

Report

Topical cannabinoid receptor 1 agonist attenuates the cutaneous inflammatory responses in oxazolone-induced atopic dermatitis model

Hyun Jong Kim¹, MD, PhD, Bongwoo Kim², PhD, Bu Man Park², MS, Jeong Eun Jeon², BS, Sin Hee Lee², MS, Shivtaj Mann³, PhD, Sung Ku Ahn⁴, MD, PhD, Seung-Phil Hong⁵, MD, PhD, and Se Kyoo Jeong^{2,6}, PhD

¹Department of Dermatology, Atopy Clinic, Seoul Medical Center, Seoul, ²CRID Center, NeoPharm Co., Ltd., Daejeon, Korea, ³College of Medicine, Nova Southeastern University, Fort Lauderdale, FL, USA, ⁴Department of Dermatology, Wonju College of Medicine, Yonsei University, Kangwon, ⁵Department of Dermatology, College of Medicine, Dankook University, Chungnam, and ⁶Department of Pharmaceutics, College of Pharmacy, Chungbuk National University, Chungbuk, Korea

Correspondence

Se Kyoo Jeong, PhD
CRID Center, NeoPharm Co., Ltd.
928 Tamnip-dong
Yuseong-gu Daejeon 305-510
Korea
E-mail: jeong@aekeyung.kr

Conflicts of interest: B.M.P., J.E.J., S.H.L., and S.K.J. are current employees of NeoPharm Co., Ltd. B.W.K. was an employee of NeoPharm Co., Ltd. when working on this study. Other authors have no conflicts of interest to declare.

Summary

Background Even with the widespread clinical use of cannabinoid receptor (CBR) stimulating compounds, such as palmitoylethanolamine, the role of CBR agonists on inflammatory skin diseases is not yet fully understood. This study was performed to investigate the effects of CBR agonists on skin inflammation, using acute and chronic inflammation animal models.

Methods The effectiveness of the newly synthesized cannabinoid receptor 1 (CB1R) agonists was determined using *in vitro* assays. Markers for epidermal permeability barrier function and skin inflammation were measured, and histological assessments were performed for evaluation.

Results Topical application of CB1R-specific agonist significantly accelerated the recovery of epidermal permeability barrier function and showed anti-inflammatory activity in both acute and chronic inflammation models. Histological assessments also confirmed the anti-inflammatory effects, which is consistent with previous reports.

Conclusions All of the results suggest that topical application of CB1R-specific agonist can be beneficial for alleviating the inflammatory symptoms in chronic skin diseases, including atopic dermatitis.

Introduction

The endocannabinoid system is comprised of endogenous cannabinoids (endocannabinoids), cannabinoid-catabolizing and -metabolizing enzymes, and their G-protein coupled receptors (cannabinoid receptor 1 and 2) (CB1R and CB2R, respectively).¹ Anandamide (AEA; *N*-arachidonylethanolamine) and 2-arachidonoylglycerol were the first endocannabinoids identified and described to regulate pain in the central nervous system.² Subsequently, cannabinoid receptor (CBR) expression was found in various tissues, including skin, in which biochemical machinery to transport, metabolize, and synthesize endocannabinoids in keratinocytes (KCs) was identified³ and suggested

to regulate skin homeostasis. Expressions of CB1R and CB2R in cultured KCs were repeatedly reported,⁴ but their role and function remained controversial.

Previously, it was reported that in non-transformed immortalized KCs (HaCaT) and primary human KCs, synthetic CB1R and CB2R agonists did not induce significant changes in the viability of the respective cells.⁵ However, a recent study reported that phytocannabinoids (Δ -9 tetrahydrocannabinol) and synthetic CBR agonists inhibited the proliferation of transformed human epidermal KCs (HaCaT). While treatment of nonspecific agonists for CB1R or CB2R inhibited the proliferation of cultured KCs, the antiproliferative effect of agonists was not blocked by their respective antagonists, which implied

non-CB1R/2-dependent action mechanism for the tested compounds.⁶ More recently, it was reported that endocannabinoid AEA markedly inhibited proliferation of HaCaT and KCs, possibly due to calcium ion influx and concomitant elevation of intracellular calcium ion concentration through activation of non-selective, highly calcium ion-permeable, ion channel transient receptor potential vanilloid 1.⁴ In contrast to the conflicting effects on proliferation, inhibitory effects of AEA on KC differentiation have been repeatedly reported. In HaCaT and NHEK cells, AEA expression decreases in parallel with differentiation status. It has been suggested that AEA inhibits differentiation by increasing DNA methylation and mitogen-activated protein kinase-dependent pathway.^{3,7}

Along with the effects on proliferation and differentiation of KCs, anti-inflammatory activity of endocannabinoids or CB1R agonists in allergic contact dermatitis model has also been reported.⁸ Furthermore, CB1R agonists have also been reported to modulate secretion of pro-inflammatory chemokines, including CXCL10 and CCL10, in skin.⁹ In addition to the direct effects on inflammation, regulatory roles of CB1R on the activation and maturation of mast cells¹⁰ further suggests the potential application of CB1R or CB2R agonists for inflammatory or allergic skin diseases. In addition, important roles of CBR in itching sensation and neuroinflammation suggest that CBR modulation can be a therapeutic target for atopic dermatitis (AD), which is characterized by intense itching symptoms.^{11,12} Recently, using a CB1R and CB2 knockout (KO) mice model, Roelandt *et al.*¹³ showed CB1R to enhance epidermal barrier function and CB2R to have negative effects on barrier integrity. Deletion of CB1R delayed the recovery of epidermal permeability barrier function after acute disruption, while CB2R deletion accelerated the recovery.

Atopic dermatitis is a chronic and relapsing inflammatory skin disease characterized by chronic inflammation and intense pruritus.¹⁴ Among the various pathologic factors currently suggested, defects in skin barrier function and type 2 helper T cells (Th2)-biased cytokines expression play crucial roles for initiation and exacerbation of symptoms.^{15,16} Taking into consideration previous studies that showed an overall enhancement of skin barrier, it could be hypothesized that topical application of CBR agonists could serve as a therapeutic regimen for AD. While there were series of reports about the use of palmitoylethanolamine (PEA) on the AD, it is now considered that PEA exerts its effects through a mechanism not involving direct CB1R activation.^{17,18}

To investigate the effects of CB1R agonist on AD, we used an oxazolone-induced AD animal model.¹⁹ AEA-derived compounds were synthesized, and their CB1R and/or CB2R modulating effects were evaluated using

CB1R or CB2R transfected Chinese hamster ovary (CHO) cells. From the compounds identified as having CB1R agonistic activity, a CB1R-specific agonist was selected and evaluated for its effects on oxazolone-induced AD-like symptoms. Skin barrier function, hydration, surface pH, and skinfold thickness were measured, and histological analysis was performed.

Materials and methods

Materials

To screen CB1R or CB2R modulators, a series of compounds were synthesized by previously reported method.²⁰ AEA was purchased from Tocris Bioscience (Bristol, UK).

Cannabinoid receptor 1 and 2 modulating activity measurement

CB1R and CB2R modulating activities were measured using CB1R- or CB2R-transfected CHO cells. To construct stable cell line expressing each CBRs, CHO-K1 cells were transfected with either CB1R (RC210397) or CB2 (RC210224) cDNA constructs, according to the manufacturer's instruction (OriGene, Rockville, MD, USA). One day after transfection, medium were replaced with F12 standard media containing 100 µg/ml of G418 (Gibco BRL, Grand Island, NY, USA) to select stable clones. After two weeks, single colonies were picked and transferred to a 24-well plate and cultured until confluence. To confirm the CB1R or CB2R expressions in transfected cells, change of cytoplasmic cAMP levels by CB1R or CB2R agonists was measured using the following method.

CB1R- or CB2R-expressing CHO cells were plated to 96-well plates (5×10^3 cells/well) one day before measurement. After removal of culture medium and phosphate-buffered saline washing, cells were incubated with serum-free F12 media (Gibco RBL) containing 10 µM of forskolin (Sigma-Aldrich, St. Louis, MO, USA) and test compound for 30 minutes at 37 °C. Cytoplasmic cAMP was measured by homogeneous time-resolved fluorescence cAMP assay kit according to the manufacturer's protocol with slight modification (Cisbio Bioassay, Bedford, MA, USA). Briefly, cells were washed by ice-cold phosphate-buffered saline and lysed by cAMP-d2 conjugate containing lysis buffer. Cell lysates were incubated with anti-cAMP-Cryptate and cAMP-d2 for two hours at room temperature. Fluorescence was measured using 2104 EnVision Multilabel Reader (PerkinElmer, Waltham, MA, USA), and the measured fluorescence ratio (665 /620 nm) was converted to concentration using the cAMP standard curve.

Animal studies

All mice used in this study were kept in the animal facility of NeoPharm Co., Ltd. (Daejeon, Korea), under specific pathogen-free conditions. Balb/c and hairless mouse (SKH-1) were purchased from Hanlim Animals (Suwon, Korea). All

experiments were performed according to the guidelines of the Institutional Animal Care and Use Committee of NeoPharm Co., Ltd.

Acute permeability barrier recovery measurement

To measure the effects of CB1R agonist on the skin barrier function, recovery of epidermal permeability barrier function was assessed. After measuring the basal transepidermal water loss (TEWL) value using TEWameter MPA5 (Courage&Khazaka, Cologne, Germany), acute barrier disruption by repeated tape stripping was performed on both flanks of a female hairless mouse. Either 0.5% of AEA (Tocris) or 1% of α -oleoyl oleylamine serinol (α -OOS) was topically applied on the same sites. TEWL was measured at 3 and 6 hours after tape stripping, and recovery of TEWL was calculated.²¹

Acute inflammation model

To measure the anti-inflammatory activity of CB1R agonist, phorbol ester-induced acute inflammation model was used. Briefly, 20 μ l of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (Sigma-Aldrich) in acetone (50 μ g/ml) was topically applied on the right ear of Balb/c mice. After 10 minutes, 20 μ l of 1% of α -OOS in ethanol was applied on the same site. After six hours, an ear skin biopsy was taken by 6 mm punch, and its weight was measured. Change of ear weight was compared to normal and vehicle-only treated sites.

Oxazolone-induced atopic dermatitis model

To induce AD-like symptoms, animals were sensitized with a single topical treatment with 50 μ l of 1% oxazolone solution on both flanks. After one week, 60 μ l of 0.1% oxazolone solution was applied on the same sites once every other day for 10 times. After the sixth challenge, animals were grouped into four groups (non-treated, vehicle treated, 1% of α -OOS, and 0.01% of dexamethasone), and each sample was topically applied on the same site twice a day for nine days. The last sample treatment was performed at two days after the last oxazolone challenge. Sample application was done at 30 minutes after oxazolone treatment and at least six hours later than previous treatment. Polyethylene glycol (Mw 400) and ethanol mixture solution (PEG 400/EtOH = 70 : 30) was used for the vehicle. After the last sample treatment, animals were anesthetized with intraperitoneal injection of 4% chloral hydration solution, and functional measurements were performed. TEWL and stratum corneum hydration were measured with MPA5 (Courage&Khazaka, Cologne, Germany) with appropriate probes. Skin surface pH was measured with a flat, glass surface electrode from Mettler-Toledo (Giessen, Germany) attached to a pH meter (PH900; Courage&Khazaka). Skinfold thickness was measured using digital calipers (Mitutoyo Korea Corporation, Seoul, Korea). Skin biopsy was taken after cervical dislocation, and sections from formalin-fixed, paraffin-embedded tissue were stained for histological assessments.

Histological observation

Skin biopsy was taken after cervical dislocation and sections from formalin-fixed, paraffin-embedded tissue were stained. Immunohistochemical staining for epidermal expressions of proliferating cell nucleus antigen (PCNA), CB1R, and interleukin (IL)-4 was performed. Antimouse antibodies for PCNA, CB1R, and IL-4 were incubated, and appropriate secondary antibodies, either donkey antigoat-IgG horseradish peroxidase conjugated antibody or goat-antirat-IgG horseradish peroxidase conjugated antibody, were incubated again. DAB+ chromogen (Dako, Carpinteria, CA, USA) was used for coloration and observed under optical microscope BX51 equipped with digital camera (Olympus, Tokyo, Japan).

Statistical analysis

Statistical analysis was performed by either two-tailed Student's *t*-test or ANOVA test with post *t*-test using GraphPad InStat (GraphPad Software, San Diego, CA, USA). *P* < 0.05 was considered significant.

Results

Development of cannabinoid receptor 1 agonist

Previously, we reported on the structural characteristics of a newly synthesized pseudo-ceramide (PC-9S).²² Owing to the similarities in structure to known endocannabinoids, such as AEA or ACEA, derivatives of pseudo-ceramide PC-9S were synthesized, and their effects on CB1R and CB2R were assessed. CBR belongs to the Gi-protein coupled receptor, and as such, activation of CBR induces downregulation of adenylyl cyclase activity, which results in reduction of intracellular cAMP production. Consequently, CB1R agonists inhibit the increase of intracellular cAMP concentration induced by forskolin treatment. As a positive control, AEA was used, which also induced significant inhibition of cAMP production in CB1R KO CHO-K1 cells (EC_{50} = 7.6 μ M). Among the tested compounds, α -OOS (EC_{50} = 26.8 μ M) and α -oleoyl oleylamine ethanol amine (EC_{50} = 24.2 μ M) attenuated cAMP production (Fig. 1a). The two agonists of CB1R displayed comparable effects, and further *in vivo* studies were performed with α -OOS.

Effects on epidermal permeability barrier function

Recently, it was reported that CB1R KO mice displayed delayed epidermal permeability barrier recovery after acute abrogation, whereas CB2R KO mice showed enhanced barrier recovery. Decreased expression of differentiation marker proteins were also observed in CB1R KO mouse, compared with littermate mice.¹³ Based on those results, we investigated the effects of CB1R agonists on epidermal permeability barrier recovery rate. As

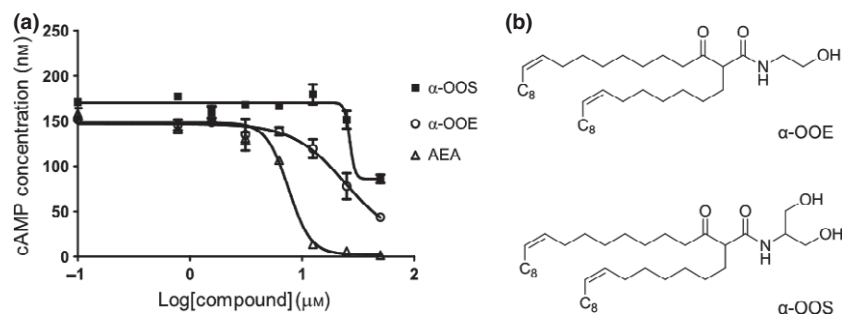


Figure 1 CB1R agonistic activity of AEA, α-OOS, and α-OOE (a) and their chemical structures (b). CBR activation was measuring the inhibition of cAMP accumulation by adenylyl cyclase activator, forskolin in CB1R-transfected CHO-K1 cells. AEA, arachidonylethanolamine; CB1R, cannabinoid receptor 1; α-OOE, α-oleoyl oleylamide ethanol amine; α-OOS, α-oleoyl oleylamine serinol

shown in Figure 2, significant increase in barrier recovery rate was observed at 0.5% AEA-treated sites, both at 3 and 6 hours post-barrier abrogation. While there was no change at 3 hours, α-OOS treatment did accelerate barrier recovery at 6 hours, which is consistent with previous results (Fig. 2).¹³

Anti-inflammatory effects of cannabinoid receptor 1 agonist

To measure the anti-inflammatory effects of α-OOS, a phorbol ester-induced acute inflammation model was used. Increase of ear weight by topical TPA was significantly

prevented by 1% α-OOS as shown in Figure 3, which suggests the anti-inflammatory effects of the CB1R agonist.

Effects in oxazolone-induced atopic dermatitis model

Enhancement of epidermal barrier function and demonstration of anti-inflammatory activity establish the basis for the utilization of CB1R agonists in treating AD symptoms. To confirm the hypothesis, we established an oxazolone-induced AD model and investigated the effects of α-OOS on various AD-like symptoms, including histological changes. Co-treatment of α-OOS and oxazolone attenuated the increase in TEWL, which was statistically significant. Topical application of dexamethasone resulted in the lowest levels of TEWL (Fig. 4a), suggesting the positive effects of topical glucocorticoid on epidermal

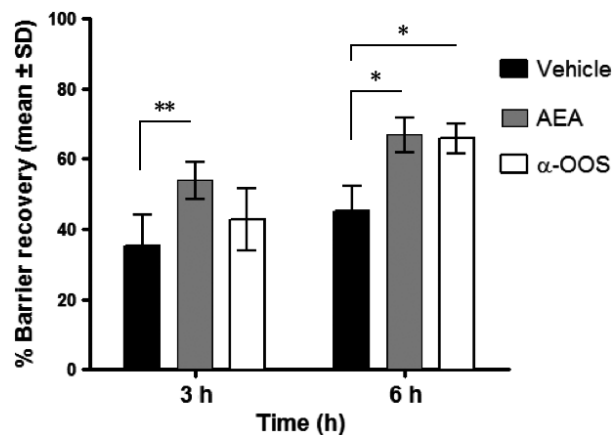


Figure 2 Topical application of cannabinoid 1 agonist accelerated the permeability barrier recovery rate. Epidermal permeability barrier was acutely disrupted by repeated tape stripping, and transepidermal water loss was measured. Topical application of cannabinoid 1 against AEA significantly accelerated barrier recovery rate at 3 h after disruption. At 6 h, both AEA- or α-OOS-treated sites showed improved barrier recovery rate, compared the vehicle-treated sites. (* $P < 0.05$, ** $P < 0.01$). AEA, arachidonylethanolamine; α-OOS, α-oleoyl oleylamine serinol

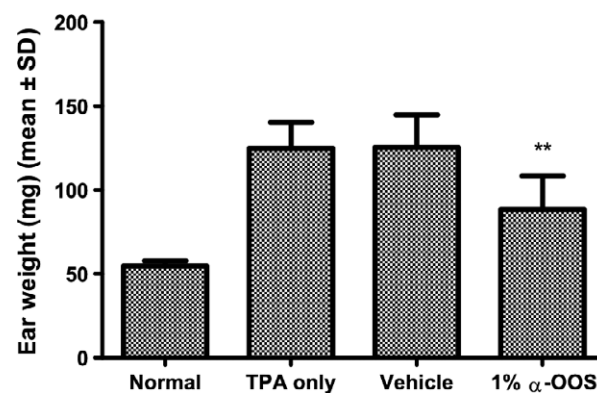


Figure 3 Anti-inflammatory effects of α-OOS in TPA-induced acute inflammation model. Topical application of 1% α-OOS significantly prevented the increase of ear weight. Statistical analysis was performed by two-tailed Student's *t*-test (** $P < 0.01$ vs. TPA only treated site). α-OOS, α-oleoyl oleylamine serinol; TPA, 12-O-tetradecanoylphorbol-13-acetate

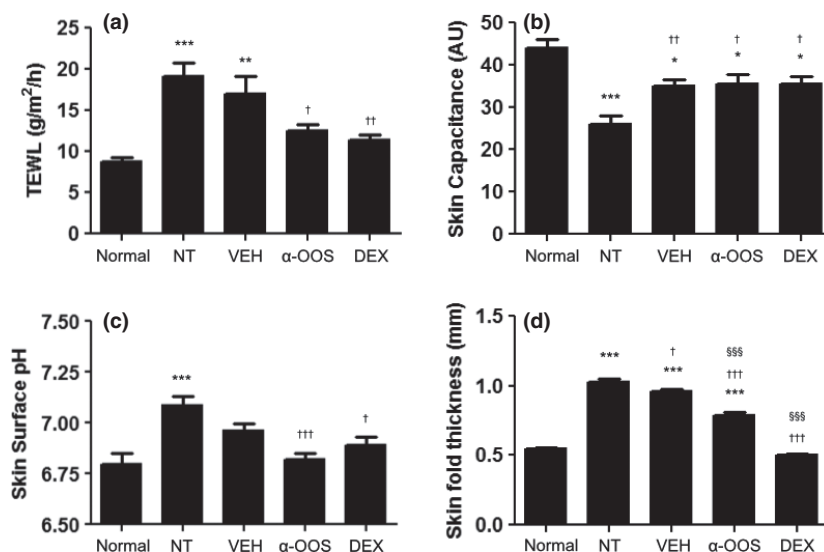


Figure 4 Change of TEWL (a), skin capacitance (b), skin surface pH (c), and skinfold thickness (d) in oxazolone model. α -OOS treatment prevented the increase of TEWL, skin surface pH, skinfold thickness and decrease of skin hydration, assessed by skin capacitance. Statistical analysis was performed by ANOVA test with post *t*-test (* $P < 0.05$ vs. normal; ** $P < 0.01$ vs. normal; *** $P < 0.001$ vs. normal; † $P < 0.05$ vs. non-treated; †† $P < 0.01$ vs. non-treated; ††† $P < 0.001$ vs. non-treated; §§§ $P < 0.001$ vs. vehicle). Vehicle-treated group also showed slight preventive effects against oxazolone-induced responses. DEX, dexamethasone; NT, non-treated; α -OOS, α -oleoyl oleylamine serinol; VEH, vehicle treated; TEWL, transepidermal water loss

permeability barrier function via downregulation of the inflammatory cascade. Next, we examined skin hydration and skin pH, which have shown to be decreased and increased, respectively, in AD. Co-treatment of α -OOS normalized skin hydration and pH, two key markers in the presentation of AD.¹⁹ However, it is important to note that an increase in skin hydration was also observed at vehicle-treated sites, suggesting that the vehicle itself may have a positive effect on skin hydration (Fig. 4b,c). Lastly, increased skinfold thickness, which also represents the inflammatory response induced by topical oxazolone, was decreased at α -OOS co-treated sites (Fig. 4d).

To further investigate the effects of CB1R agonists on skin, histologic observations were performed to observe changes in epidermal thickness normally induced by the oxazolone model. Consistent with skinfold thickness findings, co-treatment of α -OOS or dexamethasone prevented oxazolone-induced epidermal thickening (Fig. 5a). Immunohistochemical staining for PCNA further confirmed anti-inflammatory effects of α -OOS in the oxazolone model. Epidermal expression of CB1R and IL-4 levels was elevated in the oxazolone group. Co-treatment α -OOS downregulated these changes (Fig. 5b–d).

Discussion

Current use of PEA as an anti-inflammatory and antipruritic agent is based, at least in part, on the agonist

properties of PEA on CBRs in the skin.²³ While it is generally accepted that PEA does not directly activate CBR, indirect activation of CBR through the so-called entourage effect is known to be involved in the pharmacological effects of PEA.²⁴ The clinical efficacy of PEA containing adjuvant moisturizer on AD further confirms the therapeutic potential of CBR modulating compounds on inflammatory skin diseases.¹⁷ Along with PEA, many other candidate compounds are currently under development for various kinds of inflammatory diseases,²⁵ including skin diseases.²⁶ Based on the observed clinical benefits of the CB1R agonists in this study, we developed new agonists for CB1R and evaluated their therapeutic potential for inflammatory skin diseases.

We synthesized diacyl amide compounds, which are structurally similar to endocannabinoid AEA, with the variation of acyl groups' saturation status and amino acid moiety. Upon measuring the CB1R or CB2R modulating activity, several CB1R-specific agonist compounds were identified. Interestingly, among the tested compounds, only unsaturated acyl group-containing compounds showed modulating activities against CB1R or CB2R. For example, fully saturated forms of α -OOS or α -OOE, α -palmitoyl palmitamide serinol or α -palmitoyl palmitamide ethanol amine, respectively, did not show any effects on CB1R or CB2R (data not shown). Changing the amino acid moiety also affects the specificity of compound on the receptors. Further investigation about the

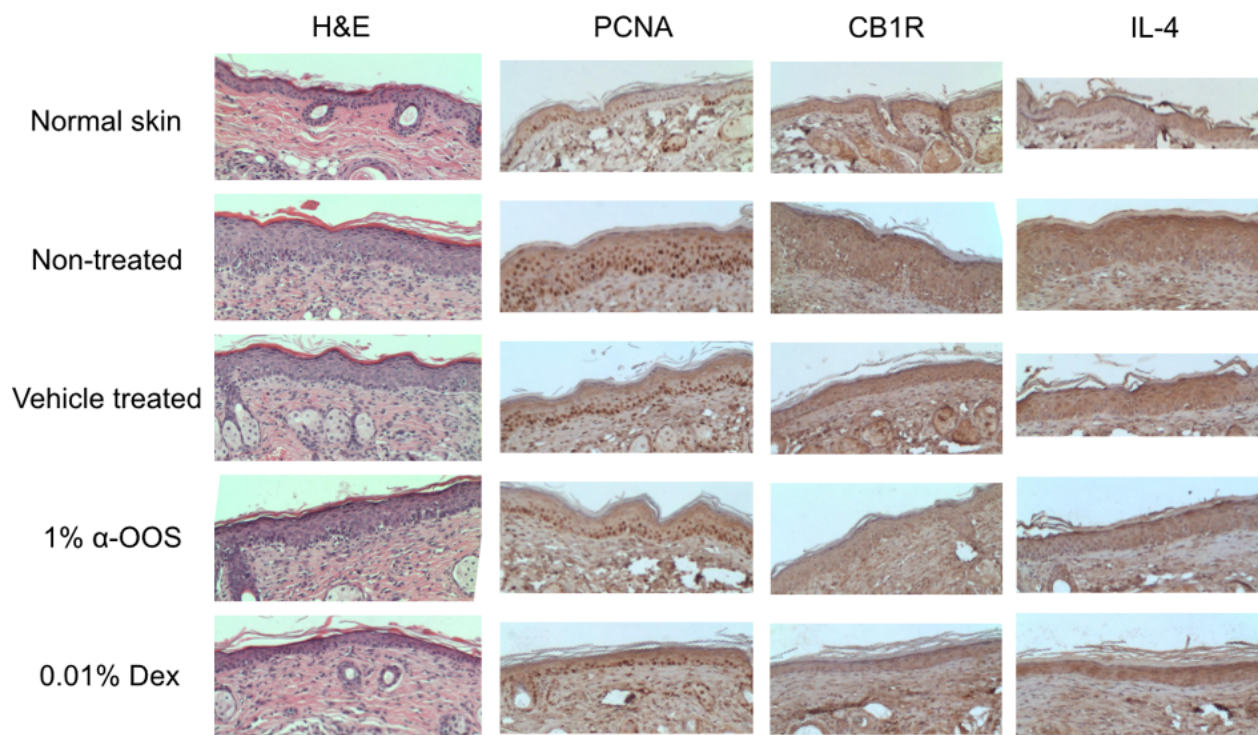


Figure 5 Change of epidermal thickness, CB1R and IL-4 expressions in epidermis. H&E staining and immunohistochemical staining for PCNA showed significant hyperproliferation in oxazolone-treated sites. Topical treatment with 1% α -OOS or 0.01% Dex decreased the skin thickness, which is further confirmed by PCNA staining. Epidermal expression of CB1R was slightly decreased in the α -OOS-treated site, and IL-4 expression was downregulated by α -OOS treatment. CB1R, cannabinoid receptor 1; Dex, dexamethasone; H&E, hematoxylin and eosin; IL, interleukin; α -OOS, α -oleoyl oleylamine serinol; PCNA, proliferating cell nucleus antigen; Veh, vehicle

specific relationship between structure vs. specificity and agonist or antagonist activity of compound are currently undergoing.

Various (patho)physiological roles of cannabinoids and their receptors in skin have been repeatedly reported,²⁶ and many of the investigations have focused on the anti-inflammatory activity.²⁷ A series of studies using CB1R and/or CB2R KO mouse models also showed the anti-inflammatory activities. In an animal model for cutaneous contact hypersensitivity, mice lacking both CB1R and CB2R showed exacerbated allergic inflammation, suggesting that CB1R and CB2R play an important role in the inflammatory response. Modulation of CBR activity, by using either agonist or antagonist, also includes similar anti-inflammatory effects of CBR activation. Interestingly, the fatty acid amide hydrolase-deficient mice, which have increased levels of endocannabinoids and consequently upregulated activation of CBR, also show reduced allergic responses.⁸ Recently, using a mouse model that lacks only CB1R in KCs, Gaffal *et al.*⁹ also showed similar anti-inflammatory activity of CB1R. Mast cells play crucial roles in many inflammatory diseases, and potential

involvement of CBR activation in mast cell maturation may underlie the anti-inflammatory action of CBR agonists.¹⁰ From our investigations, downregulation of mast cell degranulation by CB1R agonist was also observed (unpublished data).

Recently, using a CB1R and CB2R KO mouse model, Roelandt *et al.*¹³ reported the opposite effects of CB1R and CB2R on epidermal permeability barrier function and differentiation. While CB1R KO mouse showed delayed permeability barrier recovery, CB2R KO significantly accelerated barrier recovery, which suggests the positive roles of CB1R in skin barrier function. While these results further suggest the positive effects of CB1R agonist on skin barrier function, experimental results from a KO animal model do not necessarily represent the effects of the CB1R agonist study in normal animals. For example, many studies also suggested the direct and/or indirect activation of peroxisome-proliferator activated receptors (PPARs) by usual CBR agonists.⁴ It is well known that PPAR activation also results in attenuation of inflammatory responses, and potential involvement of PPAR signaling induced by CBR agonists cannot be

excluded. In addition, other receptors, including transient receptor potential vanilloid 1 and GPR55,²⁸ are also involved in CBR signaling. As a result, topical application of CBR agonists may be a more relevant model to the practical condition than the KO model for explaining the effects of CBR agonists on various skin diseases.

In this study, we showed that topical application of the CB1R-specific agonist significantly accelerated recovery of epidermal barrier function in acutely abrogated skin. Anti-inflammatory activities in both the acute irritation model using TPA and the chronic irritation model using an oxazolone application were observed, which are consistent with previous results. While further investigations should be performed to clarify the potential involvement of other signaling pathways, these results suggest that topical CB1R agonist treatment can be a potential therapeutic option for acute and chronic inflammatory skin diseases, including AD and psoriasis.

Acknowledgments

This study was supported by a grant of the Korean Health Technology R&D Project, Ministry of Health & Welfare, Republic of Korea (grant no. HN11C0048).

References

- Habayeb OMH, Bell SC, Konje JC. Endogenous cannabinoids: metabolism and their role in reproduction. *Life Sci* 2002; 70: 1963–1977.
- Thakur GA, Nikas SP, Makriyannis A. CB1 cannabinoid receptor ligands. *Mini Rev Med Chem* 2005; 5: 631–640.
- Maccarrone M. The endocannabinoid system in human keratinocytes: evidence that anandamide inhibits epidermal differentiation through CB1 receptor-dependent inhibition of protein kinase C, activating protein-1, and transglutaminase. *J Biol Chem* 2003; 278: 33896–33903.
- Toth BI, Dobrosi N, Dajnoki A, et al. Endocannabinoids modulate human epidermal keratinocyte proliferation and survival via the sequential engagement of cannabinoid receptor-1 and transient receptor potential vanilloid-1. *J Invest Dermatol* 2011; 131: 1095–1104.
- Casanova ML, Blázquez C, Martínez-Palacio J, et al. Inhibition of skin tumor growth and angiogenesis in vivo by activation of cannabinoid receptors. *J Clin Invest* 2003; 111: 43–50.
- Wilkinson JD, Williamson EM. Cannabinoids inhibit human keratinocyte proliferation through a non-CB1/CB2 mechanism and have a potential therapeutic value in the treatment of psoriasis. *J Dermatol Sci* 2007; 45: 87–92.
- Paradisi A, Pasquariello N, Barcaroli D, et al. Anandamide regulates keratinocyte differentiation by inducing DNA methylation in a CB1 receptor-dependent manner. *J Biol Chem* 2008; 283: 6005–6012.
- Karsak M, Gaffal E, Date R, et al. Attenuation of allergic contact dermatitis through the endocannabinoid system. *Science* 2007; 316: 1494–1497.
- Gaffal E, Cron M, Glodde N, et al. Cannabinoid 1 receptors in keratinocytes modulate pro-inflammatory chemokine secretion and attenuate contact allergic inflammation. *J Immunol* 2013; 190: 4929–4936.
- Sugawara K, Biro T, Tsuruta D, et al. Endocannabinoids limit excessive mast cell maturation and activation in human skin. *J Allergy Clin Immunol* 2012; 129: 726–728.
- Ong PY. Emerging drugs for atopic dermatitis. *Expert Opin Emerg Drugs* 2009; 14: 165–179.
- Paus R, Schmelz M, Biro T, et al. Frontiers in pruritus research: scratching the brain for more effective itch therapy. *J Clin Invest* 2006; 116: 1174–1186.
- Roelands T, Heughebaert C, Bredif S, et al. Cannabinoid receptors 1 and 2 oppositely regulate epidermal permeability barrier status and differentiation. *Exp Dermatol* 2012; 21: 688–693.
- Simon D, Kernland Lang K. Atopic dermatitis. *Curr Opin Pediatr* 2011; 23: 647–652.
- Boguniewicz M, Leung DYM. Atopic dermatitis: a disease of altered skin barrier and immune dysregulation. *Immunol Rev* 2011; 242: 233–246.
- Elias PM, Hatano Y, Williams ML. Basis for the barrier abnormality in atopic dermatitis: outside-inside-outside pathogenic mechanisms. *J Allergy Clin Immunol* 2008; 121: 1337–1343.
- Eberlein B, Eicke C, Reinhardt HW, et al. Adjuvant treatment of atopic eczema: assessment of an emollient containing N-palmitoylethanolamine (ATOPA study). *J Eur Acad Dermatol Venereol* 2007; 22: 73–82.
- Dalle Carbonare M, Del Giudice E, Stecca A, et al. A saturated N-acyl ethanolamine other than N-palmitoyl ethanolamine with anti-inflammatory properties: a neglected story. *J Neuroendocrinol* 2008; 20: 26–34.
- Man M-Q, Hatano Y, Lee SH, et al. Characterization of a hapten-induced, murine model with multiple features of atopic dermatitis: structural, immunologic, and biochemical changes following single versus multiple oxazolone challenges. *J Invest Dermatol* 2008; 128: 79–86.
- Park BD, Lee M, Kim Y, et al. The synthesis of new pseudoceramide using alkylketene dimer and their physical properties. *J Ind Eng Chem* 1999; 5: 228–234.
- Choi EH, Brown BE, Crumrine D, et al. Mechanisms by which psychologic stress alters cutaneous permeability barrier homeostasis and stratum corneum integrity. *J Invest Dermatol* 2005; 124: 587–595.
- Park BD, Youm JK, Jeong SK, et al. The characterization of molecular organization of multilamellar emulsions containing pseudoceramide and type III synthetic ceramide. *J Invest Dermatol* 2003; 121: 794–801.
- Petrosino S, Iuvone T, Di Marzo V. N-palmitoyl-ethanolamine: biochemistry and new therapeutic opportunities. *Biochimie* 2010; 92: 724–727.

- 24 Costa B, Comelli F, Bettoni I, *et al.* The endogenous fatty acid amide, palmitoylethanolamide, has anti-allodynic and anti-hyperalgesic effects in a murine model of neuropathic pain: involvement of CB. *Pain* 2008; 139: 541–550.
- 25 Hanus LO, Mechoulam R. Novel natural and synthetic ligands of the endocannabinoid system. *Curr Med Chem* 2010; 17: 1341–1359.
- 26 Biro T, Toth BI, Haskó G, *et al.* The endocannabinoid system of the skin in health and disease: novel perspectives and therapeutic opportunities. *Trends Pharmacol Sci* 2009; 30: 411–420.
- 27 Oka S, Wakui J, Ikeda S, *et al.* Involvement of the cannabinoid CB2 receptor and its endogenous ligand 2-arachidonoylglycerol in oxazolone-induced contact dermatitis in mice. *J Immunol* 2006; 177: 8796–8805.
- 28 Saturnino C, Petrosino S, Ligresti A, *et al.* Synthesis and biological evaluation of new potential inhibitors of N-acylethanolamine hydrolyzing acid amidase. *Bioorg Med Chem Lett* 2010; 20: 1210–1213.