Different imiquimod creams resulting in differential effects for imiquimod-induced psoriatic mouse models

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Abstract

Imiquimod (IMQ)-induced mouse psoriatic model is one of the useful models displaying most of psoriatic features. To compare the modeling efficacy of different IMQ creams, we induced the psoriatic models by topically applying two different brands of IMQ 5% creams to the shaved Balb/c mice skin and assessed the results. Balb/c female mice ($n = 24$) 8–12 weeks of age were randomly divided into experimental groups A (Likejie), B (Aldara), and control group C (Vaseline); Likejie, Aldara, or Vaseline was topically applied to the back skin for mice in groups A, B, and C, respectively, for six consecutive days. The total psoriasis area and severity index scores of groups A, B, and C were 3.25 ± 1.56 , 9.81 ± 0.84 , and 0, respectively; the Baker's scores were 2.93 ± 1.07 , 6.47 ± 1.50 , and 0, respectively; and the epidermis thickness was 49.79 ± 14.16 , 85.62 ± 17.55 , and $20.04 \pm 3.68 \mu\text{m}$, respectively. The differences between the three groups in dual were statistically significant ($P < 0.005$ for the groups in dual). Aldara group showed more characteristic alterations of psoriasiform lesions than that of Likejie both macroscopically and histopathologically. The results suggested that different brands of IMQ creams may result in differential efficacy when performing the IMQ-induced psoriasis mouse models.

Keywords: Psoriasis, imiquimod, mouse model, psoriasis area and severity index, Baker's score

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Introduction

Psoriasis is a common, chronic inflammatory skin disease affecting nearly 2–3% of the world's population.¹ It always involves the skin, and sometimes associates with arthritis, and largely, lowers quality of life. Increasing evidence suggests that psoriasis may be associated with cardiovascular diseases^{2–4} and metabolic syndromes.^{5,6} Although much evidence supports the assertion that psoriasis is a T-cell-mediated chronic inflammatory skin disease, its exact pathogenesis is not yet fully understood.⁷ The lack of an ideal animal model has greatly hindered study of the etio-pathogenesis of psoriasis.⁷ Over the past two decades, various animal models have been built including the spontaneous mouse model, the genetically engineered mouse model, the xenograft model, and the artificial induced model in addition to many others.^{7,8} Despite each model having limitations, these models make the study of psoriasis *in vitro* possible to some extent. Imiquimod (IMQ) 5% cream, a ligand for TLR7 and TLR8 and a potent immune activator, has been reported to cause psoriasis-like disease in some patients or to exacerbate the lesions in patients with well-controlled psoriasis during topical treatment for other diseases.⁹ Daily topical application of IMQ on mouse back

skin, including Aldara or other brands of IMQ cream, has been reported to cause inflamed scaly cutaneous lesions mimicking psoriasis.^{8,10–18} The aim of the present study was to compare the differential effects of IMQ-induced psoriatic mouse models by different brands of IMQ cream.

Materials and methods

Animal experiment

BALB/C female mice 8–12 weeks of age obtained from the Laboratory Animal Center (Sun Yat-sen University, China) were used in this experiment. Aldara cream (5% IMQ, 3M Pharmaceuticals, UK) and Likejie cream (5% IMQ, Keyi Pharmaceutical Co. Ltd, Hubei, China) were used to induce the psoriatic models, and Vaseline cream (Hengjian Pharmaceutical Co. Ltd, Guangdong, China) was used as a control. Aldara, Likejie, and Vaseline creams were purchased from a pharmacy.

Mice ($n = 24$) were randomly divided into experimental group A (Likejie cream), experimental group B (Aldara cream), and the control group C (Vaseline cream) by means of a random number table (eight mice in each group). An area about $2\text{ cm} \times 3\text{ cm}$ in size was shaven on the back of each mouse with an electric shaver, and the

Table 1 Baker's scoring system.¹⁹

Items		Score
Keratin	Munro abscess	2.0
	Hyperkeratosis	0.5
	Parakeratosis	1.0
Epidermis	Thinning above papillae	0.5
	Lengthening and clubbing of rete ridges	1.5
	Acanthosis	0.5
	Lack of granular layer	1.0
Dermis	Lymphocytic infiltrate	
	Mild	0.5
	Moderate	1.0
	Marked	2.0
	Papillary congestion	0.5

remaining hairs were completely removed with depilatory cream (Veet™, Reckitt Benckiser, Cedex, France) before the experiment. The method described by Van der Fits *et al.*⁸ was used for inducing the psoriatic mouse model. Both Likejia and Aldara creams were topically applied to the back skin for six consecutive days at a daily dose of 80 mg (contained 4 mg of the active compound, IMQ) in the experimental groups A and B, respectively. This dosage was determined in our previous study and could cause optimal and reproducible skin inflammation in mice. The control group was treated daily with 80 mg white Vaseline cream on the shaven skin. The day before cream usage was defined as Day 1 and the baselines of every index were evaluated on this day; 24 h after the first application of the cream was defined as Day 2. The mice were sacrificed on Day 7 by cervical dislocation.

Modified psoriasis area and severity index (PASI) score

Based on the clinical PASI score, the scoring system described by Van der Fits *et al.*⁸ was used to evaluate the severity of skin inflammation in the present study. The indexes included erythema, scales, and thickening. Each index was scored independently on a scale from 0 to 4: 0—none, 1—slight, 2—moderate, 3—marked, 4—maximum, and recorded every 24 h. The total score (erythema plus scaling plus thickening) was used to measure the severity of inflammation (scale 0–12). The area score was not taken into account, because each mouse had the same experimental area.

Pathological score

After the mouse was sacrificed, the tested skin was excised immediately and was fixed in 10% formaldehyde for 24 h. The formalin fixed skin tissue was then embedded in paraffin and sectioned vertically at 3 μm. Following this, the section was stained with Harris' hematoxylin and eosin for histological analysis. The Baker's scoring system¹⁹ (Table 1) was used to evaluate the pathological alterations on a scale ranging from 0 to 10. All the sections were scored blindly by two independent investigators and the mean score was recorded.

Measurement of the epidermic thickness

The thickness of the vertical epidermis for each mouse was evaluated under a microscope on three different fields under 100 × high power; then, the mean thickness was calculated. To evaluate the thickness, the thickest areas with typical psoriatic pathological alterations were selected for groups A and B, and the thickest areas were selected for group C.

Ethics statement

The experiments were approved by the Committee on the Ethics of Animal Experiments of The First Affiliated Hospital, Sun Yat-sen University and were conducted in compliance with the guidelines of China Council on Animal Care and Use. All mice were bred and maintained under specific pathogen-free conditions with sufficient fresh food and water *ad libitum*. During the experiment, the mice were monitored daily. After experimental completion, mice were sacrificed by cervical dislocation.

Statistical analysis

The statistical software package SPSS 13.0 (SPSS Inc., Chicago, IL, USA) was used for data analyses. Baker's scores (non-parametric distribution) were presented by median (M) and interquartile range (IQR), and Kruskal–Wallis test was used for analysis among the three groups. The total Baker's scores and modified PASI scores were expressed as mean ± standard deviation (means ± SD), respectively, and T test was used to analyze the difference between group A and group B. The epidermic thickness was expressed as mean ± standard deviation (means ± SD), one-way ANOVA was used for analyzing the differences among the three groups, and Bonferroni test was used for multiple comparisons. The Z or t value between group A and group B was expressed as Z_{A-B} or t_{A-B} , and between group B and the control group and group A and the control group as Z_{B-C} , t_{B-C} , Z_{A-C} , t_{A-C} , respectively. A significant *p* value was set at 0.05 (two-sided).

Results

Skin changes after topically applying of creams

Group A. Psoriasiform lesions had formed after topically applying Likejia for six days, showing redness, thickened skin, and scaling. The skin changes on Day 1, 3, 5, and 7 are shown in Figure 1(a) to (d). Generally, erythema appeared on Day 2 and reached its peak on Day 3, then decreased gradually for the remaining four days, except one mouse showed increasing erythema from Day 3 until Day 7. The scaling started from Day 2 to Day 4 and then decreased until the end of the experiment. The skin revealed increasing thickening from Day 2 to Day 4; after this, the thickening increased mildly up until Day 7.

Group B. All the mice showed typical psoriasis-like lesions after the application of Aldara for six days with evident erythema, scales, and thickened skin. The cutaneous changes on Day 1, 3, 5, and 7 are shown in Figure 1(e) to (h). Both erythema and skin thickening started on Day 2 and

Figure 1 (a)–(h) Imiquimod-induced inflammatory skin in mice phenotypically mimicking psoriasis. The skin presentations after topically applying of Likejia cream (a–d) and Aldara cream (e–h) on the shaved BALB/C mouse back skin on Day 1, 3, 5, and 7, respectively, suggesting that Aldara cream is a more potent inducer of psoriasiform lesions compared to Likejia. (A color version of this figure is available in the online journal.)

progressed until Day 7. The scaling started on Day 2 and increased gradually until Day 4 and then progressed rapidly until Day 7. On Day 7, the scaling resembled a mosaic of cobblestones and domed shapes were seen on the skin surface.

Group C. Little cutaneous alterations presented after treatment with Vaseline cream for six days.

The modified PASI scores showed that the erythema and skin thickness were more evident in group B than in group A from Day 4 to 7, respectively. The scales formed earlier but regressed more rapidly in group A than in group B (Figure 2). The total modified PASI scores were not significantly different between group A and group B in the initial four days, but were statistically significant from Day 5 to 7, with a higher final modified PASI score in group B

(9.81 ± 0.84) than in group A (3.25 ± 1.56) ($t_{A-B} = 10.478$, $P < 0.001$).

Histopathological alterations after the experiment

Histopathology showed that group A revealed hyperkeratosis and acanthosis, as well as moderate lymphocytic infiltrate and mild angiotectasis in the dermis; the granular layer was still present and muro abscess was absent (Figure 3(a)). Group B revealed severe acanthosis, parakeratosis, and moderate rete ridge formation, accompanied by focal munro/kogoj abscess and disappearance of the granular layer, with more evident lymphocytic infiltrate and angiotectasis in the dermis than group A (Figure 3(b)). Group C displayed normal dermis as well as epidermis (Figure 3(c)).

Figure 2 (a)–(d) Daily scores based on modified PASI. The daily scores of erythema (a), scales (b), thickness (c), and total PASI scores (d) for Likejie, Aldara, and control group, respectively (symbols indicate mean score $\pm$ standard deviation). The results show that the cutaneous erythema and thickness are more evident on the Aldara group than the Likejie group. The scales of the Likejie group present earlier but regress more rapidly than those of the Aldara group. The total PASI scores between the Aldara group (9.81 ± 0.84) and the Likejie group (3.25 ± 1.56) are significantly different on Day 7 ($t = 10.478$, $P < 0.001$). PASI: psoriasis area and severity index. (A color version of this figure is available in the online journal.)

The differences of Baker's scores among the three groups (Table 2) were statistically significant ($H = 20.096$, $p < 0.001$). Both group A (M: 2.75; IQR: 1.56) and group B (M: 6.75; IQR: 2.63) had higher total Baker's scores than group C (M: 0; IQR: 0.5), respectively ($Z_{A-C} = 3.422$, $P = 0.001$; $Z_{B-C} = 3.424$, $P = 0.001$, respectively); additionally, the score in group B (6.47 ± 1.50) was much higher than in group A (2.93 ± 1.07) ($t_{A-B} = 5.420$, $P < 0.001$).

Epidermis thickness in different groups

The thickness of epidermis for groups A, B, and C was 49.79 ± 14.16 , 85.62 ± 17.55 , and 20.04 ± 3.68 μm , respectively, with a statistically significant difference ($F = 49.600$,

$p < 0.001$). The differences among the three groups in dual were also statistically significant ($p < 0.005$ for three groups in dual). The epidermis of both group A and group B had 3–5 layers of cells while group C had only 1–2 layer(s).

Discussion

It is considered that a perfect psoriasitic animal model should include typical lesions which can be provoked by mild trauma, epidermal hyperproliferation and altered differentiation, papillomatosis, inflammatory cell infiltration including T cells, altered vascularity, and good response to antipsoriatic agents including biological products.²⁰ Although various kinds of psoriatic models are available

Figure 3 (a)–(c) Histopathological alterations. After treatment for six days, histopathology shows (a) significant hyperkeratosis and acanthosis, as well as moderate lymphocytic infiltrate and mild angiectasis in the dermis with presence of granular layer (the white hollow arrow) in the Likejia group; (b) evident acanthosis, parakeratosis, and moderate rete ridge formation, accompanied by focal Munro abscess (the white hollow arrow) and absence of granular layer in the Aldara group; and (c) normal skin in control group (a–c, hematoxylin and eosin, original magnification $\times 200$). (A color version of this figure is available in the online journal.)

at present, no animal model perfectly fulfills the criteria. IMQ-induced mouse model is considered a good model for psoriasis, can easily be obtained, and presents with the typical psoriatic phenotype.⁸ It shares most histological features of psoriasis, including cutaneous inflammation, hyperproliferation, and cutaneous infiltration of plasmacytoid dendritic cells CD4⁺ T cells, CD11c⁺ dendritic cells and neutrophils, and even an aberrant antioxidant system.^{11,21} However, the mechanisms for IMQ-induced psoriatic model are not yet fully understood.

The present study showed that both experimental groups presented psoriasis-like lesions after the application of different brands of IMQ cream. The scales in the Likejia group presented earlier with a tendency toward regression

Table 2 Pathological scores based on Baker's scoring system (M[IQR])

Items		Group A	Group B	Group C
Keratin	Munro abscess	0 (0)	1 (1.75)	0 (0)
	Hyperkeratosis	0.5 (0)	0.5 (0)	0 (0)
	Parakeratosis	0 (0.75)	1 (0)	0 (0)
Epidermis	Thinning above papillae	0 (0)	0 (0)	0 (0)
	Lengthening and clubbing of rete ridges	0.5 (0)	1 (0.38)	0 (0)
	Acanthosis	0.5 (0)	0.5 (0)	0 (0)
	Lack of granular layer	0 (0)	0.75 (0.5)	0 (0)
Dermis	Lymphocytic infiltrate	0.625 (1)	1.25 (0.5)	0 (0.5)
	Papillary congestion	0.25 (0.44)	0.5 (0)	0 (0)
Total score		2.75 (1.56)	6.75 (2.63)	0 (0.5)

The data above are presented by median (interquartile range) (M[IQR]), both group A and group B have higher total scores than group C, respectively ($Z_{A-C} = 3.422$, $P = 0.001$; $Z_{B-C} = 3.424$, $P = 0.001$, respectively). The total score of group B (6.47 ± 1.50) is significantly higher than group A (2.93 ± 1.07) ($t_{A-B} = 5.420$, $P < 0.001$).

during the experiment. However, the Aldara group had higher PASI scores and showed more characteristic lesions and histopathological features including thicker epidermis, the scales presented later than the Likejia group. The results suggested that different brands of IMQ cream can induce the mouse psoriatic-like alterations, but different agents may have differential efficacy when inducing the model. The results also showed that Aldara is a more potent inducer compared to Likejia. The reasons for such conditions remain unknown and require further study.

It was reported that the IMQ-induced psoriasis-like cutaneous inflammation in mice was mediated via the IL-23/IL-17 axis, consistent with human psoriasis.^{8,22,23} Our previous studies found that Aldara-induced psoriasisform mice had a significant increase of microvessel density in skin lesions, with dilated capillaries in the superficial and middle layers of the dermis²⁴ and over-expression of cutaneous VEGF predominately distributing the vascular endothelial cells and keratinocytes; although circulating VEGF-A levels showed no significant difference between the experimental and control groups.²⁵ Walter *et al.*²⁶ found that the vehicles in Aldara also play an important role in the modeling process, which can not only induce keratinocyte pyroptosis and parakeratosis, but also upregulate the transcripts for IL-1, G-CSF, S100A8, S100A9, IL-17A, and IL-23 *in vivo*, even in the absence of IMQ. Isostearic acid, a major component of the vehicles of Aldara, can promote inflammasome activation in cultured keratinocytes. Thus, both IMQ and its vehicles in the Aldara component were considered to be responsible for the psoriatic inflammatory response. However, Likejia is a generic drug and its chemical makeup of vehicles also includes isostearic acid, but Likejia was a less potent inducer compared to Aldara. We speculated that differential final results for our present

study may result from the possibility of the different creams' quality, or somehow inactive IMQ in Likejie, or both. Unfortunately, we cannot find clinical data for a head-to-head comparison between Likejie and Aldara in China showing whether their therapeutic effects have any differences. In the future, an experiment of a head-to-head comparison between Aldara and other IMQ creams such as Likejie may be helpful for us to solve this problem.

The present study suggests that different brands of IMQ cream may result in differential results in IMQ-induced psoriatic mouse models. Based on this, researchers should pay attention to the creams' quality when inducing the psoriatic mouse model.

Authors' contributions: All authors participated in the design, interpretation of the studies and analysis of the data, and review of the manuscript; DQL, HHW and YKZ conducted the experiments; DQL, HHW, YKZ, JHL, and FW wrote the manuscript; DQL, HHW, and FW contributed to the statistical analysis.

DECLARATION OF CONFLICTING INTERESTS

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