] and is used as another mouse

model for psoriasis.[17] Consistent with previous results, in our hands both models could successfully induce psoriatic skin lesions. In most studies, the two models show the same trend in terms of therapeutic effects.[21,22] However, our results clearly show that tapinarof has an opposite effect in the two models. The cause for this paradoxical reaction must lie in the differences between the two models.

Tapinarof can resolve skin inflammation in mice and humans by acting as an agonist for AHR, although its target cell(s) were not well characterized.[11] In DCs, AHR-signalling can inhibit the secretion of inflammatory cytokines such as IL-1β, IL-6 and IL-23, alleviating skin inflammation.[23,24] Other studies found that in T cells AHR-signalling markedly increases the proportion of Th17 cells and promotes their secretion of inflammatory cytokines, specifically IL-22.[25] Thus, depending on context, AHR-signalling can be both pro-inflammatory or anti-inflammatory.[26] In the IMQ-model, tapinarof activates AHR on DCs, inhibiting the IMQ-induced secretion of IL-23 and thereby disrupting the IL-23/IL-17/IL-22 axis of psoriatic pathogenesis at its beginning.[23,24] In the other model, IL-23 is exogenously injected into the dermis and can directly activate Th17 cells, triggering psoriatic pathogenesis. It is possible that in this model tapinarof simply has no pro-inflammatory DCs to block. Instead, tapinarof-induced AHR signalling in Th17 cells could further promote the secretion of inflammatory cytokines.[25]

The unexpected results from this study should raise our attention of choosing suitable mouse models to study the mechanism of action (MOA) in treating psoriasis. IL-23 plays a crucial role in psoriasis pathogenesis, and its application can effectively induce psoriasis-like lesions. However, direct injection of IL-23 into the dermis might bypass the pathogenesis upstream of IL-23 secretion, misleading the development of drugs targeting those processes. Topical application of IMQ might better model the whole process of the disease. Our data indicate that tapinarof might inhibit inflammatory cytokine secretion by skin DCs. This might also open up further avenues of exploration into why certain patients do or do not respond to tapinarof. Finally, increasing evidence showed that many drugs' therapeutic effects are associated with the differential metabolism by microbiota when the skin is considered an ecosystem.[27,28] It is therefore possible that the differences we observed between the models are due to different effects of the treatments on the microbiome and the compounding effect on skin immunity.[29,30]In summary, we believe that these findings may provide significant hints in understanding the mechanism of tapinarof in psoriasis.

AUTHOR CONTRIBUTIONS

Experiment and data curation and writing-draft preparation: XZ and RH. Writing-review & editing: XT, JX, and MH. Supervision: HL, JW and JX.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (81703097) and CAMS Innovation Fund for Medical Science (2019-I2M-5-066).

CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Jiucun Wang https://orcid.org/0000-0003-2765-0620
Jingjing Xia https://orcid.org/0000-0002-2892-4032

REFERENCES

1. Nair PA, Badri T. *Psoriasis*. StatPearls Publishing LLC; 2022.
2. Griffiths CEM, Armstrong AW, Gudjonsson JE, Barker J. Psoriasis. *Lancet*. 2021;397(10281):1301-1315. doi:10.1016/s0140-6736(20)32549-6
3. Lee E, Trepicchio WL, Oestreicher JL, et al. Increased expression of interleukin 23 p19 and p40 in lesional skin of patients with psoriasis vulgaris. *J Exp Med*. 2004;199(1):125-130. doi:10.1084/jem.20030451
4. Shou Y, Yang L, Yang Y, Xu J. Inhibition of keratinocyte ferroptosis suppresses psoriatic inflammation. *Cell Death Dis*. 2021;12(11):1009. doi:10.1038/s41419-021-04284-5
5. Hoegler KM, John AM, Handler MZ, Schwartz RA. Generalized pustular psoriasis: a review and update on treatment. *J Eur Acad Dermatol Venereol*. 2018;32(10):1645-1651. doi:10.1111/jdv.14949
6. Bissonnette R, Stein Gold L, Rubenstein DS, Tallman AM, Armstrong A. Tapinarof in the treatment of psoriasis: a review of the unique mechanism of action of a novel therapeutic aryl hydrocarbon receptor-modulating agent. *J Am Acad Dermatol*. 2021;84(4):1059-1067. doi:10.1016/j.jaad.2020.10.085
7. Bissonnette R, Bolduc C, Maari C, et al. Efficacy and safety of topical WBI-1001 in patients with mild to moderate psoriasis: results from a randomized double-blind placebo-controlled, phase II trial. *J Eur Acad Dermatol Venereol*. 2012;26(12):1516-1521. doi:10.1111/j.1468-3083.2011.04332.x
8. Cai L, Chen GH, Lu QJ, et al. A double-blind, randomized, placebo- and positive-controlled phase III trial of 1% benvitimod cream in mild-to-moderate plaque psoriasis. *Chin Med J (Engl)*. 2020;133(24):2905-2909. doi:10.1097/cm9.0000000000001221
9. Singh TP, Zhang HH, Hwang ST, Farber JM. IL-23- and Imiquimod-induced models of experimental psoriasis in mice. *Curr Protoc Immunol*. 2019;125(1):e71. doi:10.1002/cpim.71
10. Di Meglio P, Duarte JH, Ahlfors H, et al. Activation of the aryl hydrocarbon receptor dampens the severity of inflammatory skin conditions. *Immunity*. 2014;40(6):989-1001. doi:10.1016/j.immuni.2014.04.019
11. Smith SH, Jayawickreme C, Rickard DJ, et al. Tapinarof is a natural AhR agonist that resolves skin inflammation in mice and humans. *J Invest Dermatol*. 2017;137(10):2110-2119. doi:10.1016/j.jid.2017.05.004
12. Tortola L, Rosenwald E, Abel B, et al. Psoriasiform dermatitis is driven by IL-36-mediated DC-keratinocyte crosstalk. *J Clin Invest*. 2012;122(11):3965-3976. doi:10.1172/jci63451
13. Jean J, Soucy J, Pouliot R. Effects of retinoic acid on keratinocyte proliferation and differentiation in a psoriatic skin model. *Tissue Eng Part A*. 2011;17(13–14):1859-1868. doi:10.1089/ten.TEA.2010.0463
14. Wu J, Li S, Li T, et al. pDC activation by TLR7/8 ligand CL097 compared to TLR7 ligand IMQ or TLR9 ligand CpG. *J Immunol Res*. 2019;2019:1749803. doi:10.1155/2019/1749803

15. van der Fits L, Mourits S, Voerman JS, et al. Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis. *J Immunol*. 2009;182(9):5836-5845. doi:10.4049/jimmunol.0802999
16. Flutter B, Nestle FO. TLRs to cytokines: mechanistic insights from the imiquimod mouse model of psoriasis. *Eur J Immunol*. 2013;43(12):3138-3146. doi:10.1002/eji.201343801
17. Chan JR, Blumenschein W, Murphy E, et al. IL-23 stimulates epidermal hyperplasia via TNF and IL-20R2-dependent mechanisms with implications for psoriasis pathogenesis. *J Exp Med*. 2006;203(12):2577-2587. doi:10.1084/jem.20060244
18. Dubin C, Del Duca E, Guttman-Yassky E. The IL-4, IL-13 and IL-31 pathways in atopic dermatitis. *Expert Rev Clin Immunol*. 2021;17(8):835-852. doi:10.1080/1744666x.2021.1940962
19. Zhu X, Jiang L, Zhong Q, et al. Abnormal expression of interleukin-6 is associated with epidermal alternations in localized scleroderma. *Clin Rheumatol*. 2022;41(7):2179-2187. doi:10.1007/s10067-022-06127-w
20. Hawkes JE, Yan BY, Chan TC, Krueger JG. Discovery of the IL-23/IL-17 signaling pathway and the treatment of psoriasis. *J Immunol*. 2018;201(6):1605-1613. doi:10.4049/jimmunol.1800013
21. Zhu H, Lou F, Yin Q, et al. RIG-I antiviral signaling drives interleukin-23 production and psoriasis-like skin disease. *EMBO Mol Med*. 2017;9(5):589-604. doi:10.15252/emmm.201607027
22. Ando N, Nakamura Y, Aoki R, et al. Circadian gene clock regulates psoriasis-like skin inflammation in mice. *J Invest Dermatol*. 2015;135(12):3001-3008. doi:10.1038/jid.2015.316
23. Takemura M, Nakahara T, Hashimoto-Hachiya A, Furue M, Tsuji G. Glyteer, soybean tar, impairs IL-4/Stat6 signaling in murine bone marrow-derived dendritic cells: the basis of its therapeutic effect on atopic dermatitis. *Int J Mol Sci*. 2018;19(4):1169. doi:10.3390/ijms19041169
24. Quintana FJ, Murugaiyan G, Farez MF, et al. An endogenous aryl hydrocarbon receptor ligand acts on dendritic cells and T cells to suppress experimental autoimmune encephalomyelitis. *Proc Natl Acad Sci U S A*. 2010;107(48):20768-20773. doi:10.1073/pnas.1009201107
25. Veldhoen M, Hirota K, Westendorf AM, et al. The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins. *Nature*. 2008;453(7191):106-109. doi:10.1038/nature06881
26. Haarmann-Stemmann T, Esser C, Krutmann J. The Janus-faced role of aryl hydrocarbon receptor signaling in the skin: consequences for prevention and treatment of skin disorders. *J Invest Dermatol*. 2015;135(11):2572-2576. doi:10.1038/jid.2015.285
27. Chen H, Zhao Q, Zhong Q, et al. Skin microbiome, metabolome and skin phenome, from the perspectives of skin as an ecosystem. *Phenomics*. 2022;2(6):363-382. doi:10.1007/s43657-022-00073-y
28. Cussotto S, Strain CR, Fouhy F, et al. Differential effects of psychotropic drugs on microbiome composition and gastrointestinal function. *Psychopharmacology*. 2019;236(5):1671-1685. doi:10.1007/s00213-018-5006-5
29. Shinno-Hashimoto H, Hashimoto Y, Wei Y, et al. Abnormal composition of microbiota in the gut and skin of imiquimod-treated mice. *Sci Rep*. 2021;11:11265. doi:10.1038/s41598-021-90480-4
30. Zanvit P, Konkel JE, Jiao X, et al. Antibiotics in neonatal life increase murine susceptibility to experimental psoriasis. *Nat Commun*. 2015;6:8424. doi:10.1038/ncomms9424

How to cite this article: Zhu X, Han R, Tian X, et al. The opposite effect of tapinarof between IMQ and IL-23 induced psoriasis mouse models. *Exp Dermatol*. 2023;00:1-6. doi:10.1111/exd.14862