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Olopatadine hydrochloride accelerates the recovery of skin barrier function in mice

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antihistamine, atopic dermatitis, olopatadine hydrochloride, skin barrier, topical steroid

Conflicts of interest
The authors are employed by Kyowa Hakko Kogyo Co., Ltd (Shizuoka, Japan), the manufacturer of Allelock® (olopatadine).

Summary

Background The skin barrier function in patients with atopic dermatitis is disrupted and prolonged topical steroid therapy produces epidermal barrier disturbance. Olopatadine hydrochloride (olopatadine; Allelock®; Kyowa Hakko Kogyo Co., Ltd, Shizuoka, Japan) is an antiallergic drug with histamine H_1 receptor antagonistic action. This drug alleviates skin inflammation and decreases the number of scratching episodes in a murine model of chronic contact dermatitis.

Objectives To investigate the effects of olopatadine and a steroid on the recovery of skin barrier function after barrier disruption in mice.

Methods The skin barrier of the ears of mice was disrupted by tape stripping. The recovery of skin barrier function was monitored by measurement of transepidermal water loss (TEWL) after barrier disruption. Epidermal hyperplasia was induced by repeated tape stripping for 7 days. Olopatadine was administered orally once daily from 3 days before the first barrier disruption. Betamethasone 17-valerate (betamethasone) was applied topically once daily from 3 days before barrier disruption.

Results Tape stripping led to a significant increase in TEWL. TEWL decreased with time after tape stripping and the skin barrier function recovered by over 60% within 9 h after tape stripping. The recovery of skin barrier in olopatadine-treated mice was significantly accelerated, compared with that in vehicle-treated mice. In contrast, the skin barrier recovery in mice treated with topical betamethasone was significantly delayed, compared with that in vehicle-treated mice. Combined treatment with olopatadine and betamethasone ameliorated the delay in barrier recovery induced by topical treatment with betamethasone. In addition, olopatadine significantly prevented the increase in epidermal thickness induced by prolonged barrier disruption.

Conclusions These results suggest that systemic administration of olopatadine accelerates the recovery of skin barrier function and ameliorates the adverse effects of topical steroids on skin barrier recovery.

Atopic dermatitis (AD), allergic contact dermatitis and psoriasis vulgaris are the most common skin diseases. AD is a chronically relapsing inflammatory skin disease characterized by episodes of intense pruritus, multiple lesions with erythema, excoriation, erosions, lichenification, papules, dry skin and susceptibility to cutaneous infection. In the skin of patients with AD, skin barrier function is disrupted, with increase in transepidermal water loss (TEWL) and decrease in skin hydration.[1] The relationship of an increase of TEWL to the severity of AD symptoms has been reported.[2] Patients with AD often complain of intense itching. Scratching with fingernails causes physical damage to the skin and aggravates skin lesions.[3]

Topical steroids and emollients have been widely prescribed for the lesions in various inflammatory skin disorders including AD and contact dermatitis.[4] However, prolonged topical treatment with steroids results in well-recognized skin abnormalities such as skin atrophy and epidermal barrier disturbance.[5] For example, prolonged steroid therapy produces epidermal thinning and increases basal TEWL, indicating a defect in skin barrier function.[6,7] These adverse effects of steroids are generally attributed to their negative effects on keratinocyte proliferation and epidermal lipid synthesis.[6] A more recent study has shown that, in the normal skin of humans and mice, even short-term treatment with a potent steroid could produce deterioration in barrier homeostasis,

characterized by delayed barrier recovery and abnormal stratum corneum integrity.[8]

Histamine H_1 receptor antagonists have long been prescribed for patients with AD as an adjunct to therapy with topical agents, in the belief that they reduce pruritus by blocking the action of histamine in the skin. Histamine is also considered to contribute to the maintenance of skin barrier function in the epidermis. In an experimental model of skin barrier disruption, topical application of histamine delayed barrier recovery and topical application of the histamine H_1 receptor antagonist diphenhydramine accelerated barrier recovery.[9] However, it has not been shown whether administration of histamine H_1 receptor antagonists under commonly employed conditions (i.e. systemic oral administration) accelerates skin barrier recovery.

Olopatadine hydrochloride (olopatadine; Allelock®; Kyowa Hakko Kogyo Co., Ltd, Shizuoka, Japan) is an antiallergic agent with histamine H_1 receptor-antagonistic action. Olopatadine is indicated for the signs and symptoms of allergic rhinitis, chronic urticaria, eczema/dermatitis, prurigo, pruritus, psoriasis vulgaris and erythema multiforme. We have reported that olopatadine attenuates (i) the elevation of cytokines such as interleukin (IL)-4 and interferon-γ in the lesion and (ii) increases in the number of scratching episodes in a mouse model of chronic contact dermatitis induced by repeated challenge of hapten.[10,11] We have also demonstrated that olopatadine suppresses the rebound phenomenon after the discontinuation of topical steroid therapy in mice with chronic contact dermatitis.[12] The aims of this study were, firstly, to investigate whether systemic administration of olopatadine accelerates the recovery of skin barrier function disrupted by tape stripping in mice and, secondly, to examine the effect of olopatadine on the delay in skin barrier recovery in mice treated with topical steroid.

Materials and methods

Animals

Six-week-old male ICR mice were purchased from CLEA Japan (Tokyo, Japan). The animals were kept in a specific pathogen-free animal facility maintained at a temperature of 19–25 °C, humidity of 30–70%, and a 12-h day/night cycle, and were given access to food and water *ad libitum*. The experiments were conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals and approved by the Committee for Animal Experiments of Kyowa Hakko Kogyo Co., Ltd (Shizuoka, Japan).

Materials

Olopatadine was synthesized at Yokkaichi Plant, Kyowa Yuka Co., Ltd (Mie, Japan). Betamethasone 17-valerate (betamethasone) and chlorpheniramine maleate (chlorpheniramine) were purchased from Sigma Chemical Co. (St Louis, MO, U.S.A.). Olopatadine and chlorpheniramine were dissolved in distilled water. Betamethasone was dissolved in propylene glycol/ethanol (7 : 3).

Recovery of skin barrier function

Skin barrier function was evaluated by measurement of TEWL using a VapoMeter (Delfin, Kuopio, Finland). In mice anaesthetized with pentobarbital 50 mg kg^{-1}, the ventral surface of the right ear was treated by tape stripping with cellophane tape (Scotch; Sumitomo 3M, Tokyo, Japan) until TEWL reached 40–50 g m^{-2} h^{-1}. TEWL was measured just before, immediately after and at 1, 3, 6 and 9 h after tape stripping. The percentage of barrier recovery was calculated using the following formula: (TEWL immediately after tape stripping − TEWL at indicated time point)/(TEWL immediately after tape stripping − TEWL before tape stripping) × 100%. The areas under the curves (AUCs) for the skin barrier recovery rates were calculated using the trapezoid method. Distilled water, olopatadine at 1, 3 and 10 mg kg^{-1} daily and chlorpheniramine 10 mg kg^{-1} daily were administered orally to mice once daily from 3 days before tape stripping. To examine the effects of olopatadine on the delay in the barrier recovery in betamethasone-treated mice, distilled water or olopatadine 10 mg kg^{-1} daily was administered orally to mice in combination with topical application of either vehicle or 0·12% w/v betamethasone (0·012 mg per ear daily) once daily from 3 days before tape stripping. Distilled water, olopatadine and chlorpheniramine were administered orally at a volume of 1 mL per 100 g of body weight. Vehicle and betamethasone were applied at a volume of 10 μL per ear.

Quantification of histamine release from the skin

Skin biopsies (circles of 8 mm diameter) were taken from the ear treated with or without tape stripping and incubated in phosphate-buffered saline at room temperature for 30 min. Histamine in the supernatant was quantified with a Histamine EIA kit (MBL, Nagano, Japan), according to the manufacturer's instructions.

Epidermal hyperplasia induced by prolonged barrier disruption

The ventral surface of the right ear of mice was treated with repeated tape stripping until TEWL reached over 50 g m^{-2} h^{-1}. This procedure was carried out twice daily for 7 days. Distilled water and olopatadine at doses of 3 and 10 mg kg^{-1} daily were administered orally once daily from 3 days before the first barrier disruption. Twenty-four hours after the final barrier disruption, mice were killed and the right ear was removed. Punch biopsy specimens of the ear (circles of 8 mm diameter) were weighed, fixed with 10% v/v neutral buffered formalin and embedded in paraffin wax. Tissue sections were stained with haematoxylin and eosin for light microscopic observation. Three sections were taken from each ear specimen. On each section, 10 points were selected at

random and the thickness of the epidermis was measured with a digital high definition microscope (VH-7000C; Keyence, Osaka, Japan). The mean epidermal thickness was calculated.

Statistical analysis

Data are presented as mean ± SEM. The Aspin-Welch test or Student's t-test following the F-test was used for analysis of differences between two groups. Multiple comparisons among treatment groups were made by one-way analysis of variance, followed by the Dunnett test. $P < 0{\cdot}05$ was considered statistically significant. All statistical calculations were performed with the Statistical Analysis System (SAS release 8.2; SAS Institute, Cary, NC, U.S.A.).

Results

Olopatadine accelerates skin barrier recovery

Mean ± SEM TEWL values of the ear of mice treated with distilled water, and olopatadine at doses of 1, 3 and 10 mg kg^{-1} daily were 7·9 ± 0·6, 7·5 ± 0·4, 8·2 ± 0·5 and 7·6 ± 0·4 g m^{-2} h^{-1}, respectively, indicating that treatment with olopatadine for 3 days did not affect basal TEWL. In addition, TEWL immediately after tape stripping in olopatadine-treated mice was also comparable with that in distilled water-treated mice (Fig. 1a). TEWL decreased with time after tape stripping and skin barrier function recovered by over 60% at 9 h after tape stripping in distilled water-treated mice (Table 1). In mice treated with olopatadine at 3 and 10 mg kg^{-1} daily, the recovery of skin barrier function was significantly accelerated at 1 h and at 1, 3 and 6 h after tape stripping, respectively, compared with that in distilled water-treated mice (Fig. 1b). The AUC for skin barrier recovery rates showed that olopatadine accelerated skin barrier recovery in a dose-dependent manner (Fig. 1c). In contrast, the classical histamine H_1 receptor antagonist chlorpheniramine at 10 mg kg^{-1} daily did not have significant effects on the AUC (Fig. 1c).

Histamine release from the skin immediately after barrier disruption

To ascertain the contribution of endogenous histamine to the skin barrier recovery, we evaluated the amount of histamine released from the skin with or without tape stripping. Tape stripping significantly increased the secretion of histamine from the skin tissues within 30 min (Fig. 2).

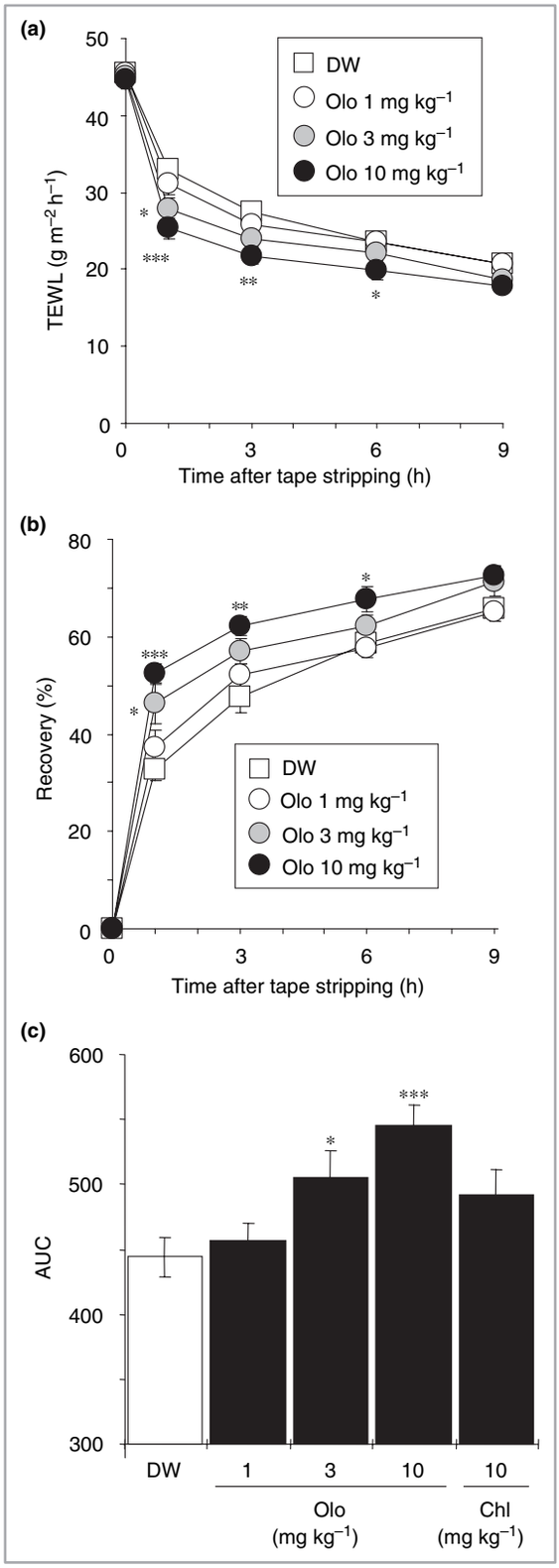

Fig 1. The effect of olopatadine (Olo) on the recovery of skin barrier function after tape stripping. Distilled water (DW) and Olo at doses of 1, 3 and 10 mg kg^{-1} daily (a–c) and chlorpheniramine (Chl) at 10 mg kg^{-1} daily (c) were administered orally from 3 days before barrier disruption. Transepidermal water loss (TEWL) was measured before, immediately after and at 1, 3, 6 and 9 h after tape stripping (a). Skin barrier recovery rate (b) and the area under the curve (AUC) for the skin barrier recovery rate (c) were determined as described in Materials and methods. Results are expressed as mean ± SEM ($n = 8$). *$P < 0{\cdot}05$, **$P < 0{\cdot}01$, ***$P < 0{\cdot}001$ as compared with the DW group by Dunnett test.

Table 1 The effect of olopatadine on skin barrier recovery after tape stripping

	Skin barrier recovery (%)			
	1 h	3 h	6 h	9 h
Distilled water	32·8 ± 2·3	47·7 ± 3·4	58·5 ± 1·4	65·7 ± 1·8
Olopatadine 1 mg kg^{-1}	37·3 ± 3·4	52·0 ± 2·5	57·5 ± 2·0	65·0 ± 2·0
Olopatadine 3 mg kg^{-1}	46·2 ± 4·2*	56·9 ± 2·6	62·2 ± 2·3	71·3 ± 3·0
Olopatadine 10 mg kg^{-1}	52·3 ± 2·3***	62·3 ± 1·9**	67·6 ± 2·6*	72·5 ± 2·1
Chlorpheniramine 10 mg kg^{-1}	44·0 ± 2·5##	56·3 ± 3·6	61·3 ± 2·7	67·3 ± 1·9

Data are presented as mean ± SEM (n = 8). *P < 0·05, **P < 0·01, ***P < 0·001 as compared with the distilled water group by Dunnett test. ##P < 0·01 as compared with the distilled water group by Student's t-test.

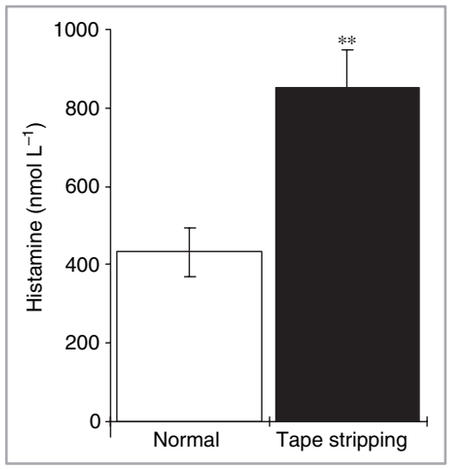

Fig 2. The release of histamine from the skin treated with tape stripping. The amount of histamine in the supernatant of the skin organ culture with or without tape stripping (Normal) was determined. Results are expressed as mean ± SEM (n = 5). **P < 0·01 as compared with the normal group by Student's t-test.

Olopatadine ameliorates the delay in barrier recovery caused by topical betamethasone

To evaluate the effects of olopatadine on the delay in barrier recovery in mice treated with topical steroids, olopatadine 10 mg kg^{-1} daily was administered orally to mice in combination with topical application of betamethasone at clinically relevant concentration (0·12% w/v). Mean ± SEM TEWL values of the ear of mice treated with vehicle and betamethasone were 7·9 ± 0·5 and 7·7 ± 0·6 g m^{-2} h^{-1}, respectively, indicating that topical treatment with betamethasone for 3 days did not affect basal TEWL. As shown in Fig. 3 and Table 2, the recovery of skin barrier function in mice treated with topical betamethasone for 3 days was significantly delayed, compared with that in vehicle-treated mice. The barrier recovery in mice treated with a combination of olopatadine and betamethasone was significantly accelerated, compared with that in mice treated with betamethasone alone (Fig. 3b).

Olopatadine suppresses epidermal hyperplasia induced by barrier disruption

Finally, we examined the effect of olopatadine on epidermal hyperplasia induced by prolonged barrier disruption. As shown in Figure 4, the thickness of the epidermis in mice treated with prolonged tape stripping for 7 days was increased 2·6-fold, compared with that in untreated mice. Olopatadine at 10 mg kg^{-1} daily significantly suppressed the increase in epidermal thickness by 40·5% (Fig. 4).

Discussion

Systemic administration of the histamine H_1 receptor antagonist olopatadine accelerated skin barrier recovery after barrier

Table 2 The effects of olopatadine and betamethasone on skin barrier recovery

	Skin barrier recovery (%)			
	1 h	3 h	6 h	9 h
Vehicle/distilled water	37·0 ± 1·3	52·1 ± 2·1	58·9 ± 2·3	64·5 ± 2·6
Vehicle/olopatadine	48·2 ± 3·0##	59·7 ± 2·2*	64·3 ± 2·5	67·7 ± 2·4
Betamethasone/distilled water	24·8 ± 2·4***	36·2 ± 3·2***	45·8 ± 2·7**	51·3 ± 3·9*
Betamethasone/olopatadine	33·4 ± 2·0$	44·3 ± 2·6*	53·3 ± 2·6	58·2 ± 4·5

Data are presented as mean ± SEM (n = 8). *P < 0·05, **P < 0·01, ***P < 0·001 as compared with the vehicle/distilled water group by Student's t-test. ##P < 0·01 as compared with the vehicle/distilled water group by Aspin–Welch test. $P < 0·05 as compared with the betamethasone/distilled water group by Student's t-test.

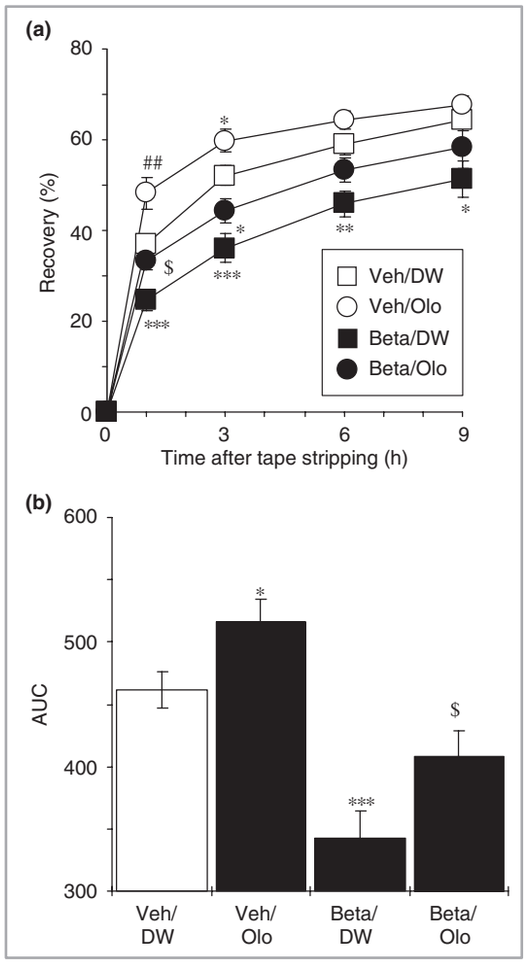

Fig 3. The effect of olopatadine (Olo) on the delay in skin barrier recovery after tape stripping in mice treated with betamethasone (Beta). Distilled water (DW) or Olo 10 mg kg^{-1} daily was administered orally to mice in combination with either topical application of vehicle (Veh/DW or Veh/Olo) or 0·12% w/v Beta (Beta/DW or Beta/Olo) from 3 days before barrier disruption. Transepidermal water loss was measured just before and at 0, 1, 3, 6 and 9 h after tape stripping. Skin barrier recovery rate (a) and the area under the curve (AUC) for the skin barrier recovery rate (b) were determined as described in Materials and methods. Results are expressed as mean ± SEM (n = 8). *P < 0·05, **P < 0·01, ***P < 0·001 as compared with the Veh/DW group by Student's t-test. ##P < 0·01 as compared with the Veh/DW group by Aspin–Welch test. $P < 0·05 as compared with the Beta/DW group by Student's t-test.

disruption. This result was consistent with the previous report that topical application of the classical histamine H_1 receptor antagonist diphenhydramine accelerated the barrier recovery,[9] suggesting that histamine and histamine H_1 receptor may contribute to the recovery of skin barrier function. In contrast, systemic administration of the classical histamine H_1 receptor antagonist chlorpheniramine had less effect on skin barrier recovery. Although chlorpheniramine at the dose used in this study has been shown significantly to suppress histamine-induced formation of paw oedema in mice,[13] it remains unclear whether systemic administration of this drug achieves sufficient drug concentration to antagonize histamine response in the epidermis. Alternatively, olopatadine appears to exert additional biological effects besides its histamine H_1 receptor antagonistic activity on skin barrier recovery. Further studies are required to elucidate whether histamine H_1 receptor antagonists other than olopatadine can accelerate the barrier recovery by systemic administration.

The facilitatory effects of olopatadine on skin barrier recovery were detected at early time points after barrier disruption by tape stripping. One of the earliest and crucial stages of the skin barrier recovery is the exocytosis of lipid-containing granules, called lamellar bodies. The lipids secreted into the intercellular domain of the stratum corneum form a water-impermeable membrane within 1 h after damage of the barrier function.[14] Previous studies showed that disruption of the skin barrier caused an immediate loss of the calcium gradient in the epidermis and that calcium ion influx into keratinocytes reduced the secretion of lamellar bodies resulting in the delay in skin barrier recovery.[15–17] In this study, we demonstrated that histamine was released from the skin organ culture immediately after barrier disruption by tape stripping. Histamine caused an elevation of intracellular calcium in keratinocytes via histamine H_1 receptor.[18] Topical application of exogenous histamine caused a delay in the skin barrier recovery.[9] These results suggest that histamine participates in the regulation of the secretion of lamellar bodies in an early stage of skin barrier recovery. The facilitatory effects of histamine H_1 receptor antagonists on the barrier recovery might be due to the acceleration of lamellar body secretion in the epidermis.

Skin atrophy and epidermal barrier disturbance are well-recognized adverse effects of prolonged topical steroid therapy.[5] Even short-term treatment with the potent steroid clobetasol at clinically relevant concentration (0·05% w/v) could produce deterioration in barrier function in the skin of humans and mice.[8] In the skin of mice treated with clobetasol, both the density of lamellar bodies and the amount of secreted lamellar bodies at the interface between the stratum corneum and the stratum granulosum were markedly reduced. Here we showed that topical treatment with betamethasone, a less potent steroid than clobetasol, could also produce a significant delay in the barrier recovery. Combined treatment with olopatadine + betamethasone ameliorated the delay in barrier recovery by betamethasone at 1 h but not at later time points after barrier disruption. Olopatadine might accelerate the secretion of lamellar bodies as described above but did not affect the reduction in lipid synthesis caused by topical steroids. Tamura *et al.*[12] reported that olopatadine suppressed the rebound phenomenon following discontinuation of topical treatment with a steroid, possibly resulting from its effects in diminishing the elevated cytokines in the lesional skin. Thus, olopatadine is expected to be a therapeutic agent that reduces the adverse effects of therapy with topical steroids.

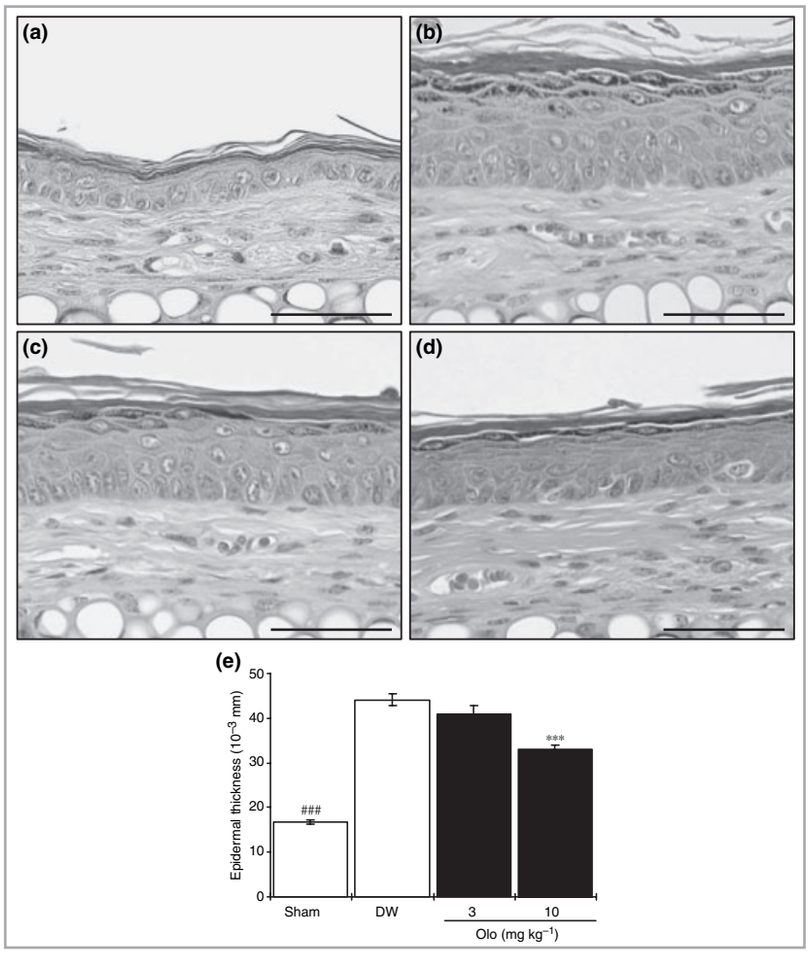

Fig 4. The effect of olopatadine (Olo) on epidermal hyperplasia induced by prolonged barrier disruption. Tape stripping was carried out twice a day for 7 days. Distilled water (DW) and Olo at doses of 3 and 10 mg kg^{-1} daily were administered orally from 3 days before the first barrier disruption. As an untreated control, mice were administered DW without tape stripping (Sham). Twenty-four hours after the final tape stripping, ear specimens were fixed and stained with haematoxylin and eosin. (a) Sham, (b) DW, (c) Olo at 3 mg kg^{-1} daily, (d) Olo at 10 mg kg^{-1} daily. Scale bar = 50 μm. The thickness of the epidermis was measured as described in Materials and methods (e). Results are expressed as mean ± SEM (n = 7–8). ***$P < 0.001$ as compared with the DW group by Dunnett test. ###$P < 0.001$ as compared with the DW group by Student's t-test.

Olopatadine prevented the epidermal hyperplasia induced by prolonged barrier disruption. Although the magnitude of epidermal hyperplasia was directly correlated with both the degree and the duration of barrier disruption, occlusion with a water-impermeable membrane did not prevent the epidermal hyperplasia, indicating that the epidermal hyperplasia did not appear to be directly related to an increase in TEWL in this model.[19] Olopatadine might suppress epidermal hyperplasia by a mechanism independent of its facilitatory effect on skin barrier recovery. The mechanisms by which prolonged barrier disruption induces the epidermal hyperplasia have remained unclear. Barrier disruption by tape stripping leads to an increase in the production of epidermal cytokines, such as tumour necrosis factor-α, granulocyte/macrophage colony-stimulating factor (GM-CSF), IL-8, IL-1α, IL-1β and IL-6.[20,21] Among these cytokines, GM-CSF, IL-6 and IL-8 have been shown to stimulate keratinocyte proliferation.[22–24] Keratinocytes, which comprise 95% of the cells in the epidermis, are one of the important sources of cytokines in the skin. Histamine has been shown to induce production of GM-CSF, IL-6 and IL-8 in human keratinocytes.[18] Olopatadine inhibited production of these cytokines induced by histamine in keratinocytes.[18] Thus, increased production of cytokines in keratinocytes induced by histamine could stimulate the proliferation of keratinocytes resulting in epidermal hyperplasia. Olopatadine might not only accelerate barrier recovery but also provide other potential benefits after epidermal injury, resulting from its inhibitory effects on the production of cytokines by keratinocytes.

Scratching with fingernails causes physical damage to the skin, resulting in an increase of TEWL, and aggravates skin lesions in patients with AD.[25] So, suppression of scratching behaviour results in a reduction of TEWL. Several reports implicated skin dryness itself and/or skin barrier disruption in dry skin-associated pruritus.[26,27] However, in this study, tape stripping treatment did not cause scratching behaviour in mice as reported previously.[28] Therefore, the facilitatory effect of olopatadine on skin barrier recovery was not due to suppression of scratching behaviour. Thus, olopatadine might not only suppress scratching behaviour but also might accelerate skin barrier recovery after physical barrier disruption by scratching behaviour in patients with AD.

In conclusion, olopatadine was demonstrated to be an anti-allergic drug accelerating skin barrier recovery and to ameliorate the adverse effects of topical steroids on the skin barrier recovery.

References

1 Jensen JM, Folster-Holst R, Baranowsky A *et al.* Impaired sphingomyelinase activity and epidermal differentiation in atopic dermatitis. *J Invest Dermatol* 2004; **122**:1423–31.

2 Kim DW, Park JY, Na GY *et al.* Correlation of clinical features and skin barrier function in adolescent and adult patients with atopic dermatitis. *Int J Dermatol* 2006; **45**:698–701.

3 Kimura T, Miyazawa H. The 'butterfly' sign in patients with atopic dermatitis: evidence for the role of scratching in the development of skin manifestations. *J Am Acad Dermatol* 1989; **21**:579–80.

4 Wahlgren CF. Itch and atopic dermatitis: an overview. *J Dermatol* 1999; **26**:770–9.

5 Ulrich RH, Thomas R, Robert A *et al.* Adverse effects of topical glucocorticosteroids. *J Am Acad Dermatol* 2006; **54**:1–15.

6 Woodbury R, Kligman AM. The hairless mouse model for assaying the atrophogenicity of topical corticosteroids. *Acta Derm Venereol (Stockh)* 1992; **72**:403–6.

7 Sheu HM, Lee JY, Chai CY *et al.* Depletion of stratum corneum intercellular lipid lamellae and barrier function abnormalities after long-term topical corticosteroids. *Br J Dermatol* 1997; **136**:884–90.

8 Kao JS, Fluhr JW, Man MQ *et al.* Short-term glucocorticoid treatment compromises both permeability barrier homeostasis and stratum corneum integrity: inhibition of epidermal lipid synthesis accounts for functional abnormalities. *J Invest Dermatol* 2003; **120**:456–64.

9 Ashida Y, Denda M, Hirao T. Histamine H1 and H2 receptor antagonists accelerate skin barrier repair and prevent epidermal hyperplasia induced by barrier disruption in a dry environment. *J Invest Dermatol* 2001; **116**:261–5.

10 Tamura T, Matsubara M, Takada C *et al.* Effects of olopatadine hydrochloride, an antihistamine drug, on skin inflammation induced by repeated topical application of oxazolone in mice. *Br J Dermatol* 2004; **151**:1133–42.

11 Tamura T, Amano T, Ohmori K *et al.* The effects of olopatadine hydrochloride on the number of scratching induced by repeated application of oxazolone in mice. *Eur J Pharmacol* 2005; **524**:149–54.

12 Tamura T, Matsubara M, Hasegawa K *et al.* Olopatadine hydrochloride suppresses the rebound phenomenon after discontinuation of treatment with a topical steroid in mice with chronic contact hypersensitivity. *Clin Exp Allergy* 2005; **35**:97–103.

13 Tamura T, Masaki S, Ohmori K *et al.* Effect of olopatadine and other histamine H1 receptor antagonists on the skin inflammation induced by repeated topical application of oxazolone in mice. *Pharmacology* 2005; **75**:45–52.

14 Elias PM, Feingold KR. Coordinate regulation of epidermal differentiation and barrier homeostasis. *Skin Pharmacol Appl Skin Physiol* 2001; **14**:28–34.

15 Menon GK, Price LF, Bommannan B *et al.* Selective obliteration of the epidermal calcium gradient leads to enhanced lamellar body secretion. *J Invest Dermatol* 1994; **102**:789–95.

16 Lee SH, Elias PM, Proksch E *et al.* Calcium and potassium are important regulators of barrier homeostasis in murine epidermis. *J Clin Invest* 1992; **89**:530–8.

17 Denda M, Fuziwara S, Inoue K. Influx of calcium and chloride ions into epidermal keratinocytes regulates exocytosis of epidermal lamellar bodies and skin permeability barrier homeostasis. *J Invest Dermatol* 2003; **121**:362–7.

18 Matsubara M, Tamura T, Ohmori K *et al.* Histamine H1 receptor antagonist blocks histamine-induced proinflammatory cytokine production through inhibition of Ca^{2+}-dependent protein kinase C, Raf/MEK/ERK and IKK/I kappa B/NF-kappa B signal cascades. *Biochem Pharmacol* 2005; **69**:433–49.

19 Denda M, Wood LC, Emami S *et al.* The epidermal hyperplasia associated with repeated barrier disruption by acetone treatment or tape stripping cannot be attributed to increased water loss. *Arch Dermatol Res* 1996; **288**:230–8.

20 Wood LC, Jackson SM, Elias PM *et al.* Cutaneous barrier perturbation stimulates cytokine production in the epidermis of mice. *J Clin Invest* 1992; **90**:482–7.

21 Wang XP, Schunck M, Kallen KJ *et al.* The interleukin-6 cytokine system regulates epidermal permeability barrier homeostasis. *J Invest Dermatol* 2004; **123**:124–31.

22 Kawada A, Hiruma M, Noguchi H *et al.* Granulocyte and macrophage colony-stimulating factors stimulate proliferation of human keratinocytes. *Arch Dermatol Res* 1997; **289**:600–2.

23 Grossman RM, Krueger J, Yourish D *et al.* Interleukin 6 is expressed in high levels in psoriatic skin and stimulates proliferation of cultured human keratinocytes. *Proc Natl Acad Sci USA* 1989; **86**:6367–71.

24 Tuschil A, Lam C, Haslberger A *et al.* Interleukin-8 stimulates calcium transients and promotes epidermal cell proliferation. *J Invest Dermatol* 1992; **99**:294–8.

25 Watanabe M, Tagami H, Horii I *et al.* Functional analyses of the superficial stratum corneum in atopic xerosis. *Arch Dermatol* 1991; **127**:1689–92.

26 Long CC, Marks R. Stratum corneum changes in patients with senile pruritus. *J Am Acad Dermatol* 1992; **27**:560–4.

27 Yosipovitch G, Boner G. Pruritus and skin hydration during dialysis. *Nephrol Dial Transplant* 1997; **12**:1769–70.

28 Miyamoto T, Nojima H, Shinkado T *et al.* Itch-associated response induced by experimental dry skin in mice. *Jpn J Pharmacol* 2002; **88**:285–92.

