J. Cosmet. Sci., 71, 425–437 (November/December 2020)

Soothing Effect of *Pogostemon cablin* Extract (Patchouli), via Cannabinoid Receptor 2 Modulation in Human Skin

FLORIAN LABARRADE, ARMELLE PERRIN, YOLENE FERREIRA, GILLES OBERTO, CATHERINE GONDRAN, CORINNE MOREL, and KARINE CUCUMEL, *Ashland Specialties France, Global Skin Research Center, Nice 06904, France (F.L., A.P., Y.F., G.O., C.G., C.M., K.C.)*

Accepted for publication August 6, 2020.

Synopsis

Skin functions as a neuro-immuno-endocrine tissue with well-defined neuronal networks and functions. The endocannabinoid system has been proven to be an important, homeostatic regulator for homeostatic and inflammatory events. The system comprises endogenous or exogenous ligands and receptors (CB1 and CB2). In the present study, we evaluated the soothing properties of a *Pogostemon cablin* (patchouli) extract. Agonist AM1241 and antagonist AM630 were used for CB2 receptor activation/inhibition. Expression of CB2 receptor and β-endorphin was monitored by immunohistochemistry. Skin inflammation was induced with ultraviolet B (UVB) or lipopolysaccharide (LPS), and the following markers were used to highlight the anti-inflammatory properties of the extract: transient receptor potential vanilloid 1 (TRPV1), interleukin receptors 1 (IL1R1), and the interleukin 6 signal transducer (IL6ST). Our results demonstrated the implication of the CB2 receptor in the skin inflammation process. The expression of CB2 receptor and β-endorphin was increased 48 hours after application of the extract. Furthermore, patchouli extract application helped to reduce IL1R1, IL6ST, and TRPV1 expression, in skin exposed to UVB or LPS. In conclusion, the application of the patchouli extract helps maintain skin integrity and reduce skin discomfort *via* modulation of CB2 receptor stimulation and the subsequent β-endorphin release.

INTRODUCTION

Skin serves as the first protective barrier between the body and the environment, with both passive and active roles against chemical, physical, and microbial insults (1). Human skin produces numerous neuropeptides that play a role in various cutaneous functions and diseases, acting as neuromodulators, neurotransmitters, and hormones. Many different biologic actions of neuropeptides in skin have been demonstrated, such as inflammatory, proliferative, and reparative processes after injury (2). Cannabinoids represent a broad class of chemical compounds derived from the cannabis plant. Cannabinoids are chemical

Address all correspondence to Florian Labarrade at flabarrade@ashland.com.

compounds that are related to the main psychoactive ingredient in marijuana: Δ9-tetrahydrocannabinol (3). Phytocannabinoids are present in a large variety of plants and can interact with cannabinoid receptors or share chemical structure with cannabinoids, or both (4). The endocannabinoid system comprises endogenous ligands such as the anandamide, enzymes, transporter apparatus, and two known receptors (CB1 and CB2). The various downstream effects of the cannabinoids are mediated through these two G protein–coupled receptors. The selective activation of cannabinoid receptor 2 is devoid of psychoactive side effects associated with cannabinoid receptor 1 stimulation. Skin inflammatory reaction is characterized by redness, swelling, heat, and pain. Even if this is an important host defense mechanism against invading pathogens, persistent or over-inflammation can result in skin damage. Distribution of CB1 and CB2 in human skin is described in many cell types and structures, and many studies have reported the promising role of cannabinoids in the treatment of dermatologic conditions, among them skin inflammation (5–10). A downstream signaling mechanism of cannabinoid receptor 2 activation is the β-endorphin release, contributing to the peripheral analgesic effect (11). β-endorphin is a proopiomelanocortin (POMC)-derived opioid neuropeptide, and on a molar basis, β-endorphin is 18–33 times more potent than morphine (12). CB2 receptor activation alone is enough for attenuation of the neuropathic pain, by attenuating inflammation, and changes in the interleukin signal (13). A large body of evidence now exists to substantiate that certain endocannabinoids activate transient receptor potential vanilloid 1 (TRPV1). TRPV1 is a key receptor of various sensory phenomena (pain, heat, and itch). It is found in nonneuronal cell types, including human skin epidermal keratinocytes (14–17). TRPV1 also acts as a nociceptive sensor and can potentiate the inflammatory process. Indeed, elevated TRPV1 expression was identified in UV-irradiated photo-aged and intrinsically aged skin (18,19). Cannabinoids also exert potent anti-inflammatory effect; however, the mechanism by which cannabinoid receptor type 2 reduces inflammation and promotes tissue repair in the course of human skin wound healing is still not completely clear. Several plants produce molecules with cannabinoid activity (20), such as β-caryophyllene (BCP), a phytocannabinoid with a strong affinity to cannabinoid receptor 2 (21), but not to cannabinoid receptor type 1 (22). BCP is one of the major active components of essential oils derived from a large number of spice and food plants. Patchouli oil has a variety of pharmacological activities, including anti-inflammatory (23,24). BCP as a plant volatile compound is commonly found in patchouli leaves, with other sesquiterpenes (25,26). Activity of BCP can be partially described as a cannabinoid receptor 2 agonist, suggesting its role as an anti-inflammatory agent (27).

In this study, we investigated the key functions of cannabinoid signaling to control local inflammation responses in the skin, with CB2 receptor agonist AM1241, antagonist AM630, and a patchouli extract. Our findings suggest that our patchouli extract possesses a soothing effect *via* the modulation of the CB2 receptor pathway.

MATERIALS AND METHODS

PLANT MATERIALS AND EXTRACTION

The aerial parts comprising the stem and leaves were collected in the field from sustainable farming in Colombia, dried, and ground by cryogenic grinding. Pure plant extract was obtained using a supercritical CO_2 extraction technique, including ethanol as a cosolvent.

This process allowed us to obtain a nonpolar fraction (containing essential oil), and a polar fraction containing molecules such as phytosterols, flavonoids, and fatty esters. Pure extract was diluted in octyldodecanol, and was used at 1% on *ex vivo* skin and at 0.001% on cultured keratinocytes.

ANTIBODIES

Primary antibodies used were anti-CNR2 (Thermo Scientific, Waltham, MA), anti–β-endorphin (LSBio, Seattle, WA), anti–interleukin receptor 1 (IL1R1) (Rockland, Limerick, PA), anti–interleukin 6 signal transducer (IL6ST) (Santa Cruz Biotechnology, Heidelberg, Germany), and anti-TRPV1 (Thermo Scientific). Alexa Fluor® coupled secondary antibodies were used (Molecular Probes, Eugene, OR).

REAGENTS

Selective and competitive antagonists of cannabinoid receptor 2: AM630 and selective agonist of cannabinoid receptor 2: AM1241 were purchased from Sigma Chemical Co. (St. Louis, MO). AM630 and AM1241 were applied on skin biopsies at 1 mM, 3 h (topical and culture medium application) before UVB irradiation with 200 mJ/cm^2 (Bio-Link Irradiator 254 nm, Fisher Scientific, Illkirch, France). Lipopolysaccharide (LPS) (Sigma-Aldrich, St. Louis, MO) was used at 0.5 mg/mL overnight.

CELL CULTURE

Normal human epithelial keratinocytes were isolated from skin obtained from plastic surgery of healthy females who had given written informed consent. Keratinocytes were cultured in keratinocyte serum–free medium, with provided human recombinant epidermal growth factor and bovine pituitary extract (Gibco, Auckland, New Zealand), and 0.1 mg/mL Primocin™ (Invivogen, San Diego, CA).

β-ENDORPHIN SYNTHESIS IN CULTURED KERATINOCYTES

Human keratinocytes were grown in tissue culture dishes (100 mm). After the addition of the patchouli extract, plates were incubated for 24 h with the patchouli extract at 0.001% [diluted in 0.001% dimethyl sulfoxide (DMSO)]. Cells were harvested, and β-endorphin was measured by enzyme-linked immunosorbent assay (Elabscience, Houston, TX).

HEMOTOXYLIN AND EOSIN STAINING

Slides containing 4 μm skin paraffin sections were deparaffinized and rehydrated in the following baths: 2 × 2 min in xylene, 2 × 2 min in 100% ethanol, 1 × 2 min in 95% ethanol, 1 × 2 min in 80% ethanol, and 1 × 5 min in H_2O. Hematoxylin staining: 1 × 3 min hematoxylin and rinsed in water for 1 × 5 min. Eosin staining and dehydration:

1 × 2 min in eosin, 1 × 2 min in 95% ethanol, 2 × 2 min in 100% ethanol, and 2 × 2 min in xylene. Coverslips were mounted on glass slides using Eukitt® (xylene based) as mounting media (O. Kindler, Germany) and dried overnight.

IMMUNOHISTOLOGICAL FLUORESCENCE

Human skin samples were obtained from plastic surgery of healthy females who had given written informed consent. After removal of subcutaneous fat, the tissue was used to obtain 6 mm punch biopsies that were incubated with the patchouli extract (or its placebo) at 1% for 48 h. Biopsies were then fixed in formaldehyde and processed in an automated Shandon Hypercenter XP (Shandon Ltd., Runcor, United Kingdom) for paraffin embedding. Sections of 4 μm thickness were cut with a microtome (Shandon) and collected on polylysine-coated glass slides (Menzel Gläser, Braunschweig, Germany) for immunohistochemistry. Heat and pepsin enzymatic antigen retrieval was performed before incubation with CB2 receptor and β-endorphin antibodies. Heat antigen retrieval was performed before incubation with IL1R1 primary antibody. Pepsin enzymatic antigen retrieval was performed before incubation with TRPV1 primary antibody. No antigen retrieval was performed before incubation with IL6ST primary antibody. After incubation with the secondary antibody, cell nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI). Skin sections were viewed under a microscope (Axiovert 200M, Carl Zeiss, Oberkochen, Germany) and photographed with a CCD camera (EXI blue, Qimaging, Surrey, BC).

SUBJECT

To demonstrate the potential effect of patchouli extract on sensitive skin, a stinging test was performed as the reference test for sensitive skin. However, other parameters known to be altered on sensitive skin, like skin barrier function, were evaluated on these 26 volunteers on the forearm at the same time as the stinging test (data not shown). One month before the beginning of the clinical test, panels of subjects who are sensitive to 0.01% of capsaicin diluted in 10% ethanol (which cause sensory irritation) and not to 10% ethanol, applying on nasolabial fold during 10 s, were preselected by a trained expert. Just after the exposure, the subject recorded the sensations of stinging each minute during 10 min on a 0- to 4-interval scales (0 = no sensation, 1 = slight stinging, 2 = moderate stinging, 3 = intense stinging, and 4 = very intense stinging). At the end of the 10 min, the 11 values recorded by the subject were summed up to a final score. If this score was superior to 10, the volunteer was declared like having sensitive skin. Only volunteers having a score superior to 10 were identified as having sensitive skin and were enrolled in the study. At the beginning, 26 volunteers were enrolled in the study and were divided in two groups of 12 volunteers homogenous in age and gender to be sure that these two parameters did not influence the results. However, during the test period, six volunteers had to be excluded of the stinging test results, because of big variation in their stinging sensation compared to other trials done at the same period. For five of these volunteers, the variation was explained by a bad cold, and for the last one, no explanation was found. Thus, the stinging result was performed on 20 volunteers, divided in two groups of 10 volunteers homogenous in age and gender.

FACE CAPSAICIN–INDUCED STINGING TEST

For this clinical study, 20 volunteers were enrolled after having read the information related to the study and signed the informed consent form. All volunteers had sensitive skin and were divided in two groups of 10, homogeneous in age and gender. For 28 d, on all the faces, one group applied a placebo cream and one group applied a cream containing the patchouli extract at 1%. The study was performed in double blind. The stinging test was carried out on the first day of the study (D0), before cream application and 28 d after cream applications (D28). The test consisted in applying 0.01% of capsaicin diluted in 10% ethanol to one nasolabial fold and 10% ethanol to the other during 30 s. Just after the exposure, the subjects recorded the sensations of stinging each minute during 10 min on a 0- to 4-interval scales (0 = no sensation, 1 = slight stinging, 2 = moderate stinging, 3 = intense stinging, and 4 = very intense stinging). At the end, the 11 scores were summed to give a score of skin sensitivity.

STATISTICAL ANALYSIS

All experiments have been repeated at least twice with cells and skin coming from different plastic surgery, and the quantification of the fluorescence was performed by measuring the fluorescent intensity of the staining, which was normalized by the epidermis or cell area. Statistical analyses were performed using Excel 365 (Microsoft, Redmond, WA). Difference between two means was performed with Student's *t*-test. A p-value ≤ 0.05 was considered statistically significant (*), p-value ≤ 0.01 as very significant (**), and p-value ≤ 0.005 as highly significant (***). For the clinical test, statistical analysis was performed using JMP® 14 (SAS Inc, Cary, NC) software. The homogeneity of the gender between the two groups was performed using the χ^2 test, and the homogeneity of the age was confirmed using the Mann–Whitney test. Concerning the statistical analysis of stinging values, the scores, which were used as a basis, concerned the difference (D28–D0) for the patchouli extract and placebo group using Wilcoxon test (after verifying the data followed a normal distribution or not with the Shapiro–Wilk test).

RESULTS

THE CB2 RECEPTOR ACTIVATION SHOWS RESISTANCE TO UV-INDUCED SKIN INFLAMMATION

Because of the potential role of cannabinoids in human skin inflammation, we investigated whether the antagonist AM630 would exhibit an amplified susceptibility to UVB-induced inflammation in human skin biopsies. Histologic analysis indicated that UVB-treated human skin showed a pronounced inflammation with the emergence of structural damages and sunburn cells (Figure 1A). Exposure to AM630 (3 h) before UVB irradiation showed a very damaged structure with an important epidermal erosion, whereas the epidermis exposed to AM1241 (3 h) was far more resistant to UVB-induced inflammation (Figure 1A). In a second time, skin was treated with the patchouli extract during 48 h after UVB irradiation. The biopsies were treated or not during 3 h with AM630 prior UVB irradiation. The patchouli extract preserved the skin from UVB, compared with the placebo condition (Figure 1B). The antagonist AM630 was able to partially block the protective effect of the patchouli extract on skin preservation.

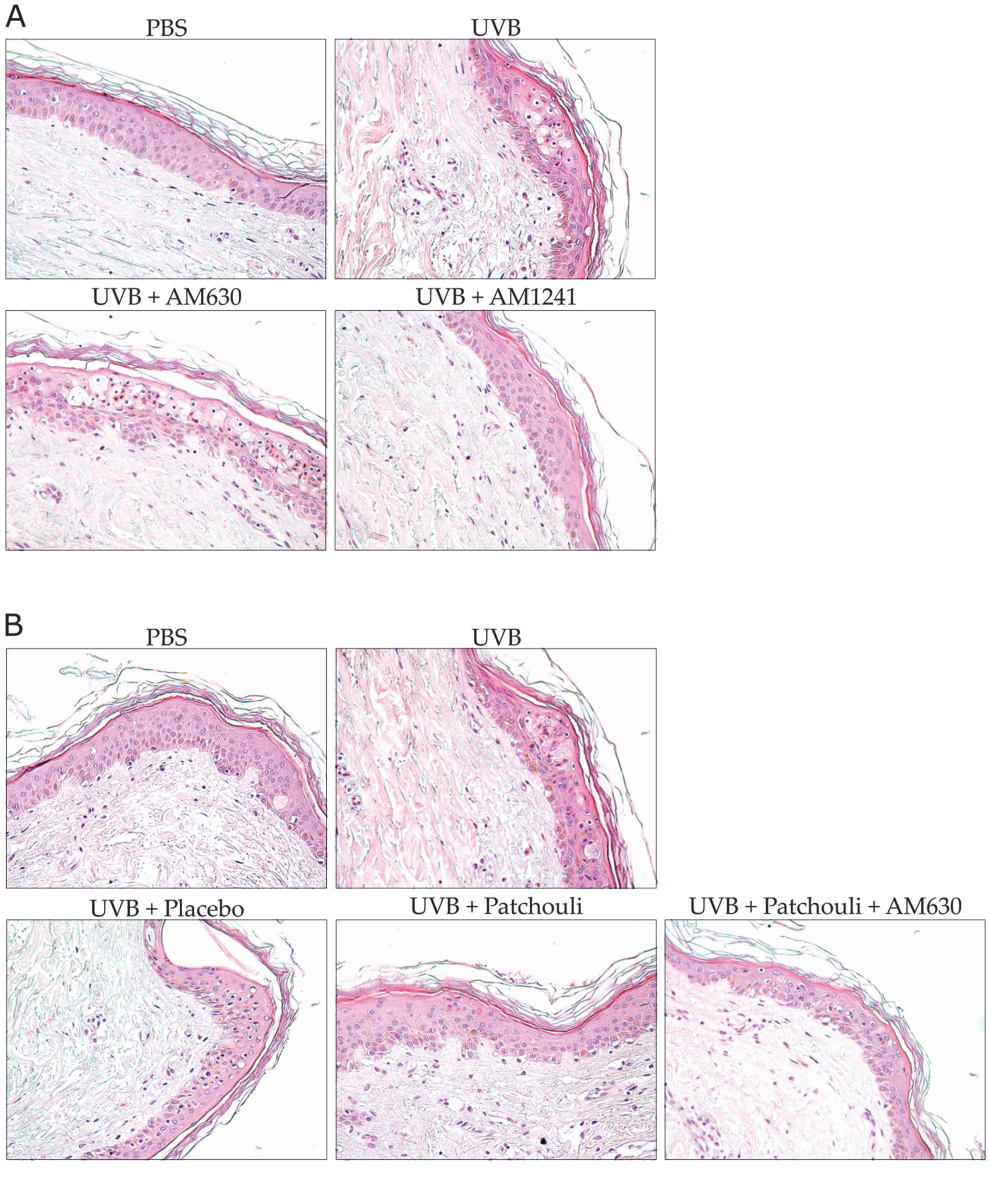

Figure 1. Stimulation or inhibition of cannabinoid receptor 2 in UVB-irradiated human skin. (A) Hematoxylin–eosin staining of human skin sections of skin treated with PBS buffer, AM630, AM1241, 3 h before UVB irradiation. Biopsies were maintained in culture 48 h after UVB irradiation, and then formalin-fixed and paraffin-embedded. (B) Hematoxylin–eosin staining of human skin sections treated or not with AM630, 3 h before UVB irradiation. Topic application of placebo and patchouli extract was performed after the irradiation. Biopsies were maintained in culture 48 h after UVB irradiation, and then formalin-fixed and paraffin-embedded (objective ×20).

PATCHOULI EXTRACT INCREASES CANNABINOID RECEPTOR 2 AND THE β-ENDORPHIN OPOID

Several lines of evidence demonstrate that phytocannabinoids can exert various biological effects in the skin. Immunohistochemistry for cannabinoid receptor 2 on human skin sections (Figure 2A) showed primary localization of the receptor in the epidermis, although some

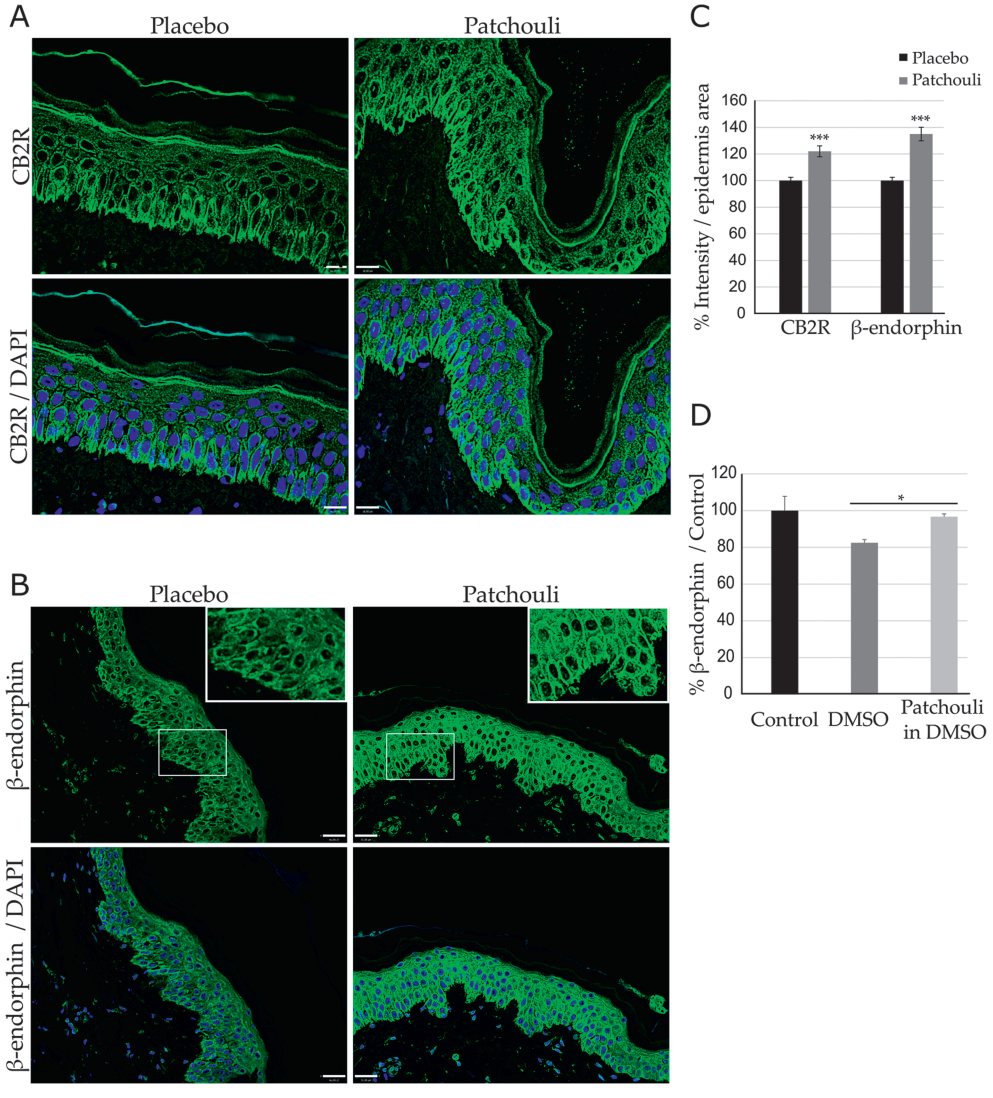

Figure 2. Expression of cannabinoid receptor 2 and β-endorphin in normal skin. (A) Immunohistochemistry of cannabinoid receptor 2 (green) and DNA stained with DAPI (blue), 48 h after topic application of placebo or patchouli extract (×40 objective lens, scale bar = 16 μm). (B) Immunohistochemistry of β-endorphin (green) and DNA stained with DAPI (blue), 48 h after topical application of placebo or patchouli extract (×20 objective lens, scale bar = 31 μm). (C) Quantification of the cannabinoid receptor 2 and β-endorphin immunohistochemistry. (D) Detection of β-endorphin expression in keratinocytes by ELISA, 24 h after patchouli extract addition in the culture media. (n = 3 mean ± Standard Error of the Mean; ***$p \leq 0.005$; **$p \leq 0.01$; *$p \leq 0.05$).

cells of the dermis were also positive. Basal layers of the epidermis showed the strongest fluorescent intensity (Figure 2A). The patchouli extract increased the expression level of the receptor (Figure 2C; +22% *$p < 0.05$). A downstream signal of the CB2 receptor is the release of the β-endorphin opioid. The patchouli extract significantly increased the protein level of β-endorphin in skin (Figure 2B and C). Moreover, the β-endorphin release could be observed in the cell interspaces (Figure 2B close-ups) compared with the

placebo condition. Synthesis of β-endorphin by keratinocytes was confirmed by ELISA (Figure 2D); keratinocytes expressed more β-endorphin when treated with the patchouli extract (+17% $*p < 0.05$). The DMSO (DMSO used at 0.001%) showed a little variation in β-endorphin levels compared with control condition.

PATCHOULI EXTRACT MODULATES EXPRESSION OF TRPV1, INTERLEUKIN RECEPTOR 1, AND IL6ST

The skin expresses an abundance of Transient Receptor Potential (TRP) channels modulating its development, integrity, and function. TRPV1 receives substantial attention as a candidate target for pain control, and itching sensation in skin. Cannabinoids have been used to treat pain for millennia; we tested the hypothesis that the patchouli extract could decrease the expression of TRPV1 and exerted a peripheral soothing effect. UVB and LPS induced TRPV1 immunohistochemistry in human skin (Figure 3A). The treatment with patchouli extract reduced the expression of TRPV1 in skin in response to UVB- or LPS-induced inflammation (Figure 3A). IL-1 is a highly active and pleiotropic pro-inflammatory cytokine. The receptor that mediates all known biologic activities of IL-1 is the IL-1 receptor type 1 (IL1R1). We investigated the capacity of the patchouli extract to reduce the expression of IL1R1. Immunohistochemistry results showed that the patchouli extract reduced IL1R1 expression, in skin inflammation models induced either by UVB (Figure 3A) or LPS (Figure 3B). Interleukin-6 (IL-6) is a pleiotropic cytokine, with diverse roles in driving chronic inflammation. IL-6 activities are predominantly exerted through a process known as trans-signaling that uses a protein called IL6ST for initiating the IL6 signal transmission. The effect of the patchouli extract on the local concentrations of IL6ST in skin was investigated. Results showed the capacity of the extract to reduce IL6 signaling through IL6ST diminution in the epidermis irradiated with UVB (Figure 3A and C).

CALMING EFFECTS OF PATCHOULI EXTRACT ON CAPSAICIN-INDUCED FACIAL STINGING IN VOLUNTEERS WITH SENSITIVE SKIN.

All 20 subjects reported a stinging/burning sensation after application of capsaicin. Twenty-eight d of cream applications, with patchouli extract, resulted in significant lower values for burning/stinging sensations in comparison to the values obtained for the placebo group (Figure 4). On the patchouli extract–treated sides, a significant decrease in the TEWL was observed after 28 d of application compared with the placebo sides, demonstrating that the patchouli extract can improve the skin barrier function (data not shown).

DISCUSSION

In this study, we highlight the key function of cannabinoid signaling, to control local immune responses in the human skin. Anti-inflammatory effects of cannabinoid CB2 receptor activation was observed with the agonist AM1241. Recent data with AM630 were available on rodent skin model (28). Our results add comprehension to the human skin physiology, by providing direct evidence supporting the tight control of the cutaneous endocannabinoid system, in the cellular response to UVB. Several previous studies showed endocannabinoid system have been involved in major roles in nociception or inflammatory reactions by receptor activation (29,30). We hypothesized that the extract

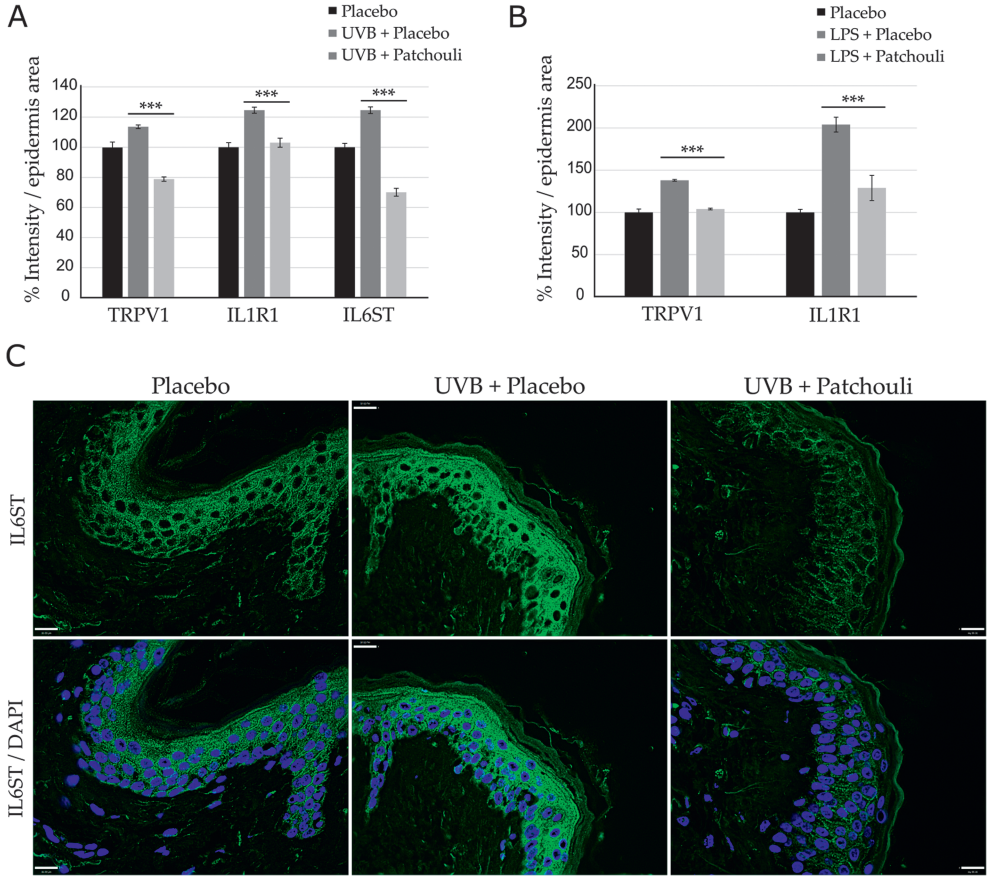

Figure 3. Anti-inflammatory effect of the patchouli extract. (A) Expression level of TRPV1, IL1R1, and IL6ST in human skin exposed to 200 mJ/cm^2 and treated with placebo or patchouli extract for 48 h. The expressions of the proteins were measured by immunohistochemistry. (B) Expression level of TRPV1, and IL1R1 in human skin exposed to 0.5 mg/mL *Escherichia coli* LPS for 1 night and treated with placebo or patchouli extract for 48 h. The expressions of the proteins were measured by immunohistochemistry. (C) Immunohistochemistry staining of IL6ST (green) and DNA stained with DAPI (blue), on human skin biopsies irradiated with 200 mJ/cm^2 UVB and treated with placebo or patchouli extract for 48 h (×40 objective lens, scale bar = 16 µm) ($n = 3$ mean ± SEM; ***$p \leq 0.005$; **$p \leq 0.01$; *$p \leq 0.05$).

could counter the inflammation induced by UVB or LPS, by stimulating CB2 receptor activity on epidermal cells and decreasing the release of inflammatory cytokines. The selective AM1241 activator, and the patchouli extract helped prevent an acute inflammation in skin without producing observable adverse effects. CB2 receptor antagonist AM630 clearly reversed the anti-inflammatory effect of patchouli in *ex vivo* skin. The patchouli extract contains many anti-inflammatory molecules such as sesquiterpenes and polyphenols, which could involve other anti-inflammatory signaling pathways independent of CB2.

Activation of peripheral CB2 receptor was shown to produce a particularly potent analgesic effect on inflammatory pain (31,32). The opioid peptide β-endorphin is derived from the precursor POMC. Patchouli application associated with CB2 receptor stimulation has effect on the opioid expression. The β-endorphin was detected in the skin biopsies, in the

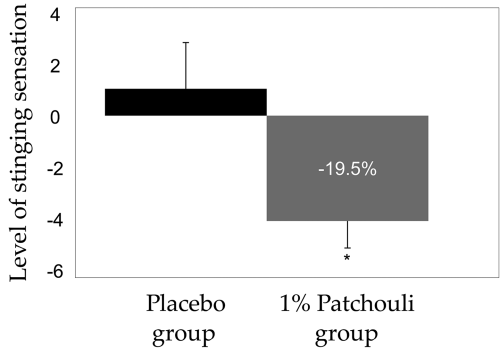

Figure 4. Evaluation of skin sensitivity by stinging test with capsaicin after 28 of cream applications. Decrease in capsaicin-induced stinging sensation after face application of a cream containing patchouli extract at 1% ($n = 10$ mean ± SEM; $p \leq 0.05$).

epidermis and some cells of the dermis. Application of the patchouli extract significantly increased the protein level of β-endorphin and the release by keratinocytes. Our results suggest that β-endorphin release contributes to the soothing effects of the patchouli extract. Our results are consistent with those of a previous study, where AM1241 stimulated β-endorphin release from immortalized human keratinocyte HaCaT cell line (33). It is also possible that other mediators, in addition to β-endorphin, might be released after the activation of CB2 receptors, contributing to the soothing effect.

TRPV1 acts as a key peripheral integrator of pain, itch, and heat sensation in skin. TRPV1 was found in human skin and might be associated with inflammation. Pharmacological blockade of TRPV1 has recently emerged as a potential novel therapeutic possibility in managing inflammatory diseases (34). In our experimental model, using UVB- and LPS-induced inflammation, skin treated with the patchouli extract showed lessened production of TRPV1 and pro-inflammatory cytokine receptors, including IL6ST and IL1R1. These results demonstrate that the activation of the CB2 receptor, at least in part, might participate in keratinocyte protection through the inhibition of cytokine receptors. The patchouli extract helped prevent the activation of TRPV1 and interleukin inflammasome pathway, and, in parallel, limited the formation of sun burn cells, as demonstrated by the suppression of tissue damage (Figure 1A and B). Likewise, the patchouli extract formulated at 1% helped to improve skin sensitivity by reducing stinging sensation induced by the activation of TRPV1 *via* capsaicin (Figure 4). The use of the patchouli extract associated with protective action although CB2 receptor activation could be a promising strategy for the treatment of sensitive skin.

CONCLUSIONS

In summary, our results confirm that CB2 agonist has strong anti-inflammatory activity in the human skin exposed to UVB or LPS. This effect, at least in part, is associated with the reduction in TRPV1, IL1R1, and IL6ST levels and the release of the β-endorphin opioid. A *Pogostemon cablin* extract (patchouli) containing the phytocannabinoid BCP, and other anti-inflammatory molecules present in the patchouli extract such as the polyphenols, was associated with a reduction in the level of inflammatory markers, *via* cannabinoid

receptor 2 modulation. This extract could be used to lessen skin discomfort, especially in case of fragile skin and itching sensation.

ACKNOWLEDGMENTS

We are grateful to Laura Mouret for the analysis of bioactive compounds present in the patchouli extract, and to Ludivine Mur and Alexia Lebleu for the technical assistance. We wish to thank Anne Clay Viardot for reading the manuscript and for the English language review.

Patents: Ashland owns patent applications related to a method for obtaining an extract of patchouli leaves and cosmetics uses thereof, published under numbers FR3091993A1 and W02020156981.

Funding: This research was funded by Ashland Inc.

Author contributions: Florian Labarrade, Yolene Ferreira, Gilles Oberto, and Catherine Gondran conceived and designed the experiments, and the methodology; Florian Labarrade, Armelle Perrin, Yolene Ferreira, and Gilles Oberto performed the experiments and analyzed the data; and Florian Labarrade wrote the paper, with Catherine Gondran revising it critically. Corine Morel was in charge of patchouli extract procurement and analysis. Catherine Gondran and Karine Cucumel supervised the study and handled project administration. All authors read and approved the final manuscript.

REFERENCES

(1) A. Oláh, A. G. Szöllősi, and T. Bíró, The channel physiology of the skin, *Rev. Physiol. Biochem. Pharmacol.*, **163**, 65–131 (2012).
(2) T. C. Theoharides, J. M. Stewart, A. Taracanova, P. Conti, and C. C. Zouboulis, Neuroendocrinology of the skin, *Rev. Endocr. Metab. Disord.*, **17**, 287–294 (2016).
(3) P. Pacher, S. Bátkai, and G. Kunos, The endocannabinoid system as an emerging target of pharmacotherapy, *Pharmacol. Rev.*, **58**, 389–462 (2006).
(4) J. Gertsch, R. G. Pertwee, and V. Di Marzo, Phytocannabinoids beyond the Cannabis plant – do they exist? *Br. J. Pharmacol.*, **160**, 523–529 (2010).
(5) S. Ständer, M. Schmelz, D. Metze, T. Luger, and R. Rukwied, Distribution of cannabinoid receptor 1 (CB1) and 2 (CB2) on sensory nerve fibers and adnexal structures in human skin, *J. Dermatol. Sci.*, **38**, 177–188 (2005).
(6) J. Zhang, L. Chen, T. Su, F. Cao, X. Meng, L. Pei, J. Shi, H. L. Pan, and M. Li, Electroacupuncture increases CB2 receptor expression on keratinocytes and infiltrating inflammatory cells in inflamed skin tissues of rats. *J. Pain*, **11**, 1250–1258 (2010).
(7) J. L. Zheng, T. S. Yu, X. N. Li, Y. Y. Fan, W. X. Ma, Y. Du, R. Zhao, and D. W. Guan, Cannabinoid receptor type 2 is time-dependently expressed during skin wound healing in mice, *Int. J. Leg. Med.*, **126**, 807–814 (2012).
(8) C. D. Río, E. Millán, V. García, G. Appendino, J. DeMesa, and E. Muñoz, The endocannabinoid system of the skin. A potential approach for the treatment of skin disorders, *Biochem. Pharmacol.*, **157**, 122–133 (2018).
(9) J. S. Mounessa, J. A. Siegel, C. A. Dunnick, and R. P. Dellavalle, The role of cannabinoids in dermatology, *J. Am. Acad. Dermatol.*, **77**, 188–190 (2017).
(10) R. Milando and A. Friedman, Cannabinoids: potential role in inflammatory and neoplastic skin diseases, *Am. J. Clin. Dermatol.*, **20**, 167–180 (2019).
(11) F. Gao. L. H. Zhang, T. F. Su, L. Li, R. Zhou, M. Peng, C. H. Wu, X. C. Yuan, N. Sun, X. F. Meng, B. Tian, H. L. Pan, and M. Li, Signaling mechanism of cannabinoid receptor-2 activation-induced β-endorphin release, *Mol. Neurobiol.*, **53**, 3616–3625 (2016).

(12) H. H. Loh, L. F. Tseng, E. Wei, and C. H. Li, Beta-endorphin is a potent analgesic agent, *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 2895–2898 (1976).
(13) H. Qian, J. Yi, J. Zhou, Y. Zhao, Y. Li, Z. Jin, and Y. Ding, Activation of cannabinoid receptor CB2 regulates LPS-induced pro-inflammatory cytokine production and osteoclastogenic gene expression in human periodontal ligament cells, *Open J. Stomatol.*, **3**, 44–51 (2013).
(14) S. Ständer, C. Moormann, M. Schumacher, J. Buddenkotte, M. Artuc, V. Shpacovitch, T. Brzoska, U. Lippert, B. M. Henz, T. A. Luger, D. Metze, and M. Steinhoffand, Expression of vanilloid receptor subtype 1 in cutaneous sensory nerve fibers, mast cells, and epithelial cells of appendage structures, *Exp. Dermatol.*, **13**, 129–139 (2004).
(15) M. Denda, S. Fuziwara, K. Inoue, S. Denda, H. Akamatsu, A. Tomitaka, and K. Matsunaga, Immunoreactivity of VR1 on epidermal keratinocyte of human skin, *Biochem. Biophys. Res. Commun.*, **285**, 1250–1252 (2001).
(16) E. Bodó, T. Bíró, A. Telek, G. Czifra, Z. Griger, B. I. Tóth, A. Mescalchin, T. Ito, A. Bettermann, L. Kovács, and R. Paus, A hot new twist to hair biology: involvement of vanilloid receptor-1 (VR1/TRPV1) signaling in human hair growth control, *Am. J. Pathol.*, **166**, 985–998 (2005).
(17) B. I. Tóth, S. Benko, A. G. Szöllosi, L. Kovács, E. Rajnavölgyi, and T. Bíró, Transient receptor potential vanilloid-1 signaling inhibits differentiation and activation of human dendritic cells, *FEBS Lett.*, **583**, 1619–1924 (2009).
(18) Y. M. Lee, Y. K. Kim, and J. H. Chung, Increased expression of TRPV1 channel in intrinsically aged and photoaged human skin in vivo, *Exp. Dermatol.*, **18**, 431–436 (2009).
(19) Y. M. Lee, S. M. Kang, and J. H. Chung, The role of TRPV1 channel in aged human skin, *J. Dermatol. Sci.*, **65**, 81–85 (2012).
(20) S. A. Bonini, M. Premoli, S. Tambaro, A. Kumar, G. Maccarinelli, M. Memo, and A. Mastinun, Cannabis sativa: a comprehensive ethnopharmacological review of a medicinal plant with a long history, *J. Ethnopharmacol.*, **227**, 300–315 (2018).
(21) K. Fidyt, A. Fiedorowicz, L. Strządała, and A. Szumny, β-caryophyllene and β-caryophyllene oxide-natural compounds of anticancer and analgesic properties, *Cancer Med.*, **5**, 3007–3017 (2016).
(22) V. Di Marzo and F. Piscitelli, The endocannabinoid system and its modulation by phytocannabinoids, *Neurotherapeutics*, **12**, 692–698 (2015).
(23) Y. Xian, J. Suo, X. D. Huang, S. Z. Hou, J. N. Chen, M. R. Ye, and Z. R. Su, A pharmacological study on anti-inflammatory effects of refined Huodan recipe, *Chin. J. Exp. Tradit. Med. Formul.*, **13**, 54–56 (2007).
(24) S. C. Zhao, Q. Jia, and F. L. Liao, The anti-inflammatory and analgesic pharmacological study of Patchouli extract, *Chin Tradit Pat Med.*, **29**, 285–287 (2007).
(25) M. K. Swamy and U. R. Sinniah, A comprehensive review on the phytochemical constituents and pharmacological activities of *Pogostemon cablin* benth.: an aromatic medicinal plant of industrial importance, *Molecules*, **20**, 8521–8547 (2015).
(26) H. S. Kusuma and M. Mahfuda, The extraction of essential oils from patchouli leaves (*Pogostemon cablin* Benth) using a microwave air-hydrodistillation method as a new green technique, *RSC Adv.*, **7**, 1336–1347 (2017).
(27) A. Oláh and T. Bíró, Targeting cutaneous cannabinoid signaling in inflammation - a "high"-way to heal? *EBioMedicine*, **16**, 3–5 (2017).
(28) S. Ghosh, L. E. Wise, Y. Chen, R. Gujjar, A. Mahadevan, B. F. Cravatt, and A. H. Lichtman, The monoacylglycerol lipase inhibitor JZL184 suppresses inflammatory pain in the mouse carrageenan model, *Life Sci.*, **92**, 498–505 (2013).
(29) J. C. Ashton and M. Glass, The cannabinoid CB2 receptor as a target for inflammation-dependent neurodegeneration, *Curr. Neuropharmacol.*, **5**, 73–80 (2007).
(30) N. D. Berger, V. M. Gadotti, R. R. Petrov, K. Chapman, P. Diaz, and G. W. Zamponi, NMP-7 inhibits chronic inflammatory and neuropathic pain via block of Cav3.2 T-type calcium channels and activation of CB2 receptors, *Mol. Pain*, **10**, 77 (2014).
(31) A. Quartilho, H. P. Mata, M. M. Ibrahim, T. W. Vanderah, F. Porreca, A. Makriyannis, and T. P. Malan, Jr., Inhibition of inflammatory hyperalgesia by activation of peripheral CB2 cannabinoid receptors, *Anesthesiology*, **99**, 955–960 (2003).
(32) B. B. Yao, G. C. Hsieh, J. M. Frost, Y. Fan, T. R. Garrison, A. V. Daza, G. K. Grayson, C. Z. Zhu, M. Pai, P. Chandran, A. K. Salyers, E. J. Wensink, P. Honore, J. P. Sullivan, M. J. Dart, and M. D. Meyerand, In vitro and in vivo characterization of A-796260: a selective cannabinoid CB2 receptor agonist exhibiting analgesic activity in rodent pain models, *Br. J. Pharmacol.*, **153**, 390–401 (2008).

(33) M. M. Ibrahim, F. Porreca, J. Lai, P. J. Albrecht, F. L. Rice, A. Khodorova, G. Davar, A. Makriyannis, T. W. Vanderah, H. P. Mata, and T. Philip Malan, Jr., CB2 cannabinoid receptor activation produces antinociception by stimulating peripheral release of endogenous opioids. *Proc. Natl. Acad. Sci. U.S.A.*, **102**, 3093–3098 (2005).
(34) J. G. Bonchak and R. A. Swerlick, Emerging therapies for atopic dermatitis: TRPV1 antagonists. *J. Am. Acad. Dermatol.*, **78**(Suppl. 1), S63–S66 (2018).