Transdermal cannabidiol reduces inflammation and pain-related behaviours in a rat model of arthritis

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Abstract

Background—Current arthritis treatments often have side-effects attributable to active compounds as well as route of administration. Cannabidiol (CBD) attenuates inflammation and pain without side-effects, but CBD is hydrophobic and has poor oral bioavailability. Topical drug application avoids gastrointestinal administration, first pass metabolism, providing more constant plasma levels.

Methods—This study examined efficacy of transdermal CBD for reduction in inflammation and pain, assessing any adverse effects in a rat complete Freund’s adjuvant-induced monoarthritis knee joint model. CBD gels (0.6, 3.1, 6.2 or 62.3 mg/day) were applied for 4 consecutive days after arthritis induction. Joint circumference and immune cell invasion in histological sections were measured to indicate level of inflammation. Paw withdrawal latency (PWL) in response to noxious heat stimulation determined nociceptive sensitization, and exploratory behaviour ascertained animal’s activity level.

Results—Measurement of plasma CBD concentration provided by transdermal absorption revealed linearity with 0.6–6.2 mg/day doses. Transdermal CBD gel significantly reduced joint swelling, limb posture scores as a rating of spontaneous pain, immune cell infiltration and thickening of the synovial membrane in a dose-dependent manner. PWL recovered to near baseline level. Immunohistochemical analysis of spinal cord (CGRP, OX42) and dorsal root ganglia (TNFα) revealed dose-dependent reductions of pro-inflammatory biomarkers. Results

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Co-first authors.

Conflicts of interest
None declared.

Author contributions
D.C.H. prepared and applied the treatments, tested blood drug concentrations, performed data analysis and wrote portions of the first draft of this manuscript. L.Z. performed the drug application, behavioural tests, data analysis, statistics and produced the figures, as well as drafted portions of and edited this manuscript. F.M. performed the blinded behavioural tests, read and edited this manuscript. S.M.A. performed the blinded behavioural tests and read this manuscript. S.L.M. performed the immunostaining, data analysis, produced the figures, as well as read and edited this manuscript. A.L.S. designed the study, supplied the drug, read and edited this manuscript. K.N.W. designed the study, read and edited this manuscript. All authors discussed the results and commented on the -manuscript.
showed 6.2 and 62 mg/day were effective doses. Exploratory behaviour was not altered by CBD indicating limited effect on higher brain function.

**Conclusions**—These data indicate that topical CBD application has therapeutic potential for relief of arthritis pain-related behaviours and inflammation without evident side-effects.

1. Introduction

Almost 50 million (22.2%) adult Americans over 18 were diagnosed with arthritis in 2007–2009, most prominently osteoarthritis and the autoimmune disease rheumatoid arthritis. A projected increase to 67 million is anticipated by 2030 (Centers for Disease Control and Prevention (CDC), 2010). The most effective treatment for rheumatoid arthritis is injectable fusion-proteins which sequester the most prominent proinflammatory cytokine tumour necrosis factor α (TNFα). These chimeric antibodies may halt progression of the disease, but side-effects include immune suppression (Crawford and Curtis, 2008; Furst, 2010; Hastings et al., 2010). Neurogenic drive also contributes to severity of arthritic inflammation (Sluka et al., 1994), and may contribute to its reoccurrence.

Cannabinoids and cannabinoid receptors are potential targets for reducing pain and inflammation (Clayton et al., 2002; Richardson et al., 2008; Zuardi, 2008). Cannabis sativa contains approximately 80 different cannabinoids of which Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD) are primary (Mechoulam and Shvo, 1963; Mechoulam, 1970; Turner et al., 1980). These compounds are chemically similar to endogenous endocannabinoid lipid derivatives including anandamide (arachidonylethanolamide) and 2-arachidonoylglycerol. At present THC and CBD are available combined in the oral spray Sativex® (GW Pharmaceuticals plc, Cambridge, UK) which is prescribed to adult patients for neuropathic pain. Psychoactive THC side-effects are experienced, however, and long-term use of cannabis sativa has been shown to increase risk of developing psychosis and schizophrenia (Berman et al., 2004; Malone et al., 2010). Research is ongoing to find better, effective cannabinoids and better routes of application.

CBD, while structurally similar to THC, is a non-psychoactive cannabinoid with therapeutic potential for treatment of neuropathic pain, cancer pain, multiple sclerosis and inflammation (Mechoulam and Hanus, 2002; Mechoulam et al., 2002; Burstein and Zurier, 2009). Oral bioavailability of CBD is very limited, due to first pass metabolism during digestion (Mechoulam and Hanus, 2002). The THC-like cannabinoids act at CB1 or CB2 receptors, whereas CBD-like cannabinoids have little binding affinity, leaving their role in inhibition incompletely understood. Data suggest in vitro application of CBD inhibits signalling through GPR55 and TRP channel superfAMILY members and in vivo oral administration is dose-dependently reducing pro-inflammatory cytokine release (Coffey et al., 1996; Malfait et al., 2000; Akopian et al., 2008; Whyte et al., 2009). More recently, CBD was successfully delivered transdermally in different species for anti-inflammatory activity (Lodzki et al., 2003; Stinchcomb et al., 2004; Paudel et al., 2010). In this study, in vivo efficacy of transdermal CBD delivery to reduce inflammation and pain-related behaviours is tested in a rat adjuvant-induced monoarthritis model with both inflammatory and neurogenic properties.
2. Materials and methods

2.1 Animals

All animal procedures were approved by the University of Kentucky IACUC committee and were conducted according to guidelines for the ethical treatment of experimental animals published by the Internal Association for the Study of Pain. All experiments were conducted using 260–280 g male Sprague–Dawley rats (Harlan Laboratories, Indianapolis, IN, USA) housed in individual cages on a 12 h/12 h dark/light reversed cycle and allowed access to food and water *ad libitum*. A total of 54 rats were used in the experiments described here of which 21 were used as naive controls and 23 were subjected to adjuvant-induced arthritis. To induce monoarthritis, animals were anesthetized (2–4% isoflurane) and one knee joint injected with 100 μL complete Freund’s adjuvant (CFA) (DIFCO Laboratories, Detroit, MI, USA) emulsion (2 mg/mL diluted in 1:1 normal saline:peanut oil). Rats were returned to their home cages and monitored daily. Joint circumference and pain-related behaviours were assessed prior to CFA injection and daily beginning on day 3 after CFA (days 3–7).

2.2 Gel preparation

All gels, including vehicle controls, were prepared by weighing the desired amount of CBD (gift from NIDA) and dissolving it in ethanol (72.5% w/w). Once dissolved, nanopure water (Barnstead NANOpure® Diamond™ ultrapure filtration system, Dubuque, IA, USA) was added followed by isopropyl myristate (Fisher Scientific, Fairlawn, NJ, USA). Carbopol® 980 polymer (Noveon Inc., Cleveland, OH, USA) was added (0.9% w/w) and the solution sonicated for 10 min to ensure complete incorporation of the Carbopol® 980. Polymerization of Carbopol® 980 to form the hydroalcoholic gel was initiated by adding sodium hydrosxide (0.1 N). Gels were then sonicated for 10 min, loaded into 1 mL syringes and sealed. Gels made just prior to the initial dosing were used for the entire week since no degradation was observed and plasma CBD concentration remained constant.

2.3 Assessment of joint inflammation

Hindlimbs were fully extended while the circumference of the knee joint was determined using a flexible tape measure wrapped around the centre of the joint. Skin temperature over the joint was measured at the patella with an infrared temperature probe (Fluke, Wilmington, North Carolina, USA). Measurements were taken on day 0, 3 and 7.

2.4 Behavioural assays

2.4.1 Spontaneous pain rating—As a measure of spontaneous pain, limb posture was scored daily in the morning while animals were in their home cages by a scientist blinded to the animal’s treatment. A subjective pain-related behavioural scale was used (Sluka et al., 1993) with 0 – normal; 1 – curling of the toes, 2 – eversion of the paw; 3 – partial weight bearing; 4 – non-weight bearing and guarding and 5 – avoidance of any contact with the hindlimb.

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2.4.3 Hindpaw thermal hypersensitivity—Hindpaw heat sensitivity was assessed prior to CFA injection as well as daily starting on day 3 after inflammation, 4 h after each gel application. Paw withdrawal latency (PWL) was measured as described in our previous study (Zhang et al., 2002). Briefly, rats were acclimated for 20–30 min to individual plastic chambers (10 × 10 × 25 cm) on a glass-top table (2 mm thick). The light beam from a high-intensity projector lamp bulb (Quartzline Lamp, GE, Cleveland, OH, USA) was projected through a 5 × 10 mm aperture attached to an on/off switch with a digital timer. Maximal cutoff time for paw withdrawal reflex was set at 15 s to avoid skin damage. Both hindpaws were tested independently for 5 trials at 5 min intervals by an examiner blinded to the treatment group. The mean responses ± standard error of the mean (SEM) are reported.

2.4.4 Exploratory activity—Open-field exploratory activities were assessed in a Flexfield Animal Activity System (San Diego Instruments, San Diego, CA, USA) with Photobeam Activity System software coupled to a 486 computer (Hewlett Packard, Palo Alto, CA, USA). Animal behaviours include time spent in total exploratory activity (active time, distance travelled, total beams broken, rearing events, rearing time) and resting were recorded in a 40 × 40 × 40 cm transparent plexiglass box for 45 min prior to and at the end of the experiment (Zhang et al., 2004).

2.5 Gel administration

On day 3 after induction of monoarthritis, the back of each animal was shaved and vehicle gel or gel containing 1 or 10% CBD was applied by rubbing it into the skin for 30 s. The area of gel application and volume used corresponded to the calculated final dose of CBD, i.e. 0.62 mg CBD was dosed by applying 75 μL of 1% CBD gel on 3.5 cm² of shaved skin of the animals’ backs, 3.1 mg CBD by giving 375 μL of 1% CBD gel on 17.5 cm², 6.2 mg equalled 750 μL of 1% CBD gel on 35.0 cm², and 62 mg by rubbing 750 μL of 10% CBD gel onto the skin (Table 1). Joint inflammation and nociceptive behaviour were assessed starting 4 h after gel application. On the final day of gel application after completion of all behavioural assays, rats were killed by pentobarbital overdose, blood samples were collected for CBD plasma quantification and transcardially perfused with 4% paraformaldehyde.

2.6 Plasma extraction

Plasma concentration of transepidermally absorbed CBD was determined. As described by Paudel et al. (2010), plasma (50 μL) was combined with 500 μL of acetonitrile (ACN) and ethyl acetate (1:1, v/v; VWR, West Chester, PA, USA), vortexed for 30 s and centrifuged at 10,000 × g for 20 min. The supernatant was transferred into a 3 mL silanized glass test tube and evaporated under nitrogen in a 37 °C water bath. It was then reconstituted in 100 μL of ACN, vortexed and sonicated for 5 min. Samples were then transferred to autosampler vials with silanized low volume inserts and 20 μL injected on the HPLC column for analysis by LC/MS.

2.7 Analytical LC/MS method

The LC/MS system used to analyse samples was comprised of a Waters Alliance 2695 pump, an autosampler, a Micromass ZQ detector and a 996 photodiode array detector with MassLynx software (Milford, MA, USA). A Symmetry® C18 column (150 × 2.1 mm, 5 μm)
TRPV1 activation/desensitization, as suggested here by the fact that this effect was reversed by both a low concentration of a CB2 receptor antagonist (AM630) and by a high concentration of a TRPV1 antagonist (I-RTX). Although this latter effect is not surprising due to the aforementioned capability of CBD to stimulate and desensitize TRPV1 (Iannotti et al., 2014), this phytocannabinoid exhibits only low affinity for CB2 (Pertwee, 2008). Therefore, we hypothesized that endogenous ligands could mediate the anti-inflammatory effect of CBD at this receptor thus explaining why such effect was antagonized by AM630. Indeed, CBD, at a -20 μM concentration (IC50 = 27.5 μM) inhibits both AEA cellular uptake and enzymatic hydrolysis (Bisogno et al., 2001), and these effects could explain both the present finding of its stimulatory action on AEA levels and indirect activation of CB2 receptors. Consequently, our hypothesis that CBD acted via elevation of AEA levels in HcaC2 cells was supported by our present finding that both AEA and a synthetic inhibitor of its degradation, URB597, similar to CBD, were able to reduce the production of both MCP-2 and other proinflammatory cytokines (i.e., IL-6 and IL-8) produced by poly(I:C)-stimulated keratinocytes. Interestingly, the stimulatory action of CBD on AEA levels was only observed after 6 hours, and this could explain why the CB2 antagonist here did not attenuate the anti-inflammatory effects of CBD after 12 and 24 hours. Importantly, whereas the effects of AEA and URB597 on MCP-2 production were comparable to those of CBD, these on IL-6 and IL-8 were statistically significant but less efficacious. This observation supports the aforementioned suggestion that these cytokines play different roles in the sensitization phase of ACD, at least in the in vitro model used here, thus possibly explaining why CB2 and TRPV1 antagonists did not attenuate the effect of CBD on these inflammatory mediators.

In conclusion, in the present study, we demonstrated that, in an in vitro model of ACD, 1) CBD inhibits the production of MCP-2 as well as IL-6, IL-8, and TNF-α; 2) the endogenous levels of AEA are increased after CBD treatment; and 3) the anti-inflammatory effect of CBD during the early sensitization phase (i.e., after 6 hours) is antagonized both by a selective CB2 antagonist—and hence potentially mediated by the endogenous agonist for CB2 receptors, AEA—and a selective TRPV1 antagonist—likely because the phytocannabinoid can directly activate and desensitize the TRPV1 channel. Given the established safety profile of CBD in humans (Leweke et al., 2012; Pertwee, 2015), these data warrant further experiments on the preclinical testing of this compound in animal models of ACD.

Authorship Contributions

Participated in research design: Petrosino, Iuvone, Di Marzo.

Conducted experiments: Petrosino, Verde, Vaia, Allarà, Iuvone.

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Cannabinoid and Allergic Contact Dermatitis


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