HHS Public Access

Author manuscript

J Invest Dermatol. Author manuscript; available in PMC 2012 March 01.

Published in final edited form as:

J Invest Dermatol. 2011 September ; 131(9): 1845–1852. doi:10.1038/jid.2011.144.

Utility of a topical peroxisome proliferator-activated receptor-α ligand with glucocorticoids in a hapten-induced murine model with features of atopic dermatitis

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Abstract

Although topical glucocorticoids (GCs) display potent anti-inflammatory activity in inflamed skin, they also can exert numerous harmful effects on epidermal structure and function. In contrast, topical applications of ligands of peroxisome proliferator-activated receptor-α (PPARα) not only reduce inflammation, and also improve cutaneous barrier homeostasis. Therefore, we examined whether sequential topical GCs followed by topical Wy14643 (a ligand of PPARα) might be more effective than either alone for atopic dermatitis (AD) in a hapten (oxazolone)-induced, murine model with multiple features of AD (Ox-AD). Despite expected anti-inflammatory benefits, topical GC alone induced: i) epidermal thinning; ii) reduced expression of involucrin, loricrin and filaggrin; and iii) allowed outside-to-inside penetration of an epicutaneous tracer. While Wy14643 alone yielded significant therapeutic benefits in mice with mild or moderate Ox-AD, it was less effective in severe Ox-AD. Yet, topical applications of Wy14643 after GC was not only significantly effective comparable to GC alone, but it also prevented GC-induced structural and functional abnormalities in permeability barrier homeostasis. Moreover, rebound flares were largely absent after sequential treatment with GC and Wy14643. Together, these results show that GC and PPARα ligand therapy together is not only effective but also prevents development of GC-induced side effects, including rebound flares, in murine AD.

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Conflict of Interest

The authors state no conflict of interest.

INTRODUCTION

Two major pathological features of atopic dermatitis (AD) are cutaneous permeability dysfunction and allergic inflammation, which drive each other in a classic vicious cycle (Elias PM *et al*., 2008). Therefore, treatment of AD ideally should incorporate methods that address both of these disease components. Topical glucocorticoids (GCs) have potent anti-inflammatory effects, and represent standard treatment for AD, particularly in severe cases. However, improvement of AD symptoms comes at a price. As inflammation recedes, numerous harmful effects on epidermal structure and function emerge, which, in turn, could account for the commonly-observed clinical phenomena of tachyphylaxis and rebound flare-ups following cessation of GC therapy (Hiratsuka *et al*., 1996; Kawakami *et al*., 2001; Fukaya *et al*., 2000). Specifically, GCs abrogate cutaneous permeability barrier homeostasis (Kao *et al*., 2003); suppress expression of epidermal antimicrobial peptide (Aberg *et al*., 2007); inhibit the expression of epidermal differentiation-linked structural proteins, such as involucrin, filaggrin and loricrin (Demerjian *et al*., 2009); and inhibit epidermal proliferation in normal skin. Skin atrophy (Schacke *et al*., 2002), therefore, results not only from loss of dermis, but also as a consequence of multiple, negative effects on epidermis.

Activators of peroxisome proliferator-activated receptors (PPARs) α, β/δ, and γ, and liver X receptors (LXRs) which belong to the superfamily of nuclear hormone receptors, display potent, but largely positive effects on epidermal structure and function in normal and diseased skin (rev. in Schmuth *et al*., 2008). Earlier studies have shown that PPAR and LXR activators display substantial anti-inflammatory activity in murine models of both irritant and acute allergic contact dermatitis (Sheu *et al*., 2002; Fowler *et al*., 2003), and reverse epidermal hyperplasia while normalizing epidermal differentiation in a hyperproliferative disease model in mice (Komuves *et al*., 2000). These earlier results suggest that PPAR and LXR activators could mitigate several features of inflammatory dermatoses; and conversely, that their activators could be useful for the treatment of such diseases, including AD. Expression of PPARα is reduced in atopic lesional skin, and topical treatment with a PPARα activator prevents emergence of murine AD (Staumont-Salle *et al*., 2008), suggesting that a reduction in PPARα signaling might contribute to the pathogenesis of AD. More recently, we showed that topical activators/ligands of PPARα display potent anti-inflammatory benefits in another murine model of AD (Hatano *et al*., 2010).

Co-treatment with certain PPAR activators reverses a variety of adverse effects of the topical GC treatment on normal murine epidermis (Demerjian *et al*., 2009); namely, co-applications of a PPARα ligand normalized the expression of differentiation-linked structural proteins (involucrin, filaggrin and loricrin); keratinocyte proliferation and epidermal thickness; and permeability barrier homeostasis. Therefore, we postulated that combination treatment of AD with both GC and a PPARα activator could be not only at least as effective as treatment with GC alone, but that it also could prevent emergence of GC-related, epidermal side effects. Thus, in the present study, we compared the efficacy of sequential combination therapy with a super-potent GC (clobetasol) plus a PPARα ligand, with the GC and PPARα ligand alone. We chose PPARα, rather than a PPARβ/δ or LXR ligand, as our therapeutic target, because first, more is known about its potential role in the pathogenesis of AD (Staumont-Salle *et al*., 2008), and because certain PPAR activators

improve inflammation and barrier function in our previously-established, hapten-induced murine model of AD (Man and Hatano *et al*., 2008). We report here that treatment with the activator of PPARα ligand, Wy14643, by itself, significantly improves mild-to-moderate disease but is less effective in more severe dermatitis. Second, we found that treatment with the super-potent GC, with Wy14643 not only was highly effective, even in severe dermatitis, but it also prevented the emergence of GC-induced, epidermal side-effects and rebound flares of dermatitis.

RESULTS

Efficacy of the PPARα activator, super-potent glucocorticoid and combination therapy

We first examined the efficacy of clobetasol propionate, a super-potent GC, and Wy14643 in Ox-AD mice with features of AD of increasing severity, assessed as changes in clinical appearance, histological features, T cell infiltration, and basal TEWL. (Recent studies have demonstrated that TEWL correlates well with clinical [inflammatory] status in AD) (Sugarman et al., 2003; Angelova-Fischer et al., 2005). Topical application of Wy14643 alone for 4 days improved clinical appearance and reduced TEWL in Ox-AD mice with 'moderate' dermatitis (initial TEWL values ≤ 25 g/m^2/h), but exacted little effects in Ox-AD mice with initial TEWL values ≥25 g/m^2/h (Fig. 1a). By contrast, topical application of clobetasol propionate alone for 4 days was uniformly effective, even in animals with severe dermatitis. While topical application of Wy14643 alone for 4 days was less effective than GC alone for severe Ox-AD, the sequential combination of GC plus Wy14643 (i.e., experimental day 1, GC alone; from day 2 to day 4, Wy14643 alone) reduced TEWL even when lesions were severe, and it did so to the same extent as GC alone. The sequential combination of GC plus vehicle was not effective for severe dermatitis (Fig. 1b). In parallel with alterations of TEWL, infiltration of CD3-positive cells also declined after treatment either with GC alone or with the sequential combination of GC and Wy14643. Infiltration of CD3-positive cells also declined after treatment with the combination of GC and vehicle (Fig. 1c), but to a lesser extent than either GC alone or the combination of GC and Wy14643.

The sequential combination of GC and the PPARα activator did not dysplay any emergence of GC-induced epidermal side effects

In parallel with the apparent therapeutic benefits described above, GC alone and the sequential combination of GC plus Wy14643 significantly normalized epidermal hyperplasia in Ox-AD mice, while, in contrast, thinning of the epidermis was readily apparent in Ox-AD mice that had been treated with GC alone (Fig. 2). In contrast, lesions treated with the sequential combination of GC and Wy14634 did not display epidermal thinning (Fig. 2). As reported previously (Man and Hatano *et al*., 2008), expression of three differentiation-linked structural proteins (involucrin, loricrin and filaggrin) was abnormal and/or reduced in the outer epidermis of Ox-AD mice, and GC alone further reduced expression of these proteins (Fig. 1S). Yet, expression of the differentiation-linked proteins normalized after sequential treatment with GC and Wy14643..

The sequential combination of GC and the PPARα activator dysplayed superior permeability barrier homeostasis to GC alone

We used three different methods to assess changes in permeability barrier status in treated Ox-AD mice. A quantitative, dye-penetration assay revealed that 'outside-to-inside' permeability improved significantly in lesions that had been treated with the sequential combination of GC and Wy14643 but not in lesions that had been treated with GC alone (Fig. 3a). Results with the electron-dense tracer, lanthanum nitrate, for 'inside-to-outside' penetration assessment supported the dye penetration assay (Figs. 3b, 3c and 3d). Finally, we compared the kinetics of recovery of permeability barrier function (% change in TEWL over time) at the end of each type of treatment; i.e., on experimental day 5, 48 h after the last Ox challenge dose. As shown in Figure 1b, values of TEWL at the end of the treatment period were similar in Ox-AD mice that had been treated with the sequential combination of GC and Wy14643 vs. sites treated with GC alone (Fig. 4a). However, 24 h after further acute abrogation of the barrier by tape stripping, TEWL declined to normal levels in Ox-AD mice that had been treated with the combination of GC and the PPARα activator, while TEWL remained higher than normal 48 h after tape stripping in Ox-AD mice that had been treated with GC alone (Fig. 4a). Accordingly, barrier recovery was greater in Ox-AD mice that had been treated with the sequential combination of GC and PPARα activator than it was in Ox-AD mice that had been treated with GC alone, at each time point examined (Fig. 4b).

The sequential combination of GC and the PPARα ligand prevents rebound flares of Ox-AD

We examined whether sequential application of GC and Wy14643 protects Ox-AD mice against the development of rebound flares, which are observed after treatment with GC alone. Eczematous lesions reappeared in mice within 4 days after discontinuation of treatment with GC alone. In contrast, re-development of such lesions was significantly reduced in Ox-AD mice that had been treated sequentially with GC plus Wy14643 (Fig. 5a). In parallel with these clinical observations, we found that both basal TEWL levels and the infiltration of CD3-positive cells were higher in mice treated with GC alone than in mice that had been treated with the sequential combination of GC and Wy14643 (Figs. 5b and 5c). These results show that application of the PPARα activator after application of GC inhibits the re-emergence (= rebound flares) that occur after termination of treatment of Ox-AD with GC alone.

DISCUSSION

Immunologic abnormalities and skin barrier dysfunction both contribute to the pathogenesis of AD (Elias *et al*., 2008), and effective therapy should address both issues. Treatment with PPARs and LXRs ligands appears promising because these agents not only have anti-inflammatory activity but they also display positive effects on cutaneous permeability-barrier homeostasis (Schmuth et al., 2008; Sheu et al., 2002; Fowler et al., 2003; Fluhr et al., 2009). Some ligands of PPARα, β/δ (but not γ) and LXR, including the PPARα ligand Wy14643, have been shown to be effective in the murine model of AD used in the present study (Hatano *et al*., 2010), and involvement of PPARα in the pathogenesis of AD has been

suggested from studies in another murine model of AD (Staumont-Salle *et al*., 2008). However, the PPARα ligand by itself displayed limited therapeutic efficacy in severe lesions in our AD model, presumably reflecting its lower anti-inflammatory potency in comparison to the 'super-potent' GC, clobetasol propionate. Yet, the potency of GC comes at a price, because important side effects appear as inflammation recedes.

Demerjian *et al*. demonstrated recently that PPARα ligands prevent the epidermal abnormalities that are induced by super-potent GC, such as epidermal thinning and aberrant permeability-barrier homeostasis (Demerjian *et al*., 2009). Therefore, we postulated that the combination of GC plus a PPARα ligand could prove both more effective and safer for the treatment of AD than treatment with either agent alone. While the sequential combination of GC and Wy14643 was as effective for severe dermatitis lesions as GC alone, epidermal thinning, which was prominent after treatment with GC alone, was not observed after co-treatment of severe dermatitis with GC plus Wy14643. In addition, the reduction in expression of three differentiation-linked structural proteins, namely, involucrin, loricrin, and filaggrin, induced during GC therapy alone, was prevented by the sequential application of GC and Wy14643, echoing previous results in similarly co-treated normal mouse skin (Demerjian *et al*., 2009). Thus, it appears that sequential applications of GC and the PPARα ligand not only maintains therapeutically efficacy, but it also blunts the harmful effects of GC alone on epidermal structure and function.

Both the quantitative, dye penetration assay and ultrastructural observations of lanthanum permeation revealed that barrier function is restored by co-treatment with the combination of GC and Wy14643, but not by GC alone. The beneficial effects of the sequential combination treatment on barrier homeostasis were consistent with the observed normalization of the expression of epidermal differentiation-linked structural proteins. Consistent with the abundant evidence that activators of PPARα have positive effects on barrier homeostasis (Schmuth *et al*., 2008), barrier recovery was also enhanced by sequential application of GC and Wy14643. Together these observations on barrier homeostasis likely account for the demonstrated ability of the PPARα ligand to prevent exacerbation of AD symptoms after discontinuation of GC therapy.

Taken together, the results of the present study suggest that combined sequential treatment with GC and Wy14643 could provide greater therapeutic benefits than treatment of AD with GC alone. Prior efforts to reduce the adverse effects of topical GC on cutaneous structure and function have involved moisturizers (Cork *et al*., 2003; Chamlin *et al*., 2002; Wirén *et al*., 2009), topical calcineurin inhibitors (Meurer *et al*., 2002; Furue *et al*., 2004), and an oral antihistamine, olopatadine hydrochloride (Tamura *et al*., 2005), both in patients with AD and in murine AD models. However, PPARα ligands, such as Wy14643, could appear to provide a superior choice for the prevention of the adverse effects of topical GC, because PPARα ligands have both anti-inflammatory effects and potent positive effects on cutaneous barrier homeostasis (Sheu *et al*., 2002; Komuves *et al*., 2000; Fluhr *et al*., 2009). Moisturizers have less anti-inflammatory activity than PPARα activators, and while topical calcineurin inhibitors display significant anti-inflammatory effects, they compromise both epidermal permeability-barrier functions and antimicrobial barrier function in mice (Kim *et al*., 2009). The oral administration of olopatadine hydrochloride has a positive effect on

permeability barrier homeostasis and inflammation (Amano *et al*., 2007; Tamura *et al*., 2008), an observation that is consistent with the known ability of antihistamines (H1 and H2 blockers) to improve barrier function (Ashida *et al*., 2001). However, it remains to be determined whether *topical* administration of olopatadine hydrochloride would also be effective for the treatment of AD, and in addition, it is unclear whether they can prevent the emergence of GC-related side effects.

According to the 'outside-inside' view of AD pathogenesis (Elias et al., 2008), normalization of barrier function should reduce the two major drivers of inflammation in AD, namely, the generation of cytokines which originate from perturbed corneocytes, and the transepidermal penetration of pro-inflammatory xenobiotes, such as haptens and microbial pathogens. Indeed, rebound flare-up was prevented only in Ox-AD mice in which the permeability barrier had been restored by sequential treatment with GC and the PPARα ligand. Thus, agents that have positive effects on permeability homeostasis should help us to prevent the negative effects of topical GC, including rebound flare-up.

In conclusion, the present study suggests that the sequential combination of topical GC and a PPARα ligand, Wy14634, might be an effective strategy for the treatment of human AD. The activators of PPAR or LXR that are most suitable for application with GC remain to be identified before this therapeutic strategy can be tested in a clinical setting. Finally, our study suggests that the Ox-AD mouse model might be useful for assessment of mechanisms involved in rebound flare-ups.

MATERIALS AND METHODS

Animals and materials

Female hairless (Hr-/Kud) mice (KYUDO Co., Fukuoka, Japan) were used at 12 to 48 weeks of age. All animals were housed under conventional conditions and had free access to a commercial diet and water. WY14643 (PPARα activator), clobetasol propionate, oxazolone, MCDB153, Evans blue, and lanthanum nitrate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Affinity-purified rabbit primary antibodies, specific, respectively, for mouse filaggrin, loricrin and involucrin, were purchased from BabCo (Richmond, CA, USA). Biotinylated second antibodies, raised in goat against rabbit IgG, and an ABC-peroxidase kit were purchased from Vector Laboratories (Burlingame, CA, USA). A rabbit anti-human antibody against CD3 was purchased from Dako (Glostrup, Denmark).

Development and treatment of hapten -induced dermatitis with features of atopic dermatitis in mice

All animal procedures were approved by the "Ethics of Animal Experimentation Committee" of Oita University. Development of a hapten (oxazolone)-induced, murine model with multiple features of AD (Ox-AD) was described in our previous studies (Hatano *et al*., 2010; Man and Hatano *et al*., 2008). Animals were sensitized by two consecutive days of topical treatment with 50 μl of 5% oxazolone in acetone. After one week, mice were treated topically on both flanks with 60 μl of 0.5% oxazolone in ethanol once every other

day for an additional 4 weeks (total of 12 challenges). To achieve more severe lesions, the concentration of oxazolone used for elicitation of AD was higher than that (i.e., 0.1%) used in our previous studies (Hatano *et al*., 2010; Man and Hatano *et al*., 2008). After the tenth challenge, when the phenotype of AD-like, chronic allergic dermatitis had been established, the therapeutic effects of a topical super-potent, class 1 glucococorticoid (GC), namely, clobetasol propionate, and of a synthetic PPARα ligand, namely, Wy14643, were assessed by the method described in our previous report (Hatano *et al*., 2010) and as described in the legend to Table 1. One hour after the eleventh challenge, twice-daily applications of 60 μl of 10 mM WY14643 in the vehicle, of 0.05% clobetasol propionate in the vehicle or of vehicle alone (a mixture of propylene glycol and ethanol, 7:3, v/v) were given for 4 days until experimental day 4. The twelfth challenge with oxazolone was administered one hour before the first application of GC, Wy14643 or vehicle on that day. As shown in Table 1, in some experimental groups, GC, Wy14643, or vehicle was applied for 4 consecutive days and in other groups, GC was applied only on the first day of the experiment, with 3 subsequent consecutive days of treatment with Wy14643 or vehicle. In some experiments, applications of oxazolone alone (i.e., without GC, Wy14643 or vehicle) was continued on the same areas on experimental day 5 and day 7 after therapeutic procedures had been discontinued.

Measurement of permeability barrier function

Basal transepidermal water loss (TEWL) was measured on individual flanks with a skin evaporative water recorder (Tewameter® TM210; Courage & Khazawa, Koln, Germany) immediately before each application of oxazolone and 48 h after the final application of oxazolone. The kinetics of permeability barrier recovery were examined as described previously (Kurahashi *et al*., 2008). Barrier disruption was achieved by sequential applications of cellophane tape (Nichiban, Tokyo, Japan). The procedure was stopped when TEWL reached 52 to 62 $g/m^2/h$, as measured with the skin evaporative water recorder. Barrier recovery was monitored immediately after and 3, 6, 24, and 48 h after further disruption of sites of skin lesions. Recovery rates were calculated as described previously (Hou *et al*., 1991).

Immunohistochemistry

Full-thickness skin was harvested for immunohistochemical staining of filaggrin, loricrin, involucrin and for counting of CD3-positive T cells 48 h after the last application of oxazolone. Immunohistochemical staining was performed as described previously (Man and Hatano *et al*., 2008; Hatano *et al*., 2009). In brief, 5-μm paraffin-embedded sections were incubated with primary antibodies overnight at 4°C. After three washes, sections were incubated with second antibodies for 30 min. Staining was detected with the ABC-peroxidase kit.

Quantitative morphology

The number of CD3-positive cells in a 250 μm × 250 μm area of dermis was determined in ≥ 40 fields of dermis in each experimental group. The thickness of layers of epidermal nucleated cells, as observed in sections stained with hematoxylin and eosin, was measured at ≥ 30 points, at intervals of 200 μm, in each experimental group. These quantitative

morphological examinations were performed under the condition in which an investigator could not find each sample belong to which experimental group.

Quantitative evaluation of outside-to-inside barrier function

Quantitative evaluation of outside-to-inside penetration of the skin was assessed with Evans blue dye. Skin samples, 16 mm in diameter, were collected from flanks 48 h after the last application of oxazolone and each sample was floated on MCDB 153 medium that contained 1.8 mM $CaCl_2$ with the outer epidermal surface of each sample exposed to the air. Then 100 μl of 2% Evans blue in PBS were pipetted onto the outer epidermal surface of each skin explant. The dye was allowed to penetrate the skin for 4 h at room temperature, and then the surface of the skin was washed with PBS and gently wiped with a Kimwipe® (NIPPON PAPER CRECIA Co., Tokyo, Japan). After the washing procedures had been repeated three times, the center of each explant was biopsied with a 4-mm punch and each 4-mm disk was placed into 100 μl of 1 N KOH. After overnight incubation at 37°C, each sample was neutralized by the addition of 900 μl of a mixture of 0.6 N H_3PO_4 and acetone (5:13, v/v). After vigorous vortexing for a few seconds, the mixture was centrifuged at 3,000 rpm for 15 min in KUBOTA RA-150AM (KUBOTA Co., Tokyo, Japan) and absorbance of supernatants was measured at 360 nm.

Electron microscopic observations of lanthanum nitrate penetration

The penetration of an electron-dense, water-soluble, low-molecular-weight tracer, lanthanum nitrate, from the outside toward the inside of the skin was assessed as described previously (Scharschmidt et al., 2009). In brief, skin samples, 16 mm in diameter, were collected from flanks 48 h after the last application of oxazolone and each sample was floated on MCDB 153 medium that contained 1.8 mM $CaCl_2$, with the outward-facing, epidermal side of each sample exposed to air. Then 100 μl of 4% lanthanum nitrate in PBS were pipetted onto the outer epidermal surface of each explant. The dye was allowed to penetrate the skin for 3 h at room temperature. Then aldehyde-fixed biopsy specimens were fixed in either 0.25% ruthenium tetroxide or 1% aqueous osmium tetroxide that contained 1.5% potassium ferrocyanide, as described previously (Hou et al., 1991). Ultrathin sections were examined with an electron microscope (Zeiss 10A; Carl Zeiss, Thornwood, NY) operated at 60 kV. Images were captured with Digital Micrograph 3.10.0 software (Gatan, Inc., Pleasanton, CA).

Statistical analysis

The statistical significance of differences was evaluated by Student’s t-test under normal distribution. A value of $p<0.05$ was considered statistically significant. Each result is given as a mean value ± SE, with the number of samples, n.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement

The authors are very grateful to Ms. Satoko Sato for her skilled technical assistance.

This study was supported by a grant from the Japanese Ministry of Education, Culture, Sports, Science and Technology (no.20591323 to Y.H.), and by grants from the U.S. National Institutes of Health (no. AR 19098 and AI 05931), and from the Medical Research Service, U.S. Department of Veterans Affairs.

Abbreviations

AD	atopic dermatitis
Ox	oxazolone
PPAR	peroxisome proliferator-activated receptor
LXR	liver X receptor
GC	glucocorticoid
TEWL	transepidermal water loss

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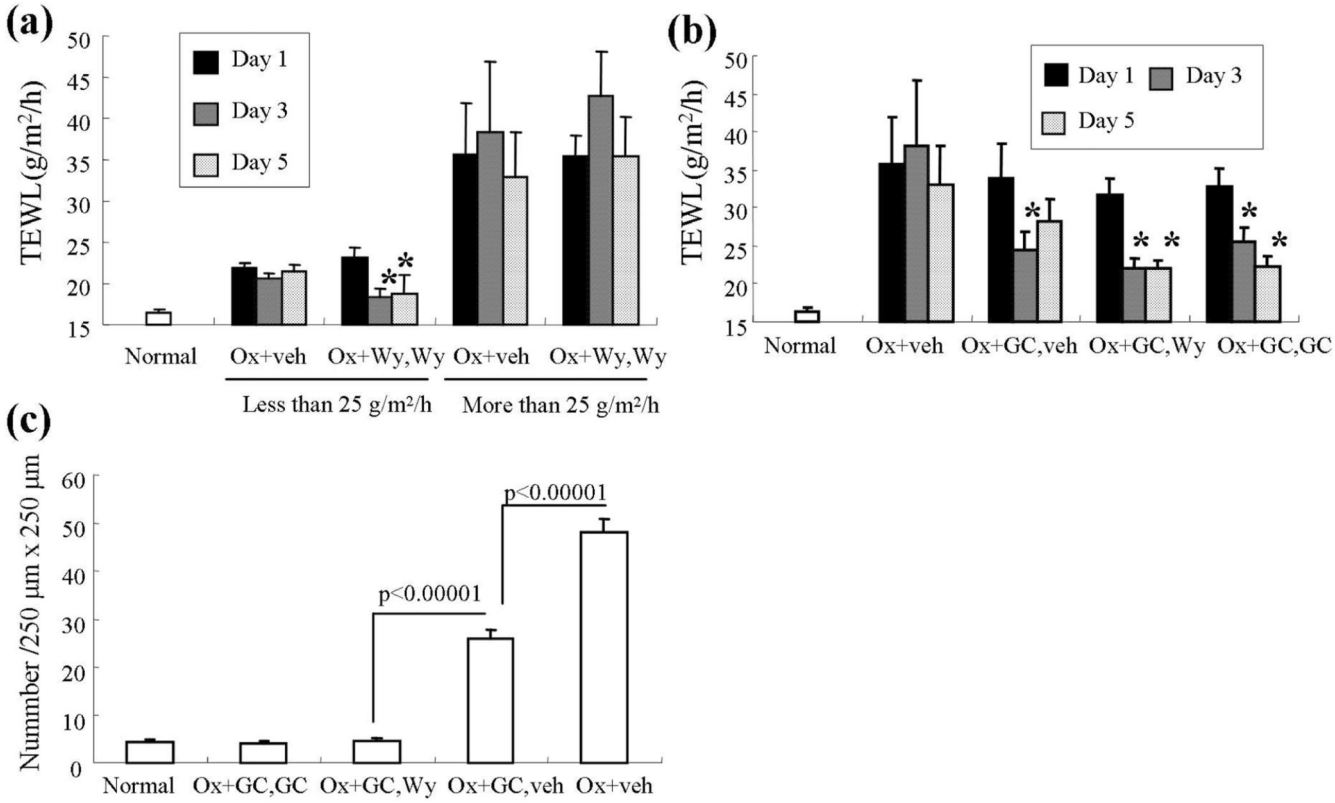

Figure 1. Effects of treatment with a topical glucocorticoid (GC) and/or a PPARα activator on transepidermal water loss (TEWL) and the infiltration of CD3-positive cells

Transepidermal water loss [(a) and (b)] was measured as described in the text. In (b) and (c), Ox-AD mice in which TEWL was more than 25 g/m^2/h on day 1 were used for the experiments. * $p<0.05$ vs. day 1. Numbers of CD3-positive cells on day 5 are shown in (c). n = 8 (four mice) in (a), n=6–10 (three to five mice) in (b) and n=40–80 (three to four mice) in (c). Abbreviation of label of each experimental group is according to the description in Table 1.

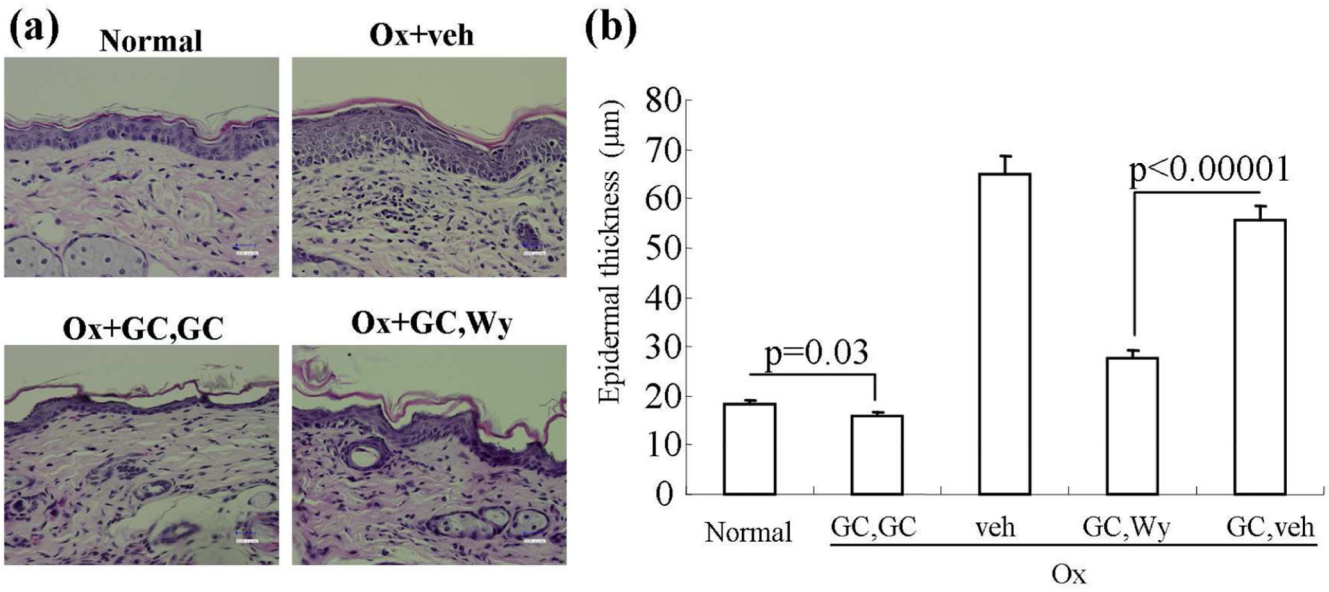

Figure 2. Morphological changes in the epidermis

Skin samples were collected on experimental day 5 and were stained with hematoxylin and eosin as shown in (a). Bars =20 μm. Epidermal thickness was measured as described in the text and is shown in (b). n= 30–60 (three to four mice). Abbreviation of label of each experimental group is according to the description in Table 1.

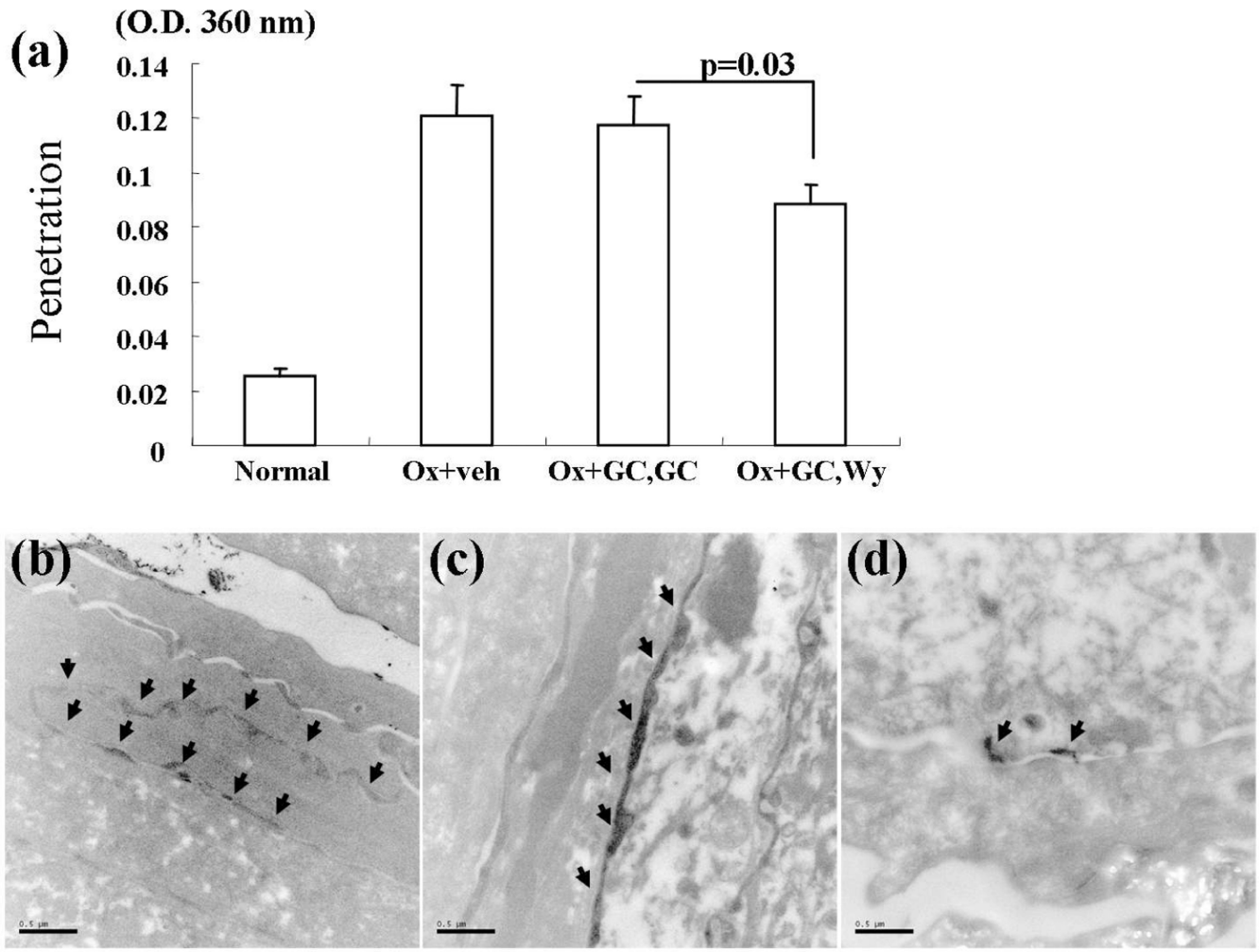

Figure 3. Assessment of outside-to-inside permeability-barrier function

The results of a quantitative penetration assay with Evans blue [(a); n=8 (four mice)] and electron micrographs [(b), (c) and (d)] are shown. See text for details. (b) Ox+veh, (c) Ox +GC,GC and (d) Ox+GC,Wy. Block arrows indicate electron-dense material, namely, lanthanum nitrate, that has penetrated intercorneocyte spaces at the stratum corneum and stratum granulosum interface. Bars = 0.5 μm. Abbreviation of label for each experimental group is according to the description in Table 1.

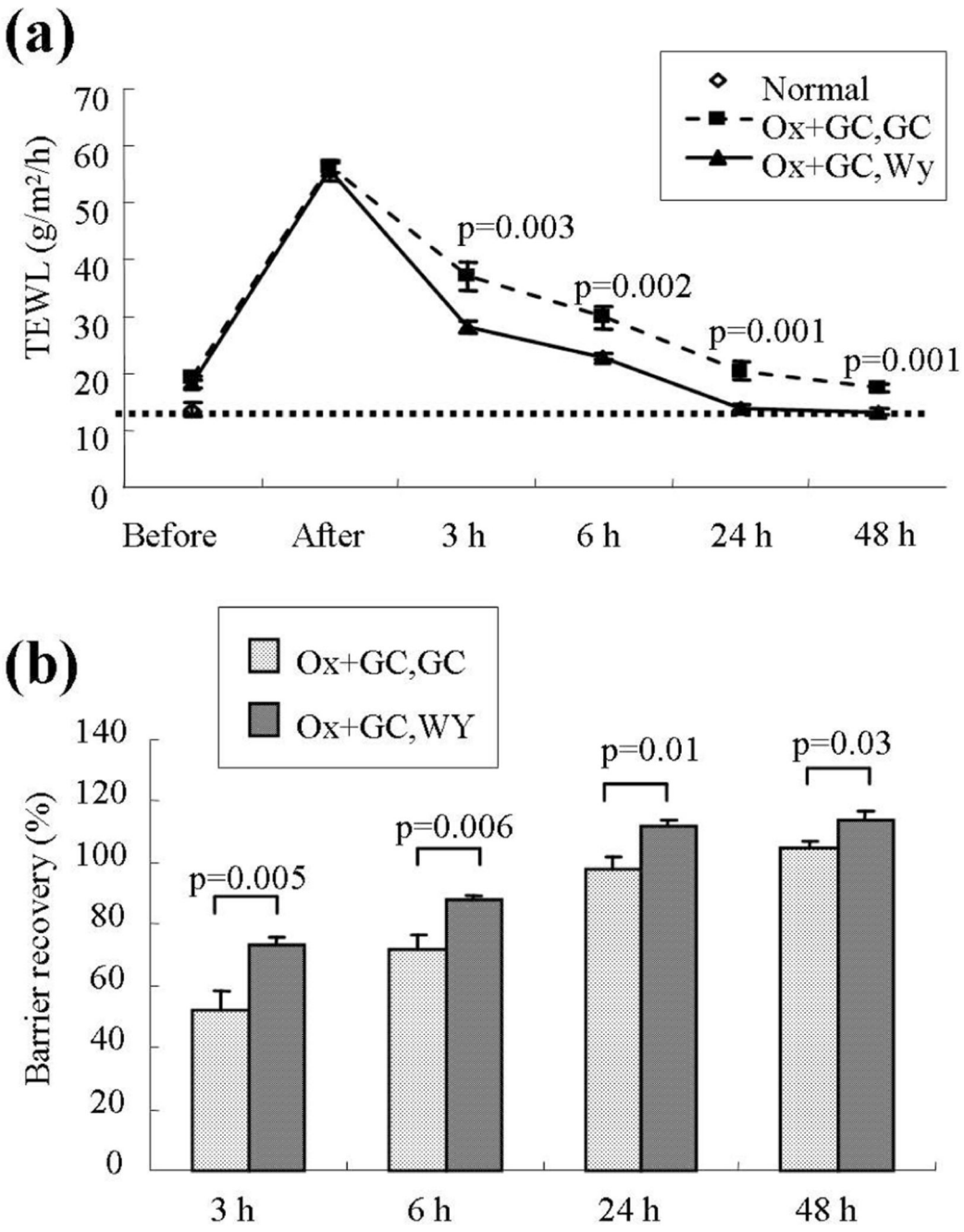

Figure 4. Recovery of permeability barrier function

On experimental day 5, the permeability barrier was disrupted by tape stripping as described in the text. Transepidermal water loss (TEWL) was examined before and after tape stripping, at each indicated time point, as described in the text. The horizontal dotted line indicates the mean values in normal control mice. The p-values in (a) refer to differences between Ox+GC,GC and Ox+GC,Wy. n = 10 (five mice). Abbreviation of label of each experimental group is according to the description in Table 1.

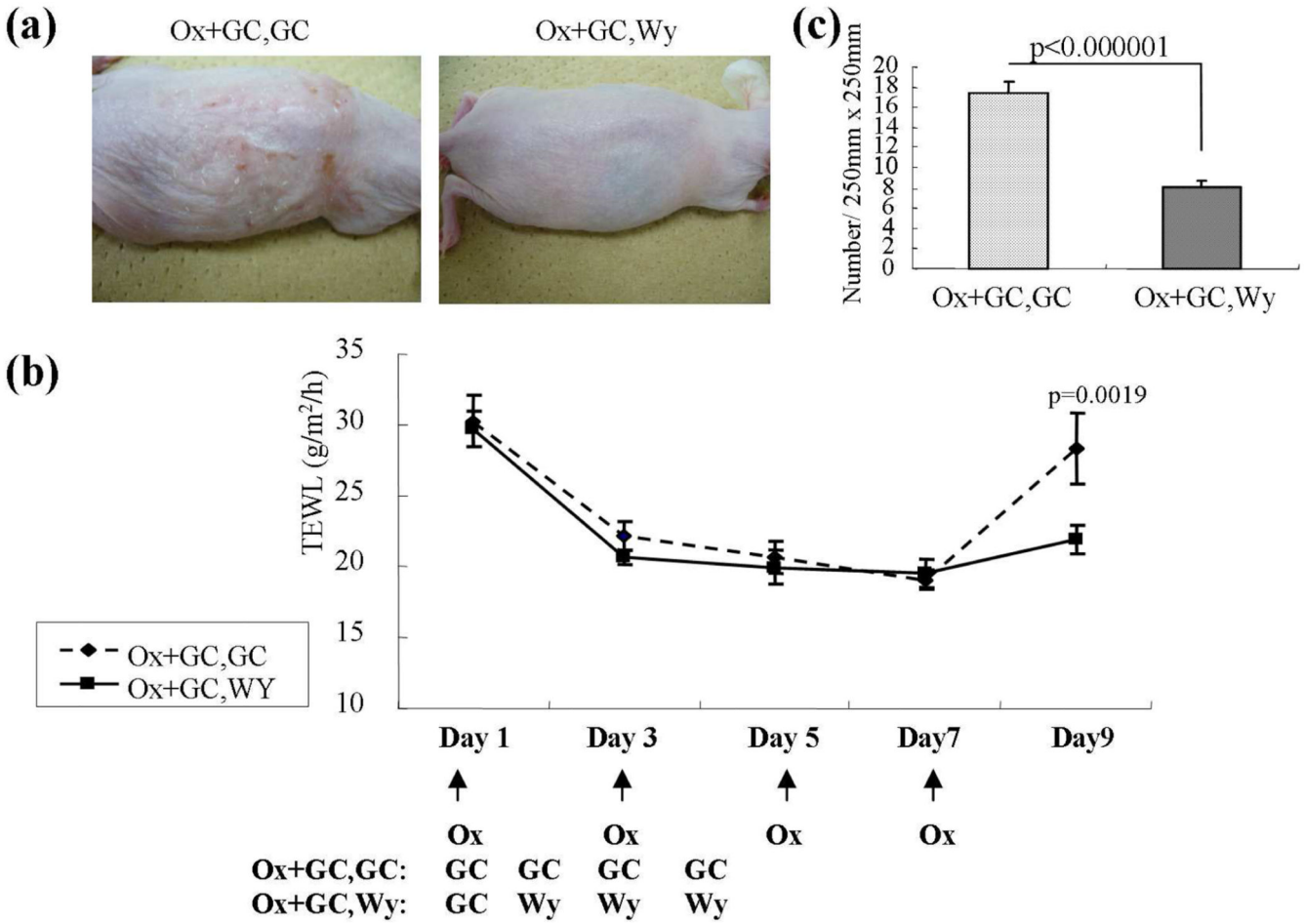

Figure 5. Rebound of dermatitis after discontinuation of therapy

The every other day skin challenge with Ox alone was continued (day 5 and day 7) on the same area without GC nor Wy treatment. (a) Clinical appearance of mice on experimental day 9. (b) Alteration of transepidermal water loss (TEWL) (c) Density of CD3-positive cells in skin samples collected on day 9. The p-values refer to results from Ox+GC,GC and Ox +GC,Wy. n = 12 (six mice) in (b), n = 24 (three mice) for GC, GC in (c) and n = 26 (three mice) for GC,Wy in (c). Abbreviation of label of each experimental group is according to the description in Table 1.

Table 1

Treatment of hapten-induced atopic dermatitis in a murine model

Experimental groups	Day1 (11th Ox[1])	Day2	Day3 (12th Ox)	Day4
Ox+veh	veh	veh	veh	veh
Ox+Wy,Wy	Wy	Wy	Wy	Wy
Ox+GC,GC	GC	GC	GC	GC
Ox+GC,veh	GC	veh	veh	veh
Ox+GC,Wy	GC	Wy	Wy	Wy

[1]Ox, Oxazolone; veh, vehicle; Wy, Wy14643; and GC, glucocorticosteroid See text for details. This table indicates the experimental groups in the present study. The abbreviation of each group is provided in the text and the figure legends.