Available online at www.sciencedirect.com
SCIENCE DIRECT
European Journal of Pharmacology 520 (2005) 164–171
www.elsevier.com/locate/ejphar

Involvement of cannabinoid CB_2 receptor-mediated response and efficacy of cannabinoid CB_2 receptor inverse agonist, JTE-907, in cutaneous inflammation in mice

Yoshifumi Ueda *, Naoki Miyagawa [1], Tomoko Matsui [1], Tetsudo Kaya [1], Hiroyuki Iwamura [1]

Japan Tobacco Inc., Central Pharmaceutical Research Institute, 1-1 Murasaki-cho, Takatsuki, Osaka, 569-1125, Japan

Received 2 August 2005; accepted 8 August 2005
Available online 8 September 2005

Abstract

Involvement of cannabinoid CB_2 receptor and effect of cannabinoid CB_2 receptor antagonist/inverse agonists on cutaneous inflammation were investigated. Mice ears topically exposed to an ether-linked analogue of 2-arachidonoylglycerol (2-AG-E) or selective cannabinoid CB_2 receptor agonist, {4-[4-(1,1-dimethylheptyl)-2,6-dimethoxy-phenyl]-6.6-dimethyl-bicyclo[3.1.1]hept-2-en-2-yl}-methanol (HU-308), had early and late ear swelling (0–24 h and 1–8 days after exposure, respectively). Both types of responses induced by 2-AG-E were significantly suppressed by oral administration of cannabinoid CB_2 receptor antagonist/inverse agonists, [*N*-(benzo[1,3]dioxol-5-ylmethyl)-7-methoxy-2-oxo-8-pentyloxy-1,2-dihydroquinoline-3-carboxamide] (JTE-907) and {*N*-[(1*S*)-endo-1,3,3-trimethylbicyclo[2.2.1]heptan-2yl]5-(4-chloro-3-methyl-phenyl)-1-(4-methylbenzyl)pyrazole-3-carboxamide}} (SR144528). In contrast, JTE-907 did not affect arachidonic acid-induced swelling. Orally administered JTE-907 (0.1–10 mg/kg) and SR144528 (1 mg/kg) also produced significant inhibition of dinitrofluorobenzene-induced ear swelling, with increased cannabinoid CB_2 receptor mRNA expression observed in the inflamed ear. These results suggest that cannabinoid CB_2 receptor is partially involved in local inflammatory responses and cannabinoid CB_2 receptor antagonist/inverse agonist has beneficial effects on ear swelling.

Keywords: JTE-907; Cannabinoid CB_2 receptor; Inverse agonist; Agonist; Ear swelling

1. Introduction

Cannabinoids, active ingredients of *Cannabis sativa linnaeus* (marijuana) and their derivatives, exert a wide variety of effects on the central nervous system and immune system (Pertwee, 2000; Piomelli et al., 2000; Porter and Felder, 2001). Two main subtypes of cannabinoid receptors, central nervous type (CB_1) and peripheral type (CB_2), have been isolated and cloned, and found to be abundant in central nervous system and peripheral tissues, respectively (Facci et al., 1995; Matsuda et al., 1990; Munro et al., 1993; Pettit et al., 1996; Schatz et al., 1997). The physiological role of cannabinoid CB_2 receptor in the immune system, however, has not been fully characterized. This group previously demonstrated that cannabinoid CB_2 receptor antagonist/inverse agonists, JTE-907 and SR144528 (Rinaldi-Carmona et al., 1998), inhibit carrageenan-induced paw edema in mice, suggesting that cannabinoid CB_2 receptor and endogenous ligands are involved in edema induction (Iwamura et al., 2001).

Arachidonic acid derivatives, 2-arachidonoylglycerol (2-AG) and anandamide have been proposed as endogenous ligands for cannabinoid CB_1 and CB_2 receptor (Devane et al., 1992; Mechoulam et al., 1995; Sugiura et al., 1995). 2-AG is more likely the physiological ligand for cannabinoid CB_2 receptor, as it acts as a full agonist at cannabinoid receptors (Gonsiorek et al., 2000; Hillard, 2000; Sugiura et al., 1999, 2000) and levels of 2-AG in various mammalian

* Corresponding author. Tel.: +81 72 681 9700; fax: +81 72 681 9783.
E-mail address: yoshifumi.ueda@ims.jti.co.jp (Y. Ueda).
[1] Tel.: +81 72 681 9700; fax: +81 72 681 9783.

0014-2999/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2005.08.013

tissues are markedly higher than anandamide (Bisogno et al., 1997; Di Marzo et al., 1999; Sugiura et al., 1998; Varga et al., 1998). Much evidence suggests a physiological role for 2-AG, as it induces migration of inflammatory and leukemia cells through a cannabinoid CB_2 receptor-dependent mechanism (Jorda et al., 2002; Kishimoto et al., 2003; Oka et al., 2004). It is therefore possible that 2-AG could be a physiological inflammatory mediator in certain circumstances. These observations led this group to investigate whether topical exposure of cannabinoid CB_2 receptor agonist on mouse ear could induce cutaneous inflammation and whether the inflammation could be influenced by cannabinoid CB_2 receptor antagonist/inverse agonists. Effect of cannabinoid CB_2 receptor antagonist/inverse agonists on allergic dermatitis caused by repeated topical treatment of dinitrofluorobenzene (DNFB) was investigated. As 2-AG is known to be rapidly metabolized (Bisogno et al., 1997; Di Marzo et al., 1998), 2-AG-E, a metabolically stable ether analogue of 2-AG with agonistic activity for cannabinoid CB_2 receptor (Sugiura et al., 2000), was used.

This report shows that 2-AG-E and synthetic cannabinoid CB_2 receptor agonist, HU-308 (Hanus et al., 1999), induce ear swelling characterized by a persistent effect for 8 days after single exposure to ear and that swelling is reduced by cannabinoid CB_2 receptor antagonist/inverse agonists, JTE-907 and SR144528. Cannabinoid CB_2 receptor antagonist/inverse agonists were also demonstrated to have an inhibitory effect on ear swelling induced by repeated exposure to DNFB in mice.

2. Materials and methods

2.1. Animals

Female BALB/c mice (obtained from Japan SLC Inc., Hamamatsu, Japan) were housed in plastic cages in a room illuminated for a 12 h cycle (8:00–20:00), with controlled temperature (25±3 °C) and humidity (55±15%). Animals were fed a standard laboratory diet and given water ad libitum. All procedures related to the use of animals in this study were reviewed and approved by the Institutional Animal Care and Use Committee at Japan Tobacco Inc.

2.2. Reagents

JTE-907 [*N*-(benzo[1,3]dioxol-5-ylmethyl)-7-methoxy-2-oxo-8-pentyloxy-1,2-dihydroquinoline-3-carboxamide], SR144528 {*N*-[(1*S*)-endo-1,3,3-trimethylbicyclo[2.2.1]heptan-2yl]5-(4-chloro-3-methyl-phenyl)-1-(4-methylbenzyl) pyrazole-3-carboxamide}}, HU-308 {4-[4-(1,1-dimethylheptyl)-2,6-dimethoxy-phenyl]-6.6-dimethyl-bicyclo[3.1.1] hept-2-en-2-yl}-methanol, and 2-AG-E were synthesized at Japan Tobacco Inc. (Osaka, Japan). FK506 was extracted from Prograf® (Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan). Prednisolone, indomethacin, arachidonic acid, and acetone were obtained from Sigma-Aldrich (St. Louis, MO, USA). DNFB was purchased from Nacalai Tesque Inc. (Kyoto, Japan) and dissolved in acetone : olive oil (3 : 1). Arachidonic acid, 2-AG-E, and HU-308 were dissolved in acetone. JTE-907, SR144528, indomethacin, prednisolone, and FK506 were suspended in 0.5% methyl cellulose, then orally administered at a volume of 10 ml/kg.

2.3. Cannabinoid agonist- and arachidonic acid-induced mouse ear swelling

Each side of the left ears of mice was topically exposed to vehicle (acetone), 2-AG-E, HU-308, and arachidonic acid (10 μl/site). Vehicle (0.5% methyl cellulose), JTE-907, SR144528, indomethacin, and prednisolone were orally administered 1 h prior to topical exposure to 2-AG-E or arachidonic acid. Time course change of ear thickness after exposure was measured using a thickness gauge (Mitutoyo Corporation, Kawasaki, Japan) in a blind-manner and expressed as the increase in ear thickness relative to the value measured immediately before exposure to 2-AG-E or arachidonic acid.

2.4. DNFB-induced allergic dermatitis in mice

Experiments were carried out by a method previously described (Nagai et al., 1997a,b, 2000). Each side of both ears of mice had 0.15% DNFB or vehicle applied at a volume of 25 μl/site, once a week for 4 or 5 weeks. JTE-907, SR144528, prednisolone, and FK506 were orally administered once a day for 6 days from 1 day after the 3rd exposure to DNFB, plus 1 h before and 23 h after the 4th exposure to DNFB. Ear thickness was measured using a thickness gauge 24 h after the 4th exposure to DNFB in a blind-manner and expressed as the increase in ear thickness relative to the value measured immediately before the 4th DNFB application.

2.5. Expression of cannabinoid CB_2 receptor mRNA in DNFB-sensitized mice

Change in cannabinoid CB_2 receptor mRNA level in cervical lymph node and ear was assessed by quantitative reverse transcriptase-polymerase chain reaction (RT-PCR). Total RNA was isolated from the ears and lymph nodes of mice 4 h after the 5th exposure to DNFB using QIAshredder (Qiagen, Hilden, Germany) and RNeasy Mini kits (Qiagen) according to the manufacturer's instructions. Reverse transcription of total RNA was carried out in the reaction mixture (consisting of 10 U/μl moloney murine leukemia virus reverse transcriptase, 10 mM dithiothreitol, 0.5 mM dNTP mix, 10 pg/μl oligo(dT) primer and first strand buffer) to obtain cDNA. Sample was incubated at 37 °C for 1 h, then 95 °C for 5 min. Relative quantification was performed on an ABI PRISM 7700 Sequence Detector (Perkin-Elmer Applied

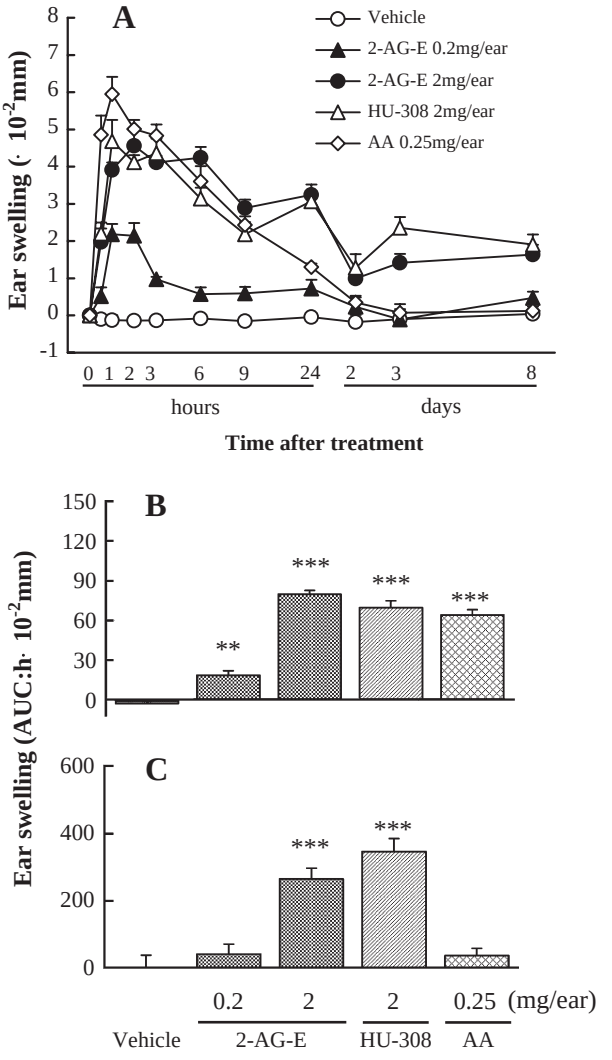

Fig. 1. Increase in ear thickness over time caused by cannabinoid ligands and arachidonic acid in mice (A). 2-AG-E, HU-308, arachidonic acid (AA) or vehicle was applied to each side of the left ear (10 μl). Results are presented as the increase in ear thickness relative to the value measured immediately before treatment. Early response or late response is shown as the area under the curve (AUC): 0–24 h and 1–8 days, respectively (B, C). Data are expressed as mean±S.E.M. (n=5–6). **, P<0.01, ***, P<0.001 vs. vehicle (Dunnett's test following one-way ANOVA [$F(4,24)=84.73$, P<0.001 (B): $F(4,24)=22.51$, P<0.001 (C)]).

Biosystems Inc., CA, USA). Oligonucleotide primer and TaqMan probes were designed using Primer Express version 1.5 software (Applied Biosystems Inc.): cannabinoid CB_2 receptor forward primer 5′-TTTCTTACCTGCCGCTCATG-3′, reverse primer 5′-CCAGCCCAGTAGGTAGTCGTTA-3′, probe 5′-(FAM)AGTCCCTGCTCTGAGCTTTTCC-CACTGATC(TAMRA)-3; glyceraldehyde-3-phosphate dehydrogenase (GAPDH) forward primer 5′-ACATGTTC-CAGTATGACTCCACTCAC-3′, reverse primer 5′-TCTCGCTCCTGGAAGATGGT-3′, probe 5′-(Tat)AACG-GCACAGTCAAGGCCGAGAAT(TAMRA)-3. GAPDH was selected as endogenous control. For amplification of cannabinoid CB_2 receptor and GAPDH, cDNA added to the PCR mixture (0.025 U/μl AmpliTaq Gold, 5.5 mM $MgCl_2$, 0.2 mM dNTP, 300 nM each primer, 100 nM probe, and TaqMan buffer A) in a final volume of 50 μl was used with the following thermal cycling conditions: 2 min/50 °C and 10 min/95 °C followed by 50 cycles of 2-step PCR (15 s/95 °C and 1 min/60 °C). Results were expressed as the relative amount of cannabinoid CB_2 receptor normalized by GAPDH.

2.6. Statistics

Data are represented as mean±S.E.M. The significance of differences between normal control and vehicle-treated ear edema groups was determined by Student's t-test. Data comparisons between the vehicle-treated and drug-treated groups were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's test. Probability of P<0.05 was considered to be significant.

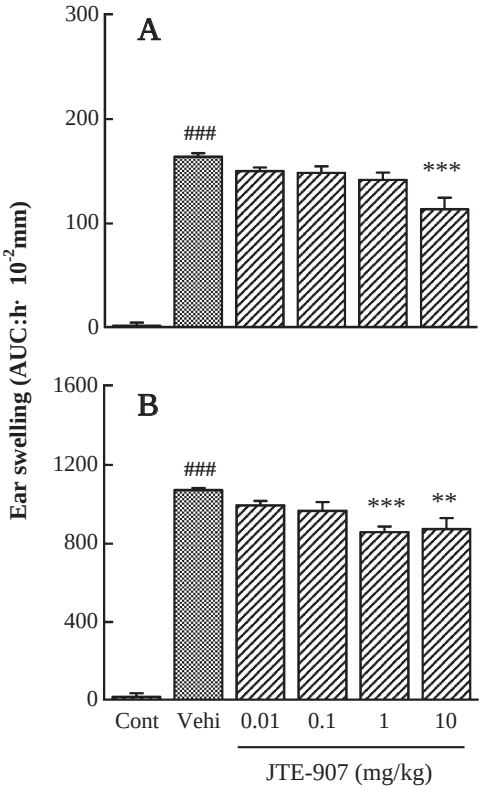

Fig. 2. Effect of cannabinoid CB_2 receptor inverse agonist, JTE-907, on 2-AG-E-induced early (A) and late (B) ear swelling response. 2-AG-E (2 mg/ear) was topically exposed to each side of the left ear of mice. Vehicle or JTE-907 was orally administered 1 h prior to 2-AG-E treatment. Ear thickness was measured using a thickness gauge in a blind-manner. Time points for measurements were pre- and 1, 2, 3, 5, 7, and 24 h, plus 2, 5, and 8 days after 2-AG-E treatment. Data are expressed as mean±S.E.M. of AUC: 0–24 h (early) and 1–8 days (late) (n=8). ###, P<0.001 vs. control (Student's t-test). **, P<0.01, ***, P<0.001 vs. vehicle (Dunnett's test following one-way ANOVA [$F(4,35)=7.35$, P<0.001 (A): $F(4,35)=5.89$, P<0.001 (B)]). Cont: control, Vehi: vehicle.

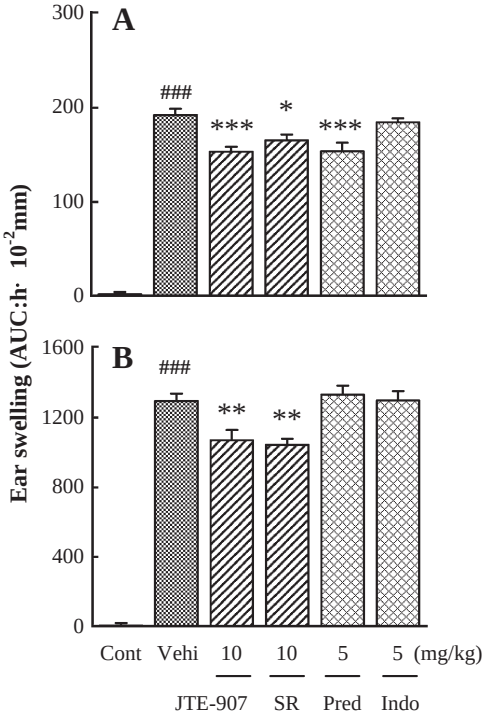

Fig. 3. Effect of JTE-907, SR144528 (SR), prednisolone (Pred), or indomethacin (Indo) on 2-AG-E-induced early (A) and late (B) ear swelling response. 2-AG-E (2 mg/ear) was topically exposed to each side of the left ear of mice. Vehicle, JTE-907, SR144528 (SR), prednisolone (Pred), or indomethacin (Indo) was orally administered 1 h prior to 2-AG-E treatment. Ear thickness was measured using a thickness gauge in a blind-manner. Time points for measurements were pre- and 1, 2, 3, 5, 7, and 24 h, plus 3, 6, and 8 days after 2-AG-E treatment. Data are expressed as mean±S.E.M. of AUC: 0–24 h (early) and 1–8 days (late) (n=8). ###, P<0.001 vs. control (Student's t-test). *, P<0.05, **, P<0.01, ***, P<0.001 vs. vehicle (Dunnett's test following one-way ANOVA [$F(4,35)=7.83$, P<0.001 (A): $F(4,35)=8.12$, P<0.001 (B)]). Cont: control, Vehi: vehicle.

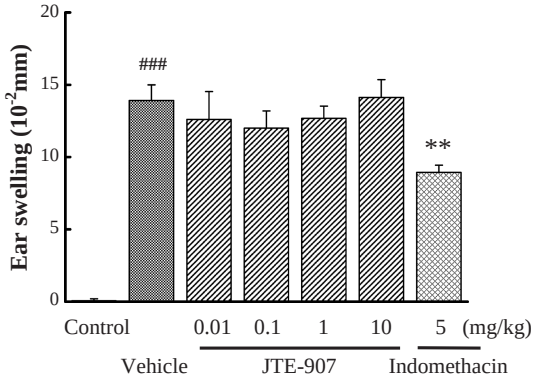

Fig. 4. Effect of JTE-907 on arachidonic acid-induced ear swelling in mice. Ear swelling was induced by topical exposure to arachidonic acid (0.25 mg/ear) on each side of the left ear. Ear swelling was measured 1 h after treatment. Vehicle, JTE-907 or indomethacin was orally administered 1 h prior to arachidonic acid exposure. Data are expressed as mean±S.E.M. (n=7). ###, P<0.001 vs. control (Student's t-test). **, P<0.01 vs. vehicle (Dunnett's test following one-way ANOVA [$F(5,36)=4.54$, P<0.01]).

3. Results

3.1. Characterization of cannabinoid CB_2 receptor-mediated ear swelling in mice

Topical exposure of cannabinoid CB_2 receptor agonists to mouse ear was compared to arachidonic acid. Mice ear exposed to arachidonic acid, 2-AG-E, and HU-308 evoked immediate ear swelling (early response) (Fig. 1A). Early responses to 2-AG-E and HU-308 (2 mg/ear) were comparable to that induced by arachidonic acid (0.25 mg/ear). Although the arachidonic acid-induced response was almost absent 2 days after treatment, 2-AG-E- and HU-308-induced ear swelling persisted for at least 8 days after treatment (late response). These early and late responses

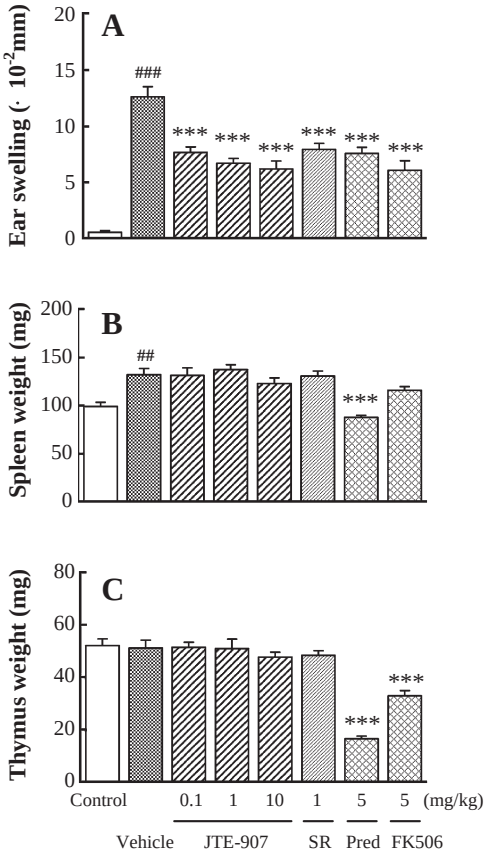

Fig. 5. Effects of JTE-907, SR144528, prednisolone, and FK506 on ear swelling (A), weight of spleen (B), or thymus (C) in mouse DNFB-induced allergic dermatitis model. Each mouse received topical exposure of 0.15% DNFB in acetone/olive oil or vehicle once a week for 4 weeks. Vehicle, JTE-907, SR144528 (SR), prednisolone (Pred), or FK506 was orally administered once a day for 6 days before, and 1 and 23 h after the 4th exposure to DNFB. Ear thickness and weight of spleen and thymus were assessed 24 h after the 4th DNFB exposure. Results are presented as the increase in ear thickness relative to the value measured immediately before the 4th DNFB exposure. Data are expressed as mean±S.E.M. (n=8). ##, P<0.01, ###, P<0.001 vs. control (Student's t-test). ***, P<0.001 vs. vehicle (Dunnett's test following one-way ANOVA [$F(6,49)=11.78$, P<0.001 (A): $F(6,49)=9.41$, P<0.001 (B): $F(6,49)=33.04$, P<0.001 (C)]).

were expressed as the area under the curve (AUC): 0–24 h and 1–8 days, respectively (Fig. 1B, C). Both early and late responses induced by 2-AG-E increased dose-dependently. No significant increase in ear thickness was observed in vehicle (acetone)-treated mice.

3.2. Effect of cannabinoid CB_2 receptor antagonist/inverse agonists on ear swelling induced by 2-AG-E or arachidonic acid

In order to assess whether cannabinoid CB_2 receptor is involved in the early and late responses induced by 2-AG-E, the effect of cannabinoid CB_2 receptor antagonist/inverse agonists, JTE-907 and SR144528, on 2-AG-E-induced ear swelling was examined. Orally administered JTE-907 and SR144528 alone did not affect ear thickness (data not shown); however, pretreatment of either compound partially but significantly suppressed both early and late responses induced by 2-AG-E (Figs. 2 and 3). In contrast, prednisolone showed a significant reduction in early response, but not in late response. Indomethacin did not show a significant effect on either early or late responses. To further characterize the difference between 2-AG-E- and arachidonic acid-mediated ear response, effect of JTE-907 on ear swelling caused by topical exposure to arachidonic acid in mice was examined. Indomethacin, as a positive control, significantly inhibited arachidonic acid-induced ear swelling, whereas JTE-907 did not show any change at doses up to 10 mg/kg (Fig. 4).

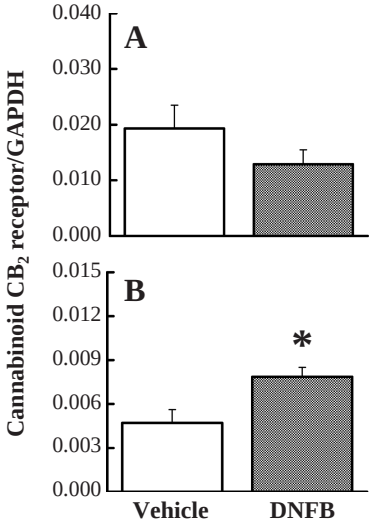

Fig. 6. Expression of cannabinoid CB_2 receptor mRNA in lymph nodes (A) and ear (B) in mouse DNFB-induced allergic dermatitis model. Expression of cannabinoid CB_2 receptor mRNA was examined 4 h after the 5th DNFB exposure. Quantitative RT-PCR analysis of cannabinoid CB_2 receptor transcript level was performed as described under Materials and methods. Results are presented normalized to the amount of GAPDH. Data are expressed as mean±S.E.M. ($n=8$). *, $P<0.05$ vs. vehicle (Student's t-test).

3.3. Effect of cannabinoid CB_2 receptor antagonist/inverse agonists on DNFB-induced allergic dermatitis in mice

To determine whether cannabinoid CB_2 receptor antagonist/inverse agonists could produce a suppressive effect on allergic dermatitis in mice, administration of JTE-907 and SR144528 was studied in a DNFB-induced allergic dermatitis model, plus compared to prednisolone and FK506. Repeated topical application of DNFB on mouse ear provokes ear swelling from 24 h after the 2nd application, and ear thickness increases in proportion to the number of exposures to DNFB (Nagai et al., 1997a,b). Orally administered JTE-907 (0.1–10 mg/kg) once a day for 6 days before, and 1 and 23 h after the 4th exposure to DNFB showed significant and dose-dependent inhibition of ear swelling at 24 h after 4th DNFB application (Fig. 5). SR144528 also showed significant suppression at 1 mg/kg. Although orally administered prednisolone and FK506 significantly suppressed ear swelling, thymus and/or spleen weight was significantly decreased. JTE-907 and SR144528 did not show any significant effect on thymus and spleen weight.

3.4. Expression of cannabinoid CB_2 receptor mRNA in lymph nodes and ear of DNFB-induced allergic dermatitis mice

Finally, to assess whether cannabinoid CB_2 receptor is expressed in lymph nodes and ear of DNFB-induced dermatitis mice, cannabinoid CB_2 receptor mRNA was measured by RT-PCR. Expression of cannabinoid CB_2 receptor mRNA was detected in both lymph nodes and ears of mice receiving application of vehicle (non-DNFB), but significantly up-regulated in mice receiving DNFB. There were no significant changes in lymph node cannabinoid CB_2 receptor mRNA expression (Fig. 6).

4. Discussion

This study demonstrated that single and direct exposure of cannabinoid CB_2 receptor agonists, 2-AG-E (Sugiura et al., 2000) and HU-308 (Hanus et al., 1999), to mice ear induced immediate and persistent cutaneous swelling. 2-AG is rapidly metabolized to arachidonic acid, which further produces active metabolites such as prostaglandins (Bisogno et al., 1997; Di Marzo et al., 1998). To avoid the complicated metabolism of 2-AG, we chose to use metabolically stable 2-AG-E. 2-AG-E-induced ear swelling was concentration-dependent. 2-AG-E (2 mg/ear) and HU-308 (2 mg/ear) induced a similar degree of early response compared to that induced by arachidonic acid (0.25 mg/ear); however, long lasting swelling (late response) was observed with 2-AG-E and HU-308, but not arachidonic acid application. 2-AG-E- and HU-308-induced swelling persisted even 8 days after single application, whereas arachidonic acid-induced swelling was almost absent 2 days after application. Although a

higher amount of arachidonic acid was not used, previous studies indicate that the swelling does not persist longer than 48 h, even with an amount greater than 0.25 mg/ear (Young et al., 1984). The 2-AG-E-induced ear swelling could include mechanisms other than cannabinoid CB_2 receptor, such as chemical irritation, because the effect of both JTE-907 and SR144528 was partial. However, the results suggest that cannabinoid CB_2 receptor is at least partially involved in early and late responses. These results also suggest that the late response was not due to arachidonic acid release, as JTE-907 did not show any effect on arachidonic acid-induced swelling.

Interestingly, the early response induced by 2-AG-E as well as 2-AG is also reported in a recent publication that shows topical treatment of 2-AG-E- and 2-AG-induced ear edema reaching a peak 1 h after the treatment (Oka et al., 2005). Although late response was not measured, the study suggested that the early response is cannabinoid CB_2 receptor-mediated through a nitric oxide releasing mechanism. The late response might be from direct action on cell migration through cannabinoid CB_2 receptor. It has been reported that 2-AG induces migration of macrophage-like cells, such as differentiated HL-60, U937, and THP-1 cells, and human peripheral blood-derived monocytes (Kishimoto et al., 2003). Migration of differentiated HL-60 cells was induced by 2-AG-E as well as 2-AG, with 2-AG-induced migration assumed to mainly involve chemotaxis rather than chemokinesis. Migration was inhibited by pretreatment with SR144528 suggesting that it is mediated through cannabinoid CB_2 receptor (Kishimoto et al., 2003). 2-AG also evoked migration of mouse splenocytes and was blocked by SR144528 (Jorda et al., 2002). These findings suggest that the early and late responses caused by topical exposure of 2-AG-E involve cannabinoid CB_2 receptor-mediated nitric oxide release and cell migration into tissue, respectively, the latter of which could be demonstrated by a histopathological approach.

Anti-inflammatory effects of cannabinoid agonists include suppression of cytokines and cell proliferation by 2-AG in vitro (Chang et al., 2001; Gallily et al., 2000; Lee et al., 1995), plus anti-edema effects from cannabinoid CB_2 receptor agonists, HU-308, GW405833, and AM1241, in vivo (Clayton et al., 2002; Hanus et al., 1999; Nackley et al., 2003, 2004; Quartilho et al., 2003). However most studies have examined anti-edema effects of systemic treatment, but not always topical treatment, with cannabinoid CB_2 receptor agonists; all topical effects of these agonists have not been shown. This discrepancy is likely to be due to target tissue or mediator differences between the treatments.

DNFB-induced allergic dermatitis model (Nagai et al., 1997a,b, 2000) is characterized by ear swelling with increased eosinophil migration into tissue. This study demonstrated that cannabinoid CB_2 receptor antagonist/inverse agonists, JTE-907 and SR144528, suppressed ear swelling in DNFB-induced allergic dermatitis, and cannabinoid CB_2 receptor mRNA expression was increased in inflamed ear. 2-AG induces migration of eosinophilic leukemia cells as well as macrophages through cannabinoid CB_2 receptor (Oka et al., 2004) and production of 2-AG increases in inflamed ear (Oka et al., 2005). Therefore, it is possible that 2-AG production in inflamed tissue induces eosinophil migration through increased cannabinoid CB_2 receptors resulting in pathological changes, such as swelling. The mechanism of anti-edema effects of cannabinoid CB_2 receptor antagonist/inverse agonists may relate to the action of antagonists against endogenous cannabinoid ligands, such as 2-AG, in inducing migration.

Prednisolone and FK506 are topically administered as therapy for allergic dermatitis, such as atopic dermatitis. Both compounds have already been demonstrated to have inhibitory effects on ear swelling induced by repeated DNFB treatment in mice (Nagai et al., 1997a; Ueda et al., 2003). It is important to note that the effect of JTE-907 and SR144528 on ear swelling was comparable to that of prednisolone and FK506. Although JTE-907, SR144528, prednisolone, and FK506 suppressed ear swelling, thymus and/or spleen weight was decreased by systemically administered prednisolone and FK506, reflecting immunosuppression. In contrast, JTE-907 and SR144528 did not affect thymus or spleen weight. These findings suggest that systemically administered cannabinoid CB_2 receptor antagonist/inverse agonist can exert an anti-allergic action like prednisolone and FK506 without causing systemic immunosuppression.

In conclusion, the present study demonstrated that topical exposure of cannabinoid CB_2 receptor agonist can induce cutaneous swelling in mice, in part via a cannabinoid CB_2 receptor dependent mechanism, and that cannabinoid CB_2 receptor antagonist/inverse agonists, JTE-907 and SR144528, inhibit allergic dermatitis caused by repeated treatment with DNFB in mice. These results suggest that cannabinoid CB_2 receptor antagonist/inverse agonists like JTE-907 and SR144528 are potentially useful for the clinical treatment of allergic dermatitis.

Acknowledgements

We would like to thank T. Kato for excellent technical assistance.

References

Bisogno, T., Sepe, N., Melck, D., Maurelli, S., De Petrocellis, L., Di Marzo, V., 1997. Biosynthesis, release and degradation of the novel endogenous cannabimimetic metabolite 2-arachidonoylglycerol in mouse neuroblastoma cells. Biochem. J. 322 (Pt 2), 671–677.

Chang, Y.H., Lee, S.T., Lin, W.W., 2001. Effects of cannabinoids on LPS-stimulated inflammatory mediator release from macrophages: involvement of eicosanoids. J. Cell. Biochem. 81, 715–723.

Clayton, N., Marshall, F.H., Bountra, C., O'Shaughnessy, C.T., 2002. CB1 and CB2 cannabinoid receptors are implicated in inflammatory pain. Pain 96, 253–260.

Devane, W.A., Hanus, L., Breuer, A., Pertwee, R.G., Stevenson, L.A., Griffin, G., Gibson, D., Mandelbaum, A., Etinger, A., Mechoulam, R., 1992. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 258, 1946–1949.

Di Marzo, V., Bisogno, T., Sugiura, T., Melck, D., De Petrocellis, L., 1998. The novel endogenous cannabinoid 2-arachidonoylglycerol is inactivated by neuronal- and basophil-like cells: connections with anandamide. Biochem. J. 331 (Pt 1), 15–19.

Di Marzo, V., Bisogno, T., De Petrocellis, L., Melck, D., Orlando, P., Wagner, J.A., Kunos, G., 1999. Biosynthesis and inactivation of the endocannabinoid 2-arachidonoylglycerol in circulating and tumoral macrophages. Eur. J. Biochem. 264, 258–267.

Facci, L., Dal Toso, R., Romanello, S., Buriani, A., Skaper, S.D., Leon, A., 1995. Mast cells express a peripheral cannabinoid receptor with differential sensitivity to anandamide and palmitoylethanolamide. Proc. Natl. Acad. Sci. U. S. A. 92, 3376–3380.

Gallily, R., Breuer, A., Mechoulam, R., 2000. 2-Arachidonylglycerol, an endogenous cannabinoid, inhibits tumor necrosis factor-alpha production in murine macrophages, and in mice. Eur. J. Pharmacol. 406, R5–R7.

Gonsiorek, W., Lunn, C., Fan, X., Narula, S., Lundell, D., Hipkin, R.W., 2000. Endocannabinoid 2-arachidonyl glycerol is a full agonist through human type 2 cannabinoid receptor: antagonism by anandamide. Mol. Pharmacol. 57, 1045–1050.

Hanus, L., Breuer, A., Tchilibon, S., Shiloah, S., Goldenberg, D., Horowitz, M., Pertwee, R.G., Ross, R.A., Mechoulam, R., Fride, E., 1999. HU-308: a specific agonist for CB(2), a peripheral cannabinoid receptor. Proc. Natl. Acad. Sci. U. S. A. 96, 14228–14233.

Hillard, C.J., 2000. Biochemistry and pharmacology of the endocannabinoids arachidonylethanolamide and 2-arachidonylglycerol. Prostaglandins Other Lipid Mediat. 61, 3–18.

Iwamura, H., Suzuki, H., Ueda, Y., Kaya, T., Inaba, T., 2001. In vitro and in vivo pharmacological characterization of JTE-907, a novel selective ligand for cannabinoid CB2 receptor. J. Pharmacol. Exp. Ther. 296, 420–425.

Jorda, M.A., Verbakel, S.E., Valk, P.J., Vankan-Berkhoudt, Y.V., Maccarrone, M., Finazzi-Agro, A., Lowenberg, B., Delwel, R., 2002. Hematopoietic cells expressing the peripheral cannabinoid receptor migrate in response to the endocannabinoid 2-arachidonoylglycerol. Blood 99, 2786–2793.

Kishimoto, S., Gokoh, M., Oka, S., Muramatsu, M., Kajiwara, T., Waku, K., Sugiura, T., 2003. 2-arachidonoylglycerol induces the migration of HL-60 cells differentiated into macrophage-like cells and human peripheral blood monocytes through the cannabinoid CB2 receptor-dependent mechanism. J. Biol. Chem. 278, 24469–24475.

Lee, M., Yang, K.H., Kaminski, N.E., 1995. Effects of putative cannabinoid receptor ligands, anandamide and 2-arachidonyl-glycerol, on immune function in B6C3F1 mouse splenocytes. J. Pharmacol. Exp. Ther. 275, 529–536.

Matsuda, L.A., Lolait, S.J., Brownstein, M.J., Young, A.C., Bonner, T.I., 1990. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346, 561–564.

Mechoulam, R., Ben-Shabat, S., Hanus, L., Ligumsky, M., Kaminski, N.E., Schatz, A.R., Gopher, A., Almog, S., Martin, B.R., Compton, D.R., Pertwee, R.G., Griffin, G., Bayewitch, M., Barg, J., Vogel, Z., 1995. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. Biochem. Pharmacol. 50, 83–90.

Munro, S., Thomas, K.L., Abu-Shaar, M., 1993. Molecular characterization of a peripheral receptor for cannabinoids. Nature 365, 61–65.

Nackley, A.G., Makriyannis, A., Hohmann, A.G., 2003. Selective activation of cannabinoid CB(2) receptors suppresses spinal fos protein expression and pain behavior in a rat model of inflammation. Neuroscience 119, 747–757.

Nackley, A.G., Zvonok, A.M., Makriyannis, A., Hohmann, A.G., 2004. Activation of cannabinoid CB2 receptors suppresses C-fiber responses and windup in spinal wide dynamic range neurons in the absence and presence of inflammation. J. Neurophysiol. 92, 3562–3574.

Nagai, H., Hiyama, H., Matsuo, A., Ueda, Y., Inagaki, N., Kawada, K., 1997a. FK-506 and cyclosporin A potentiate the IgE antibody production by contact sensitization with hapten in mice. J. Pharmacol. Exp. Ther. 283, 321–327.

Nagai, H., Matsuo, A., Hiyama, H., Inagaki, N., Kawada, K., 1997b. Immunoglobulin E production in mice by means of contact sensitization with a simple chemical, hapten. J. Allergy Clin. Immunol. 100, S39–S44.

Nagai, H., Ueda, Y., Ochi, T., Hirano, Y., Tanaka, H., Inagaki, N., Kawada, K., 2000. Different role of IL-4 in the onset of hapten-induced contact hypersensitivity in BALB/c and C57BL/6 mice. Br. J. Pharmacol. 129, 299–306.

Oka, S., Ikeda, S., Kishimoto, S., Gokoh, M., Yanagimoto, S., Waku, K., Sugiura, T., 2004. 2-arachidonoylglycerol, an endogenous cannabinoid receptor ligand, induces the migration of EoL-1 human eosinophilic leukemia cells and human peripheral blood eosinophils. J. Leukoc. Biol. 76, 1002–1009.

Oka, S., Yanagimoto, S., Ikeda, S., Gokoh, M., Kishimoto, S., Waku, K., Ishima, Y., Sugiura, T., 2005. Evidence for the involvement of the cannabinoid CB2 receptor and its endogenous ligand 2-arachidonoylglycerol in 12-*O*-tetradecanoylphorbol-13-acetate-induced acute inflammation in mouse ear. J. Biol. Chem. 280, 18488–18497.

Pertwee, R.G., 2000. Cannabinoid receptor ligands: clinical and neuropharmacological considerations, relevant to future drug discovery and development. Expert Opin. Investig. Drugs 9, 1553–1571.

Pettit, D.A., Anders, D.L., Harrison, M.P., Cabral, G.A., 1996. Cannabinoid receptor expression in immune cells. Adv. Exp. Med. Biol. 402, 119–129.

Piomelli, D., Giuffrida, A., Calignano, A., Rodriguez de Fonseca, F., 2000. The endocannabinoid system as a target for therapeutic drugs. Trends Pharmacol. Sci. 21, 218–224.

Porter, A.C., Felder, C.C., 2001. The endocannabinoid nervous system: unique opportunities for therapeutic intervention. Pharmacol. Ther. 90, 45–60.

Quartilho, A., Mata, H.P., Ibrahim, M.M., Vanderah, T.W., Porreca, F., Makriyannis, A., Malan Jr., T.P., 2003. Inhibition of inflammatory hyperalgesia by activation of peripheral CB2 cannabinoid receptors. Anesthesiology 99, 955–960.

Rinaldi-Carmona, M., Barth, F., Millan, J., Derocq, J.M., Casellas, P., Congy, C., Oustric, D., Sarran, M., Bouaboula, M., Calandra, B., Portier, M., Shire, D., Breliere, J.C., Le Fur, G.L., 1998. SR 144528, the first potent and selective antagonist of the CB2 cannabinoid receptor. J. Pharmacol. Exp. Ther. 284, 644–650.

Schatz, A.R., Lee, M., Condie, R.B., Pulaski, J.T., Kaminski, N.E., 1997. Cannabinoid receptors CB1 and CB2: a characterization of expression and adenylate cyclase modulation within the immune system. Toxicol. Appl. Pharmacol. 142, 278–287.

Sugiura, T., Kondo, S., Sukagawa, A., Nakane, S., Shinoda, A., Itoh, K., Yamashita, A., Waku, K., 1995. 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. Biochem. Biophys. Res. Commun. 215, 89–97.

Sugiura, T., Kodaka, T., Nakane, S., Kishimoto, S., Kondo, S., Waku, K., 1998. Detection of an endogenous cannabimimetic molecule, 2-arachidonoylglycerol, and cannabinoid CB1 receptor mRNA in human vascular cells: is 2-arachidonoylglycerol a possible vasomodulator? Biochem. Biophys. Res. Commun. 243, 838–843.

Sugiura, T., Kodaka, T., Nakane, S., Miyashita, T., Kondo, S., Suhara, Y., Takayama, H., Waku, K., Seki, C., Baba, N., Ishima, Y., 1999. Evidence that the cannabinoid CB1 receptor is a 2-arachidonoylglycerol receptor. Structure–activity relationship of 2-arachidonoylglycerol, ether-linked analogues, and related compounds. J. Biol. Chem. 274, 2794–2801.

Sugiura, T., Kondo, S., Kishimoto, S., Miyashita, T., Nakane, S., Kodaka, T., Suhara, Y., Takayama, H., Waku, K., 2000. Evidence that 2-arachidonoylglycerol but not *N*-palmitoylethanolamine or anandamide is the physiological ligand for the cannabinoid CB2 receptor. Comparison of the agonistic activities of various cannabinoid receptor ligands in HL-60 cells. J. Biol. Chem. 275, 605–612.

Ueda, Y., Sone, T., Inagaki, N., Nagai, H., 2003. Effects of prednisolone on the cutaneous reaction and skin barrier function in mice treated with a hapten. Biol. Pharm. Bull. 26, 618–621.

Varga, K., Wagner, J.A., Bridgen, D.T., Kunos, G., 1998. Platelet- and macrophage-derived endogenous cannabinoids are involved in endotoxin-induced hypotension. FASEB J. 12, 1035–1044.

Young, J.M., Spires, D.A., Bedord, C.J., Wagner, B., Ballaron, S.J., De Young, L.M., 1984. The mouse ear inflammatory response to topical arachidonic acid. J. Invest. Dermatol. 82, 367–371.