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Pharmacological Properties of Tapinarof in Mice as a Novel Topical Agent for Plaque Psoriasis

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Tapinarof is a non-steroidal, small molecule aryl hydrocarbon receptor (AhR) agonist that has demonstrated clinical efficacy and safety in patients with plaque psoriasis. In this study, we investigated the effects of tapinarof on interleukin (IL)-23-induced psoriasis-like dermatitis, which is a direct reflection of IL-23/type 17 helper T (Th17) axis activation considered pivotal in the pathogenesis of psoriasis, or normal skin in mice to elucidate its pharmacological properties. In mice with dermatitis, topical administration of tapinarof induced AhR activation, promoted the expression of an antioxidant molecule, and suppressed Th17 cytokine production in the skin, resulting in the reduction of skin swelling and histopathological changes. In normal mice, tapinarof did not induce the skin thinning observed with dexamethasone. These findings suggest that tapinarof represents a new topical treatment option for psoriasis carrying a lower risk of skin atrophy.

Key words tapinarof, plaque psoriasis, atrophy, IL-17A, aryl hydrocarbon receptor

INTRODUCTION

Psoriasis is a chronic immune-mediated skin disease that affects an estimated 125 million people worldwide.[1,2] Histologically, psoriatic skin lesions are characterized by hyperproliferation of keratinocytes and a mixed cellular infiltrate in both the epidermal and dermal layers of the skin. This pathological condition is caused by abnormal immune activation in the skin, triggered by various environmental stimuli and loss of immune tolerance through the recognition of psoriasis autoantigens.[3-6] Proinflammatory cytokines such as tumor necrosis factor (TNF)α and interleukin (IL)-23 are secreted from activated dendritic cell subsets. These cytokines promote polarization and clonal expansion of type 1 helper T (Th1), type 17 helper T (Th17), and type 22 helper T (Th22) cells, respectively, in psoriatic plaques. These activated T cells, in turn, induce abnormal activation and proliferation of keratinocytes through the release of interferon γ, TNFα, IL-17A, and IL-17F, thereby forming a feed-forward inflammatory response, which contributes to the pathogenesis of psoriasis.[3] Particularly, the IL-23/Th17 axis plays a crucial role in the pathogenesis of psoriasis and its importance is strongly underscored by the high efficacy of biologics targeting this axis. These include guselkumab, risankizumab, and tildrakizumab, which are monoclonal antibodies against the p19 subunit of IL-23, along with the IL-17A neutralizing antibodies, ixekizumab and secukinumab.[1,3]

While these biologic agents have significantly advanced the treatment of patients with moderate to severe psoriasis in particular, most patients present with mild and localized disease, making them suitable candidates for topical therapy.[7,8,9] The current mainstay of topical therapy includes topical corticosteroids (TCs), vitamin D analogs, or a combination of both. However, particularly in the case of TCs, their use necessitates caution due to the potential for local side effects such as skin atrophy, striae, folliculitis, telangiectasia, and purpura. These characteristics lead to restrictions on the body surface area, duration, and site of application.[7,8] On the other hand, vitamin D analogues have a slower onset of action and are less effective than TCs.[7,8] These properties of conventional topical agents including side effects, complicated regimens, and inadequate efficacies have highlighted the remaining unmet medical needs in the topical treatment of psoriasis.

The aryl hydrocarbon receptor (AhR) is widely expressed in various cells of skin tissue, including immune cells such as T cells and antigen-presenting cells, as well as fibroblasts, keratinocytes, and melanocytes, where it functions as a chemical sensor, transducing external and internal stimuli into biological responses.[10,11] Once activated, the AhR translocates to the

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nucleus, forms a heterodimer with the AhR nuclear transporter, and regulates downstream gene transcription.[10] This process leads to the upregulation of the chemical metabolizing enzymes, CYP1A1, CYP1A2, and CYP1B1, promoting rapid ligand degradation and maintaining keratinization homeostasis.[10,13] Additionally, AhR activation enhances the expression of the antioxidant molecule NAD(P)H: quinone oxidoreductase (NQO1), helping to counteract oxidative stress associated with ligand degradation.[10,14] Tapinarof is a non-steroidal small molecule that activates AhR.[12,15] Recent studies have revealed that tapinarof improves skin barrier function by increasing the expression of barrier-related proteins, including filaggrin and loricrin, through AhR agonism.[12,15] In addition, tapinarof has an antioxidant effect through the activation of nuclear factor erythroid 2-related factor 2 (NRF2) and directly increases the expression of NQO1.[12,15] Furthermore, tapinarof has been reported to suppress the production of inflammatory cytokines, including IL-17A, IL-17F, IL-19, IL-22, IL-23A, and IL-1β, and reduce psoriasis-like skin inflammation in mice induced by imiquimod through AhR agonism.[12,15] In phase III clinical trials, tapinarof cream 1% produced significant improvement in patients with moderate plaque psoriasis, maintained efficacy in a 52-week extension trial, and demonstrated high tolerability.[16,17]

While its clinical efficacy and tolerability for plaque psoriasis patients have been demonstrated, the pharmacological properties of tapinarof have not yet been fully elucidated. Further studies on its mechanism of action, particularly its effect on the IL-23/Th17 axis, are necessary. There have been no previous reports examining the safety profiles of tapinarof therapy compared to conventional therapy. Therefore, in this study, we investigated the pharmacological effects of tapinarof in mice with IL-23-induced psoriasis-like skin inflammation, which is considered to be due directly to IL-23/Th17 axis activation. We further examined the effects of tapinarof on normal mouse skin, focusing on skin atrophy, one of the major adverse effects of conventional TCs therapy.

MATERIALS AND METHODS

Compounds Tapinarof was obtained from Dermavant Sciences GmbH (Basel, Switzerland). Dexamethasone (Dex) was purchased from Sigma-Aldrich, Co. (St. Louis, MO, USA). Ethanol (EtOH) was purchased from FUJIFILM Wako Pure Chemical Co. (Osaka, Japan). Both tapinarof and Dex were dissolved in EtOH for topical administration *in vivo*.

Animals Seven-week-old female C57BL/6J mice and six-week-old female Crl:CD1(ICR) mice were purchased from The Jackson Laboratory Japan, Inc. (Yokohama, Japan) and used for experiments after a week of acclimation. All mice were maintained under specific pathogen-free conditions at a room temperature of 23 ± 3°C and air humidity of 55% ± 15% on a 12-h/12-h light/dark cycle. All experiments were conducted in accordance with ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines and in compliance with the Guidelines for Animal Experimentation of the Central Pharmaceutical Research Institute of Japan Tobacco, Inc. (Protocol No. 02800, Date: Jan 18, 2022).

Animal Study in Mice with Psoriasis-like Dermatitis The IL-23-induced psoriasis-like skin inflammation model was established with slight modifications as previously described.[18] Female C57BL/6J mice received repeated injections of 20 μL of 25 μg/mL recombinant mouse IL-23 (R&D Systems, Inc., Minneapolis, MN, USA) dissolved in Dulbecco's phosphate buffered saline (PBS) containing 0.1% bovine serum albumin (BSA) (Sigma-Aldrich, Co.) into the left ear for 3 consecutive days. Tapinarof and Dex were administered topically on both sides of the left ears at a volume of 20 μL per mouse (10 μL to each side of the ear) once daily from the day of the first IL-23 injection, from Days 1 to 3. Clinical observations were conducted once daily during the dosing period. All mice were observed for abnormalities in physical appearance, nutrition, body posture, behavior, and excrement. Ear thickness, as an index of ear swelling, was measured with a digital thickness gauge (Digimatic Indicator; Mitutoyo Co., Kawasaki, Japan) on Day 1 and 4, then expressed as the increase in thickness from the baseline measurement. After the measurement, mice were anesthetized and euthanized, and their left ears were excised and divided into three sections for analysis of gene expression, cytokine measurements, and histological evaluation. Ear sections were homogenized, and total RNA was prepared using the RNeasy Mini Kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions. IL-17A levels were assessed with the Mouse IL-17 Quantikine ELISA kit (R&D Systems, Inc., Minneapolis, MN, USA). The remaining pieces of the ears were fixed in 10% neutral-buffered formalin (FUJIFILM Wako Pure Chemical, Osaka, Japan). The tissues were embedded in paraffin wax, sectioned, and stained with hematoxylin and eosin (HE). Tissue specimens were prepared for pathological evaluation by the Biopathology Institute (Kokuto, Japan).

Skin Atrophy Assessment in Normal Mice Atrophy assessment was performed following a slightly modified procedure from a previous report.[19] Briefly, Crl:CD1(ICR) mice received daily topical applications of tapinarof and Dex, with a dosing volume of 20 μL/animal once daily applied to both sides (10 μL each on the external and internal surfaces) of the left ear for 7 days. Ear thickness was measured with a digital thickness gauge (Digimatic Indicator; Mitutoyo Co.) on Days 1, 2, 4, and 8, then change in ear thickness (skin atrophy) was expressed as the change from the baseline measurement. After the final measurement, mice were anesthetized and euthanized, and their left ears were excised. This was followed by preparation of paraffin blocks for pathological evaluation as mentioned above. Body weights were measured before the ear thickness measurement on Days 1, 2, 4, and 8 of the dosing period.

Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR) RT-qPCR was performed using the TaqMan™ RNA-to-Ct™ 1-Step Kit and the QuantStudio 7 Flex Real-Time PCR System, both of which were from Thermo Fisher Scientific Inc. (Waltham, MA, USA). All Taqman probes (*Cyp1a1*: Mm 00487218_m1, *Nqo1*: Mm 01253561_m1, and β-actin (*Actb*): Mm 02619580_m1) were also purchased from Thermo Fisher Scientific. The comparative cycle time method was used to normalize the transcript levels to those of *Actb*.

Pathological Examinations The central region of the HE-stained ear sections, which corresponded approximately to the site where the ear thickness was measured, was examined under a light microscope. In the study with psoriasis-like dermatitis model mice, the microscopic findings were graded based on the following criteria: infiltration of inflammatory cells into dermis (− = negative: no lesion, ± = very slight:

infiltration of small numbers of inflammatory cells partially or sparsely, +=slight: infiltration of small numbers of inflammatory cells diffusely, 2+=moderate: infiltration of moderate numbers of inflammatory cells with apparent thickening of the ear due to the increase in number of inflammatory cells, and 3+=severe: infiltration of large numbers of the inflammatory cells with marked thickening of the ear due to the increase in number of inflammatory cells), acanthosis (−=negative: no thickening, ±=very slight: very slight or focal thickening of epidermis: hypertrophy of the epidermal cells is absent, +=slight: slight thickening of the epidermis: hypertrophy of the epidermal cells is very slight or not seen, 2+=moderate: moderate thickening of the epidermis with slight hypertrophy of the epidermal cells, and 3+=severe: marked thickening of the epidermis with marked hypertrophy of epidermal cells), crust (−=negative: no lesion, ±=very slight: very slight or focal crust formation on the epidermis, +=slight: slight and multifocal crust formation on the epidermis with erosion, 2+=moderate: moderate and multifocal to diffuse crust formation on the epidermis with erosion and/or ulcer, and 3+=severe: marked and diffuse crust formation on the epidermis with erosion and/or ulcers). In the normal mouse study, thinning of the epidermis and atrophy of the sebaceous glands were assessed on a non-graded scale.

Statistical Analyses Data are expressed as the mean and standard deviation of the indicated number of samples. SAS System Version 9.4 (SAS Institute Inc., Cary, NC, USA) and EXSUS Version 10.1 (EPS Co., Tokyo, Japan) were used for the statistical analyses. In the IL-23-induced mouse model, the changes in the ear thickness and IL-17A levels on Day 4 were statistically analyzed using a closed testing procedure. First, the sham-treated group and the vehicle-treated group were compared. Upon finding a significant difference, comparisons were performed between the vehicle-treated group and the Dex-treated group. Finally, after also finding a significant difference, the vehicle-treated group and tapinarof-treated group were compared. Differences between two groups were assessed by Student *t*-tests or Aspin-Welch *t*-tests depending on homoscedasticity determined by F-tests. Differences among multiple groups were analyzed by Dunnett tests or Steel tests, based on homoscedasticity determined by the Bartlett test. In the normal mouse model, the between-group differences in the changes in ear thickness and body weight on Day 8 were statistically analyzed by Tukey multiple comparison tests or Steel-Dwass multiple comparison tests, again based on homoscedasticity determined by the Bartlett test. A two-tailed significance level of 5% was used.

RESULTS

Tapinarof Inhibits IL-23-Induced Skin Inflammation After Topical Administration The IL-23-induced skin inflammation model is widely used model, reflecting the central role that IL-23/Th17 axis plays in the pathogenesis of psoriasis. We examined the effects of tapinarof on psoriasis-like skin inflammation using this model (Fig. 1A). Repeated injection of IL-23 into mouse ears induced ear swelling on Day 4, and ear thickness was markedly increased (Fig. 1B). Once

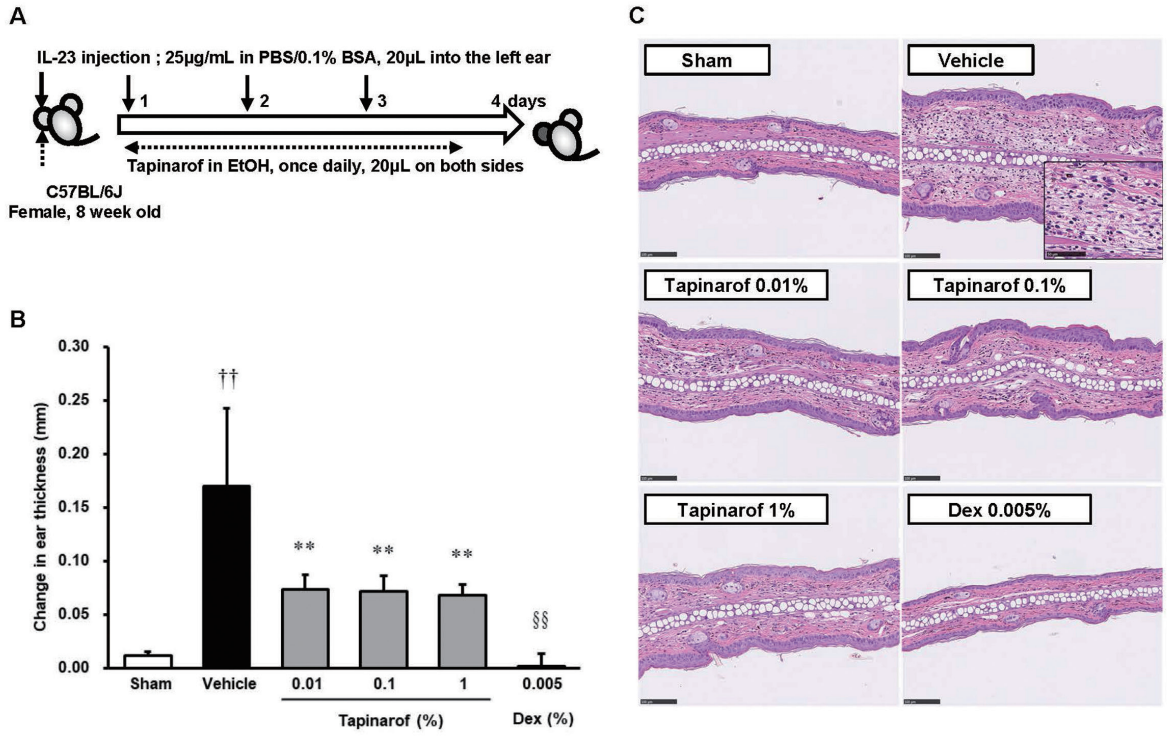

Fig. 1. Effects of Topical Administration of Tapinarof on Skin Inflammation in IL-23-Induced Psoriasis-Like Dermatitis in Mice

(A) Psoriasis-like dermatitis was induced in female C57BL/6J mice by repeated injection of 20 μL of 25 μg/mL recombinant mouse IL-23 in PBS containing 0.1% BSA into the left ear for 3 consecutive days (IL-23-induced group). For the sham-treated group, PBS containing 0.1% BSA alone was injected. In the IL-23-induced group, vehicle (EtOH), 0.01%, 0.1%, 1% tapinarof, or 0.005% Dex were administered topically once daily for 3 days. (B) Ear thickness was assessed on Day 1 and 4. Data are presented as the change in ear thickness from baseline. The results on Day 4 are shown as the mean + SD (n = 10). Significant difference; ††, $p < 0.01$; sham vs. vehicle (Aspin-Welch t-test), §§, $p < 0.01$; vehicle vs. 0.005% Dex (Student t-test), **, $p < 0.01$; vehicle vs. 0.01%, 0.1%, and 1% tapinarof (Dunnett test). (C) Representative photomicrographs of HE-stained ear skin samples. Group names are presented on each panel. Sham, normal ear skin; vehicle, slight inflammatory cell infiltration in the dermis; 0.01%, 0.1%, and 1% tapinarof, very slight infiltration of inflammatory cells; and 0.005% Dex, no abnormality. Bar = 100 μm (inside the vehicle panel enlarged view: Bar = 50 μm).

Table 1. Effects of Tapinarof and Dexamethasone on the Histopathological Changes in the Ear Skin of Mice with Psoriasis-Like Dermatitis (Microscopic Findings)

Organ		Group	Sham	Vehicle	Tapinarof	Tapinarof	Tapinarof	Dexamethasone
		Concentration (%)			0.01%	0.1%	1%	0.005%
	Findings	Number of animals	10	10	10	10	10	10
Skin (ear)			<10>	<10>	<10>	<10>	<10>	<10>
	Infiltration, inflammatory cells, dermis	–	10	0	4	5	5	10
		±	0	4	6	5	5	0
		+	0	5	0	0	0	0
		2+	0	1	0	0	0	0
		3+	0	0	0	0	0	0
	Acanthosis	–	10	3	8	8	7	10
		±	0	6	2	2	3	0
		+	0	1	0	0	0	0
		2+	0	0	0	0	0	0
		3+	0	0	0	0	0	0
	Crust	–	10	5	8	8	6	10
		±	0	3	2	2	4	0
		+	0	2	0	0	0	0
		2+	0	0	0	0	0	0
		3+	0	0	0	0	0	0

<>, Numbers of animals examined
-, No abnormal findings
±, Very slight; +, Slight; 2+, Moderate; 3+, Severe

daily topical administration of tapinarof at concentrations of 0.01%, 0.1%, and 1% was well tolerated with no abnormalities in any of the clinical observations, and on Day 4, significantly inhibited the ear swelling. Used as reference, 0.005% Dex also inhibited the ear swelling.

Tapinarof Attenuates Histopathological Changes in IL-23-Induced Skin Inflammation We further investigated the inhibitory effects of tapinarof on IL-23-induced skin inflammation in mice by conducting histopathological examination of the ear skin. In the vehicle-treated group, slight to moderate inflammatory cell infiltration in the dermis, along with very slight to slight acanthosis and crust formation in the epidermis, were observed (Table 1, Fig. 1C, Fig. S1). In the tapinarof-treated group, both the incidence and severity of the inflammatory cell infiltration, acanthosis, and crust formation decreased at concentrations of 0.01% and above. In the Dex-treated group, both the incidence and severity of the inflammatory cell infiltration, acanthosis, and crust formation were fully inhibited. Therefore, tapinarof is suggested to attenuate skin inflammation mediated by the IL-23/Th17 axis.

Tapinarof Activates AhR Pathway and Reduces IL-17A Level in Mice with Skin Inflammation We investigated the pharmacological characteristics of tapinarof in this model. AhR agonism and subsequent antioxidant activation were assessed by measuring the mRNA expression of *Cyp1a1* and *Nqo1*, respectively, in the ear skin. The results revealed a significant elevation from the lowest concentration of 0.01% in the tapinarof-treated group compared to the vehicle-treated group (Fig. 2A, B). The impact on inflammatory cell activation was evaluated by measuring IL-17A levels in the ear skin. IL-17A levels were increased in the vehicle-treated group on Day 4 (Fig. 2C). Tapinarof significantly inhibited this cytokine elevation. Therefore, we suggest that an antioxidant effect and inhibition of Th17 cytokine production through activation of the AhR pathway are involved in the suppressive effects of tapinarof on skin inflammation induced by IL-23.

Tapinarof Has No Skin-Thinning Effect in Normal Mice To investigate and compare the effects on skin atrophogenicity, tapinarof or Dex were applied dermally once daily to the ear of normal ICR mice for 7 days, and ear thickness was evaluated (Fig. 3A). The Dex-treated group showed a decrease in ear thickness in an application-period dependent manner (Fig. 3B). On Day 8, the Dex-treated group showed a significant decrease in ear thickness compared to the vehicle-treated, non-treated, or tapinarof-treated groups. Conversely, the tapinarof-treated group showed no significant difference in the change in ear thickness compared to the vehicle-treated or non-treated groups on Day 8. There was a slight difference in body weight change in the Dex-treated group compared to the vehicle-treated group on Day 8, but none of the other groups showed significant differences (Fig. S2).

Tapinarof Causes No Histopathological Change in Normal Skin in Mice We further investigated the effects of tapinarof on normal skin in mice through histopathological examination of the ear skin. There were no significant histopathological changes in the ear skin in any animals in the tapinarof-treated, vehicle-treated, and non-treated groups, whereas epidermal thinning of the ear skin and atrophy of the sebaceous glands were observed in all animals in the Dex-treated group (Table 2, Fig. 3C). Therefore, based on these results, tapinarof, unlike Dex, is suggested to be a potential topical treatment with lower risk of skin atrophy.

DISCUSSION

Tapinarof, a topical agent recently launched for the treatment of plaque psoriasis, has demonstrated efficacy and safety in clinical trials.[16,17] Recent studies have reported that tapinarof inhibits the production of Th17 cytokines and improves psoriasis symptoms through AhR activation in the imiquimod-induced mouse model.[12] However, the effect of tapinarof on the IL-23/Th17 axis has not been demonstrated. Therefore, in this study, we investigated the pharmacological effects of tapinarof using an *in vivo* mouse model that directly reflects

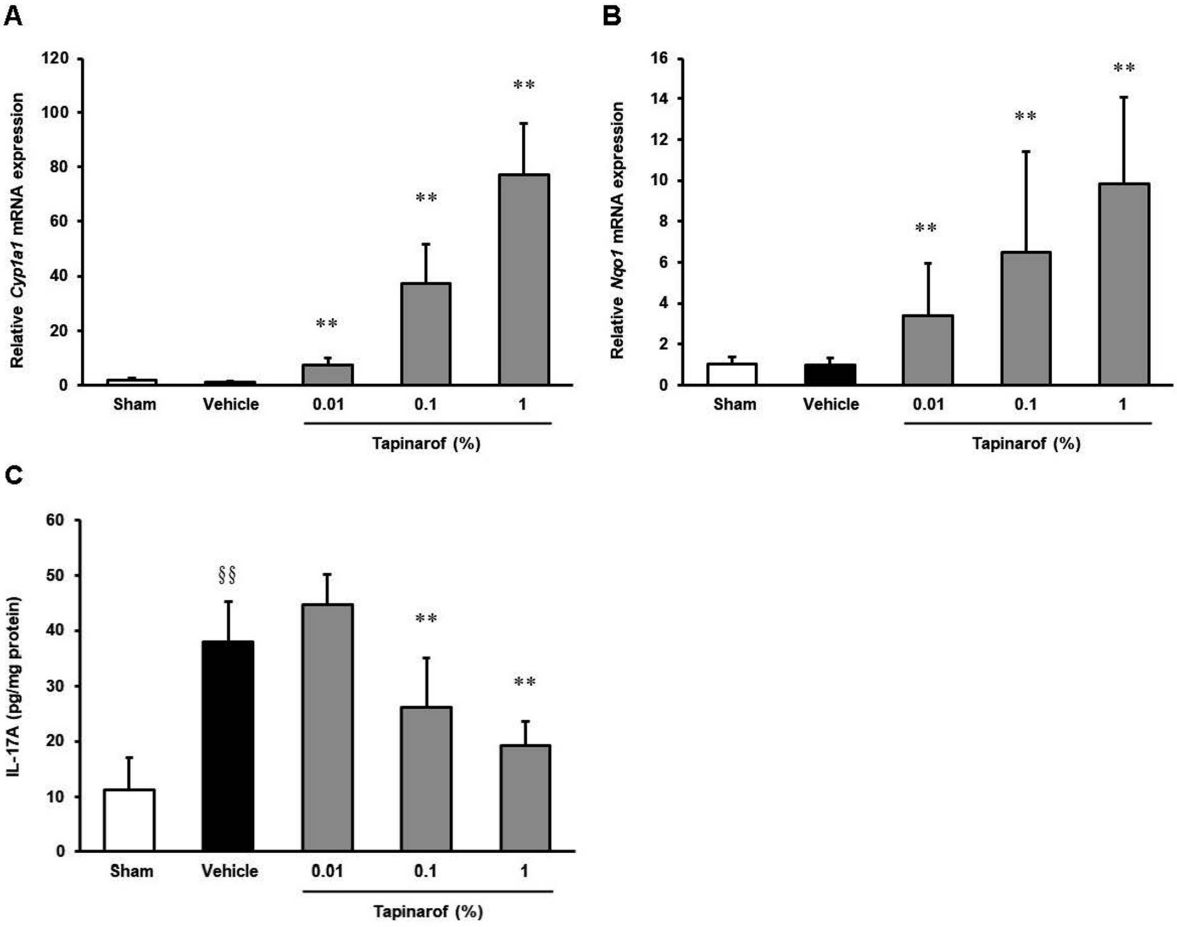

Fig. 2. Effects of Topical Administration of Tapinarof on AhR Pathway and IL-17A Levels in IL-23-Induced Psoriasis-Like Dermatitis in Mice

The relative mRNA expression of *Cyp1a1* (A) and *Nqo1* (B) in the ear tissue on Day 4 are shown as fold change to vehicle control. Data are expressed as mean + SD (n = 10). Significant difference; **, $p < 0.01$ (Dunnett test). (C) ELISA kit was used to measure the IL-17A levels in the ear tissue on Day 4. Data are shown as mean + SD (n = 10). Significant difference; §§, $p < 0.01$; sham vs. vehicle (Student t-test), **, $p < 0.01$; vehicle vs. 0.1% and 1% tapinarof (Dunnett test).

IL-23/Th17 axis activation. Our study is the first to demonstrate that tapinarof suppresses IL-23-induced psoriasis-like skin inflammation in mice. Moreover, tapinarof activated AhR pathway, upregulated the expression of antioxidant molecule NQO1, and reduced IL-17A production in the ears of mice with skin inflammation.

In an IL-23-induced psoriasis model, we demonstrated the efficacy of tapinarof, while another group previously reported that tapinarof was not effective.[20] The discrepancy between these studies may be attributed to differences in experimental design, particularly in the timing of tapinarof application. Unlike the previous group, who pre-applied tapinarof and ceased its application during IL-23 administration, we applied it concurrently with the induction of pathology by IL-23. This suggests that the concurrent presence of tapinarof during the activation of the Th17 pathway by IL-23 stimulation might be crucial for its inhibitory effects. Since the IL-23/Th17 axis is thought to be constantly activated in psoriasis patients, topical treatment with tapinarof could potentially improve the pathological conditions through inhibition of the axis.

In this model, tapinarof induced the expression of *Cyp1a1* and *Nqo1* in the ear in a concentration-dependent manner, starting from 0.01%, while inhibition of ear swelling and histopathological inflammation saturated at 0.01% or higher. This suggests that even low concentrations of tapinarof could activate AhR pathway sufficiently to suppress psoriasis symptoms. The observed efficacy at concentrations lower than the clinical use concentration of 1% may be attributed to the thinner skin of the mouse ear and differences in drug permeability resulting from the use of a different solvent. Additionally, 0.01% tapinarof did not inhibit the IL-17A production, potentially due to underestimation in the experimental design. We followed the previous method,[18] which demonstrated that an IL-17A blocking antibody suppressed epidermal thickening. In this study, ear thickness and histopathological examinations were evaluated at the central, tip part of the ear, while IL-17A production was measured by homogenizing the entire ear tissue from tip to base. The base of the ear is thicker than the tip, which could potentially result in differences in drug penetration. Histopathological observations showed a gradient of efficacy of tapinarof, decreasing from the tip toward the base. This difference in evaluation sites may have led to the weaker inhibition of IL-17A production compared to the suppression of ear thickness and skin inflammation symptoms.

We showed the effects of tapinarof on IL-17A production and the expression of *Cyp1a1* and *Nqo1*. However, the specific cell types targeted by tapinarof remain unidentified. Previous studies have reported that tapinarof increases the expression of *CYP1A1* as an indicator of AhR activation and inhibits IL-17A production in $CD4^+$ cells.[12] In this study, suppression of IL-

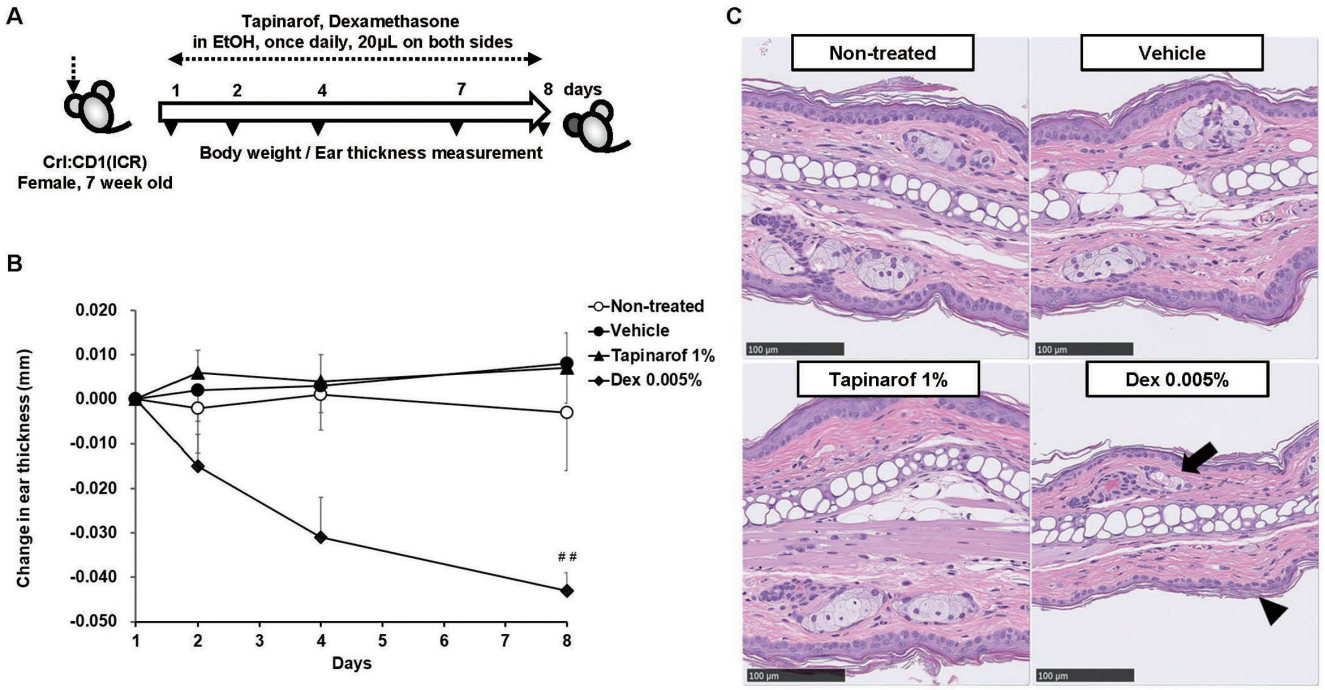

Fig. 3. Effects of Topical Administration of Tapinarof on Normal Skin in Mice

(A) Vehicle (EtOH), 1% tapinarof, or 0.005% Dex were administered topically once daily to both sides of the left ear for 7 days. (B) Ear thickness changes were assessed on Days 1, 2, 4, and 8. Data are represented as the change from baseline. The results are shown as the mean and SD (n = 8 or 10). Significant difference; ##, $p < 0.01$; 0.005% Dex vs. non-treated, vehicle, 1% tapinarof (Steel-Dwass multiple comparison test). (C) Representative photomicrographs of HE-stained ear skin samples. Group names are presented on each panel. Non-treated, normal ear skin; vehicle and 1% tapinarof, no abnormality; and 0.005% Dex, thinning of epidermis (arrowhead) and atrophy of sebaceous glands (arrow). Bar = 100 μm.

Table 2. Effects of Tapinarof and Dexamethasone on the Histopathological Changes in the Ear Skin of Normal Mice (Microscopic Findings)

Group	Non-treated		Vehicle		Tapinarof 1%		Dexamethasone 0.005%	
Number of animals	8		10		10		10	
Findings	-	P	-	P	-	P	-	P
Ear skin								
Thinning, epidermis	8	0	10	0	10	0	0	10
Atrophy, sebaceous glands	8	0	10	0	10	0	0	10

'-, findings absent; P, findings present (non graded findings)

17A production by tapinarof was observed in an *in vitro* study using human peripheral blood mononuclear cells (PBMCs) stimulated with T-cell activators (Fig. S3). The suppression of IL-17A production is thought to take place in Th17 cells. Tapinarof has also been reported to induce *CYP1A1* expression and antioxidant activity in keratinocytes.[12] In our study, we observed that tapinarof induced the expression of *NQO1* in PBMCs stimulated with T-cell activators (Urashima *et al.*, unpublished data). Based on these previous findings and preliminary data from our study, the induction of the antioxidant molecule NQO1 may occur in keratinocytes and T cells. However, considering the broad expression of AhR in the skin, similar effects may occur in other cells, warranting further investigation. Furthermore, we did not investigate the AhR dependency or the mechanisms underlying symptom improvement in this model. Previous studies using AhR knockout mice have demonstrated that the suppression of imiquimod-induced dermatitis and other inflammatory cytokines is AhR-dependent.[12] Given these findings, future research should be conducted to elucidate the precise mechanisms underlying tapinarof's effects on IL-23-induced dermatitis.

Next, we applied 1% tapinarof, which had shown sufficient efficacy, to normal mouse skin to determine its skin atrophogenic potential in comparison to 0.005% Dex. Although Dex-treated mice exhibited a slight reduction in body weight, this group showed pronounced skin thinning and atrophy of sebaceous glands. Conversely, the tapinarof-treated group did not exhibit significant skin thinning. Long-term use of TCs can cause specific adverse reactions such as skin atrophy, which could impact the quality of life and drug selection.[21] These findings suggest that tapinarof may be safer and more user-friendly than TCs in terms of skin atrophy. Tapinarof is thought to be an alternative to TCs, especially for long-term use. Nevertheless, the comparison was not conducted at a concentration of Dex showing equivalent dermatitis suppression to tapinarof. This is a limitation in the assessment of the impact on normal mouse skin. The concentration of Dex was set at the maximum concentration that did not affect the general physical condition and body weight of mice. It is necessary to verify the findings in this study that tapinarof has less

potential for skin atrophy by comparative evaluation with Dex at a concentration that exhibits a dermatitis suppression effect equivalent to that of 1% tapinarof.

The AhR pathway is involved in the regulation of the immune balance of Th17/Th22 and regulatory T (Treg) cells, and its regulation is ligand dependent. It has been shown that prolonged activation of AhR by 2,3,7,8-tetrachlorodibenzo-p-dioxin may potentiate Treg cell deviation, whereas transient AhR activation by 6-formylindolo [3,2-b] carbazole may interfere with Treg cell differentiation and shift the immune response toward Th17 cells and to Th22 cell differentiation.[10,22] Since tapinarof suppressed Th17 cytokine production in this study, tapinarof may regulate the immune balance between the Th17/Th22 and Treg cells. Although the effect of tapinarof on Treg cells was not examined in this study, it could be hypothesized that tapinarof enhances Treg cells by inhibiting the Th17 pathway. Further studies should be conducted in the future to elucidate the effect of tapinarof on Th17/Th22 and Treg balance. This will allow for a more comprehensive understanding of the pharmacological properties of tapinarof in the treatment of psoriasis.

In conclusion, this study indicates that tapinarof has antioxidant effects and suppresses IL-23/Th17 axis through AhR agonism, which may contribute to the amelioration of psoriasis symptoms. Furthermore, tapinarof has been shown to have lower potential for skin atrophogenicity than conventional TCs. These findings provide a new perspective on the pharmacological properties of tapinarof and position it as a promising novel topical treatment option for psoriasis patients.

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