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Pure Δ^9-tetrahydrocannabivarin and a *Cannabis sativa* extract with high content in Δ^9-tetrahydrocannabivarin inhibit nitrite production in murine peritoneal macrophages

Barbara Romano[a,b,*], Ester Pagano[a,b], Pierangelo Orlando[b,c,d], Raffaele Capasso[a,b], Maria Grazia Cascio[e], Roger Pertwee[e], Vincenzo Di Marzo[b,f], Angelo A. Izzo[a,b], Francesca Borrelli[a,b,*]

[a] *Department of Pharmacy, University of Naples Federico II, Naples, Italy*
[b] *Endocannabinoid Research Group, Italy*
[c] *Institute of Protein Biochemistry, National Research Council, Pozzuoli, Naples, Italy*
[d] *Institute of Applied Sciences & Intelligent Systems of National Research Council, Pozzuoli, Naples, Italy*
[e] *School of Medical Sciences, Institute of Medical Sciences, University of Aberdeen, Aberdeen, Scotland, UK*
[f] *Institute of Biomolecular Chemistry, National Research Council, Pozzuoli, Naples, Italy*

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ABSTRACT

Historical and scientific evidence suggests that *Cannabis* use has immunomodulatory and anti-inflammatory effects. We have here investigated the effect of the non-psychotropic phytocannabinoid Δ^9-tetrahydrocannabivarin (THCV) and of a *Cannabis sativa* extract with high (64.8%) content in THCV (THCV-BDS) on nitric oxide (NO) production, and on cannabinoid and transient receptor potential (TRP) channel expression in lipopolysaccharide (LPS)-stimulated murine peritoneal macrophages.

THCV-BDS and THCV exhibited similar affinity in radioligand binding assays for CB_1 and CB_2 receptors, and inhibited, via CB_2 but not CB_1 cannabinoid receptors, nitrite production evoked by LPS in peritoneal macrophages. THCV down-regulated the over-expression of inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2) and interleukin 1β (IL-1β) proteins induced by LPS. Furthermore, THCV counteracted LPS-induced up-regulation of CB_1 receptors, without affecting the changes in CB_2, TRPV2 or TRPV4 mRNA expression caused by LPS. Other TRP channels, namely, TRPA1, TRPV1, TRPV3 and TRPM8 were poorly expressed or undetectable in both unstimulated and LPS-challenged macrophages.

It is concluded that THCV – via CB_2 receptor activation – inhibits nitrite production in macrophages. The effect of this phytocannabinoid was associated with a down-regulation of CB_1, but not CB_2 or TRP channel mRNA expression.

Abbreviations: BSA, albumin bovine serum; CB_1, cannabinoid receptor type 1; CB_2, cannabinoid receptor type 2; CBC, cannabichromene; CBD, cannabidiol; CBG, cannabigerol; COX-2, cyclooxygenase-2; DAN, 2,3-diaminonaphthalene; DMSO, dimethyl sulfoxide (DMSO); DTT, 1,4-dithiothreitol; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol tetracetic acid; ELISA, enzyme-linked immunosorbent assay; FBS, fetal bovine serum; hCB_1/hCB_2-CHO cells, Chinese hamster ovarian stably transfected with complementary DNA encoding human cannabinoid CB_1 receptors and human cannabinoid CB_2 receptors; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; i.p., intraperitoneally; IL-1β, interleukin-1 beta; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NO, nitric oxide; NOS, NO synthase; NP-40, nonyl phenoxypolyethoxylethanol; PBS, phosphate buffer saline; PMSF, phenylmethylsulfonyl fluoride; Rimonabant, 5-(4-Chlorophenyl)-1-(2,4-dichloro-phenyl)-4-methyl-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide; SDS, sodium dodecyl sulphate; SEM, stardard error of the mean; SR 144528, N-[(1*S*)-*endo*-1,3,3-trimethyl bicyclo [2.2.1] heptan2-yl]-5-(4-chloro-3-methylphenyl)-1-[(4-methylphenyl)methyl]-1H-pyrazole-3-carboxamide; THC, Δ^9-tetrahydrocannabinol; THCV BDS, Cannabis extract with high content in THCV; THCV, Δ^9-tetrahydrocannabivarin; TRP, transient receptor potential channel; TRPA1, transient receptor potential cation channel subfamily A member 1; TRPM8, transient receptor potential cation channel subfamily M member 8; TRPV1, transient receptor potential cation channel subfamily V member 1; TRPV3, transient receptor potential cation channel subfamily V member 3; TRPV4, transient receptor potential cation channel subfamily V member 4.

* Corresponding authors at: Department of Pharmacy, University of Naples Federico II, via Domenico Montesano 49, 80131 Naples, Italy.
E-mail addresses: barbara.romano@unina.it (B. Romano), francesca.borrelli@unina.it (F. Borrelli).

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1. Introduction

Anedcotal and scientific evidence suggests that *Cannabis sativa* preparations exert anti-inflammatory and immunomodulatory properties [1,2]. Δ^9-tetrahydrocannabinol (THC), the main psychotropic ingredient of *Cannabis*, has been the primary focus of *Cannabis* research since 1964, when Raphael Mechoulam isolated and synthesised it [3]. Subsequently, a number of papers published starting from the seventies showed that THC, as well as *Cannabis* extracts, exerted anti-inflammatory and immunosuppressive actions [4]. Although famous for THC, *Cannabis* produces more than 100 cannabinoids, named phytocannabinoids, at least some of which could contribute to the anti-inflammatory and immunomodulatory effects of *Cannabis* preparations. Interestingly, thanks to the advances in plant breeding programmes, standardized extracts containing known amounts of phytocannabinoids from specific *Cannabis* strains can also be obtained [3]. These *Cannabis* extracts with high content in a specific phytocannabinoid can exhibit as many or even more pharmacological properties than the pure phytocannabinoid [5].

One of the minor phytocannabinoids contained in *Cannabis sativa* is Δ^9-tetrahydrocannabivarin (THCV), a propyl analogue of THC, which was isolated and identified at the beginning of the 1970s [6]. Despite this, THCV has only recently been subject to major investigation. Experimental studies have shown that THCV reduces food intake [7], inhibits hepatosteatosis [8], attenuates nausea [9], affects bladder contractility [10], ameliorates insulin sensitivity in diabetes [11] and suppresses seizures [12]. Importantly, THCV has also been shown to display anti-oedema activity in a carrageenan model of acute inflammation [13].

Macrophages play an important role in the pathogenesis of inflammation-related injury and exhibit a particularly vigorous response to lipopolysaccharide (LPS), a component of the membranes of gram-negative *bacteria* [14]. When activated, macrophages may produce massive amounts of nitric oxide (NO), a potent pro-inflammatory mediator. NO is a free radical generated enzymatically by NO synthase (NOS) through the conversion of L-arginine to citrulline. NOS in macrophages is inducible and catalyses the formation and release of a large amount of NO, which acts as a cytotoxic agent during immune and inflammatory responses [15]. Of relevance for the present investigation, it has been reported that: i) marijuana consumption affects macrophage function in humans [16], including the ability to produce NO [17]; ii) a number of non-psychotropic phytocannabinoids, such as cannabichromene (CBC) and cannabigerol (CBG), share the ability of THC to reduce nitrite over-production evoked by LPS in peritoneal macrophages [18,19].

Given the importance of macrophage-derived NO in inflammation and immunity and because *Cannabis* is known to affect macrophage function and NO production in humans, in the present study we have investigated the effect of the non-psychotropic phytocannabinoid THCV and of a *Cannabis* extract with high (64.8%) content in THCV (THCV-BDS) both on NO production, and on cannabinoid receptor and transient receptor potential (TRP) channel expression in LPS-stimulated macrophages.

2. Methods

2.1. Materials

Pure THCV and a *Cannabis sativa* extract with high content in THCV (i.e. THCV-BDS) were supplied by GW Pharmaceuticals (Cambridge, UK). Pure THCV showed a purity by HPLC >95% whereas the content (% w/w) of THCV in THCV-BDS was 64.8% (refer to Table 1 for the relative abundance of the other constituents). The concentration of THCV-BDS reported in the present paper indicated the amount of THCV contained in the extract (e.g. 1 μmol of THCV-BDS contained 1 μmol of THCV). Rimonabant (5-(4-Chlorophenyl)-1-(2,4-dichloro-phenyl)-4-methyl-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide) and SR 144528 (N-[(1*S*)-*endo*-1,3,3-trimethyl bicyclo [2.2.1] heptan2-yl]-5-(4-chloro-3-methylphenyl)-1-[(4-methylphenyl)methyl]-1H-pyrazole-3-carboxamide) were kindly supplied by SANOFI Recherche, (Montpellier, France). LPS from *Escherichia coli* serotype O111:B4, thioglycollate medium, cadmium, 2,3-diaminonaphthalene (DAN) and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) were purchased from Sigma-Aldrich (Milan, Italy). All reagents for western blot analysis and cell culture were obtained from Sigma-Aldrich (Milan, Italy), Bio-Rad Laboratories (Milan, Italy) and Microtech (Naples, Italy). For the binding experiments [^{3}H]CP55940 (160 Ci/mmol) was obtained from PerkinElmer Life Science Inc. (Boston, MA, USA). THCV, THCV-BDS, rimonabant and SR144528 were dissolved in dimethyl sulfoxide (DMSO). The drug vehicle had no significant effects *in vitro* on the responses under study (0.01% DMSO) or in the radioligand binding assays performed with hCB_1/hCB_2-CHO cells (Chinese hamster ovarian cells stably transfected with complementary DNA encoding human cannabinoid CB_1 receptors and human cannabinoid CB_2 receptors) (0.1% DMSO, v/v).

Table 1
Content of the main phytocannabinoids contained in a *Cannabis sativa* extract with high content in Δ^9-tetrahydrocannabivarin (THCV BDS).

Phytocannabinoid	Content(% w/w)
Δ^9-tetrahydrocannabivarin	64.8%
Δ^9-tetrahydrocannabinol	13.5%
Cannabinovarin	1.1%
Cannabinol	0.5%
Δ^9-tetrahydrocannabivarin acid	0.4%
Cannabigevarin	0.1%

2.2. Plant material and extraction

For the experiments reported in this paper, a specific *Cannabis sativa* strain (with a defined genotype) has been cloned to get a controlled amount of THCV [20]. *Cannabis sativa* was grown in highly secure computer-controlled glasshouses. All aspects of the growing climate, including temperature, air change and photoperiod, were computer-controlled and the plants were grown without the use of pesticides (see details at http://www.gwpharm.com). *Cannabis* dry flowers and leaves were extracted at room temperature with CO_2 to give an extract which, when evaporated to dryness, was a brownish solid. A portion of the extract was dissolved in methanol for HPLC analysis (Agilent 1100) using a C18 column (150 × 4.6 mm, 1 ml/min flow rate). The HPLC chromatogram and amounts of the main cannabinoids present in THCV-BDS are reported in Fig. 1 and Table 1.

2.3. Animals

Male ICR mice (Charles River, Calco, Italy), weighing 26–28 g, were used after 1-week acclimation period (temperature 23 ± 2 °C and humidity 60%, free access to water and standard food). All animal procedures were in conformity with the principles of laboratory animal care (NIH publication no.86–23, revised 1985) and the Italian D.L. no.116 of January 27, 1992 and associated guidelines in the European Communities Council Directive of November 24, 1986 (86/609/ECC).