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Beneficial effect of the non-psychotropic plant cannabinoid cannabigerol on experimental inflammatory bowel disease

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ABSTRACT

Inflammatory bowel disease (IBD) is an incurable disease which affects millions of people in industrialized countries. Anecdotal and scientific evidence suggests that Cannabis use may have a positive impact in IBD patients. Here, we investigated the effect of cannabigerol (CBG), a non-psychotropic Cannabis-derived cannabinoid, in a murine model of colitis. Colitis was induced in mice by intracolonic administration of dinitrobenzene sulphonic acid (DNBS). Inflammation was assessed by evaluating inflammatory markers/parameters (colon weight/colon length *ratio* and myeloperoxidase activity), by histological analysis and immunohistochemistry; interleukin-1β, interleukin-10 and interferon-γ levels by ELISA, inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) by western blot and RT-PCR; CuZn-superoxide dismutase (SOD) activity by a colorimetric assay. Murine macrophages and intestinal epithelial cells were used to evaluate the effect of CBG on nitric oxide production and oxidative stress, respectively. CBG reduced colon weight/colon length *ratio*, myeloperoxidase activity, and iNOS expression, increased SOD activity and normalized interleukin-1β, interleukin-10 and interferon-γ changes associated to DNBS administration. In macrophages, CBG reduced nitric oxide production and iNOS protein (but not mRNA) expression. Rimonabant (a CB_1 receptor antagonist) did not change the effect of CBG on nitric oxide production, while SR144528 (a CB_2 receptor antagonist) further increased the inhibitory effect of CBG on nitric oxide production. In conclusion, CBG attenuated murine colitis, reduced nitric oxide production in macrophages (effect being modulated by the CB_2 receptor) and reduced ROS formation in intestinal epithelial cells. CBG could be considered for clinical experimentation in IBD patients.

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1. Introduction

Inflammatory bowel disease (IBD) comprises the chronic relapsing inflammatory disorders Crohn's disease (CD) and ulcerative colitis (UC) [1]. It is characterized by abdominal pain, diarrhoea, bleeding and malabsorption [2]. Its incidence is increasing worldwide, and the disease remains incurable [3,4]. The incidence and prevalence of IBD has increased in the past 50 years, up to 8–14/100,000 and 120–200/100,000 cases, respectively, for UC and 6–15/100,000 and 50–200/100,000 cases, respectively, for CD [3]. Conventional therapies for IBD include aminosalicylates, corticosteroids, thiopurines, methotrexate, and anti-tumour necrosis factor agents [5]. Although these drugs may

Abbreviations: CB, cannabinoid; CBD, cannabidiol; CBG, cannabigerol; CD, Crohn's disease; COX-2, cycloxygenase-2; DNBS, 2,4,6-dinitrobenzene sulphonic acid; H2DCF-DA, 2′,7′-dichlorfluorescein-diacetate; IBD, Inflammatory bowel disease; iNOS, inducible nitric oxide synthase; MPO, myeloperoxidase; ROS, reactive oxygen species; SOD, superoxide dismutase; UC, ulcerative colitis.

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be effective, their long-term use can induce severe side effects that have detrimental impact on life quality of patients [6]. Hence, it is required to develop new approaches with fewer side effects for the treatment of IBD.

Anecdotal reports suggest that IBD patients experience relief by smoking marijuana [7,8]. Recent retrospective observational studies, by showing that Cannabis use is common in patients with IBD for symptom relief, have confirmed such reports [9,10]. Also, a pilot prospective study found that treatment with inhaled Cannabis improved quality of life in patients with long-standing CD and UC [11]. In Israel, inhaled Cannabis has been legally registered for palliative treatment of both CD and UC. Δ^9-tetrahydrocannabinol (Δ^9-THC), the main Cannabis psychotropic ingredient which activates cannabinoid (CB_1 and CB_2) receptors, and cannabidiol (CBD), the best studied among the so-called non-psychotropic cannabinoids, have been previously shown to ameliorate experimental colitis in rodents [12–14]

Cannabigerol (CBG) is a non-psychotropic cannabinoid - obtained in 1964 by Gaoni and Mechoulam when they separated a hexane extract of hashish on Florisil [15] - which does not induce Δ^9-THC-like effects *in vivo* [16]. Relatively few studies have sought to investigate the pharmacological actions of this compound [17,18]. CBG was shown to exert antiproliferative [19], antibacterial [20] and anti-glaucoma [21] actions and to antagonize the anti-nausea effect of CBD [22]. Potential targets of CBG actions include transient receptor potential (TRP) channels [23], cyclooxygenase (COX-1 and COX-2) enzymes [24], as well as cannabinoid, 5-HT_{1A} and α_2 adrenergic receptors [25].

In the present study, we investigated the effect of CBG in an experimental model of murine colitis. To further characterize CBG action, we evaluated the effect of this phytocannabinoid in peritoneal macrophages and in intestinal epithelial cells.

2. Materials and methods

2.1. Drugs and reagents

CBG [purity by high-performance liquid chromatography (HPLC), 99.0%] was kindly supplied by GW Pharmaceuticals (Porton Down, Wiltshire, UK). Rimonabant (5-(p-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-N-piperidinopyrazole-3-carboxamide hydrochloride) and SR144528 (N-[-1S-endo-1,3,3-trimethyl-bicyclo[2.2.1]heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methyl benzyl)-pyrazole-3-carboxamide) were a kind gift from Drs Madaleine Mosse 'and Francis Barth (SANOFI Recherche, Montpellier, France). Dinitrobenzene sulphonic acid (DNBS), neutral red solution, myeloperoxidase from human leucocytes, hydrogen peroxide (H_2O_2), $FeCl_2{\cdot}4H_2O$, 2′,7′-dichlorfluorescein-diacetate (H2DCF-DA), lipopolysaccharide (LPS, from Escherichia coli serotype O111:B4), thioglycollate medium, cadmium, 2,3-diaminonaphtalene (DAN), 2,6-di-tert-butyl-4-methylphenol (BHT), fluorescein isothiocyanate (FITC)-conjugated dextran (molecular mass 3–5 kDa), streptevidin, 3,3′-diaminobenzidine tetrahydrochloride, Mayer's haematoxylin solution, N,N,N',N'-tetramethylbenzidine were purchased from Sigma Aldrich S.r.l. (Milan, Italy). All reagents for cell culture and western blot analysis were obtained from Sigma Aldrich S.r.l. (Milan, Italy), Amersham Biosciences Inc. (UK), Bio-Rad Laboratories (USA) and Microtech S.r.l. (Naples, Italy). All chemicals and reagents employed in this study were of analytical grade.

CBG was dissolved in ethanol/Tween20/saline (1:1:8; for *in vivo* experiments) or ethanol (for *in vitro* experiments). Rimonabant and SR144528 were dissolved in dimethyl sulphoxide (DMSO). DNBS was dissolved in 50% ethanol (0.15 ml/mouse). The CBG vehicles (60 μl/mouse *in vivo* or 0.01% ethanol *in vitro*) had no significant effects on the responses under study.

2.2. Animals

Male ICR mice, weighing 30–35 g, were supplied by Harlan Italy (Corezzana, Milan, Italy). All animals, used after 1 week of acclimation (temperature, 23 ± 2 °C; humidity, 60%), had free access to water and food. Mice were fed *ad libitum* with standard food, except for the 24-h period immediately preceding the administration of DNBS. All experiments complied with the Italian D.L. no. 116 of 27 January 1992 and associated guidelines in the European Communities Council Directive of 24 November 1986 (86/609/ECC).

2.3. Induction of experimental colitis and pharmacological treatment

Colitis was induced by the intracolonic administration of DNBS [12]. Briefly, mice were anesthetized with inhaled 5% isoflurane (Centro Agrovete Campania, Scafati, SA, Italy) and subsequently DNBS (150 mg/kg) was inserted into the colon using a polyethylene catheter (1 mm in diameter) *via* the rectum (4.5 cm from the anus). Three days after DNBS administration, all animals were euthanized by asphyxiation with CO_2, the mice abdomen was opened by a midline incision and the colon removed, isolated from surrounding tissues, opened along the antimesenteric border, rinsed, weighed and length measured [in order to determined the colon weight/colon length ratio (mg/cm) used as an indirect marker of inflammation]. All measurements were performed by operators which were unaware of the particular treatment (blinded evaluation). For biochemistry analysis, tissues were kept at −80 °C until use, while for histological examination tissues were fixed in 10% formaldehyde.

The dose of DNBS was selected on the basis of preliminary experiments showing a remarkable colonic damage associated to high reproducibility and low mortality for the 150 mg/kg dose. The time point of damage evaluation (*i.e.*, 3 days after DNBS administration) was chosen because maximal DNBS-induced inflammation has been reported in mice after 3 days [26]. Furthermore, previous studies have shown that 3 days after intracolonic DNBS administration in mice, the inflammatory response may be modulated by administration of cannabinoid drugs such as direct cannabinoid receptor agonists or antagonists [12,26].

In the preventive protocol CBG (1–30 mg/kg) was given once a day for six consecutive days starting 3 days before DNBS administration, while in the curative protocol CBG (1–30 mg/kg) was injected for two consecutive days starting 24-h after DNBS administration.

2.4. Histology and immunohistochemistry

Histological and immunochemistry evaluations, performed 3 days after DNBS administration, were assessed on a segment of 1 cm of colon located 4 cm above the anal canal by blinded examiners. After fixation for 24 h in saline 10% formaldehyde, samples were dehydrated in graded ethanol and embedded in paraffin. Thereafter, 5-μm sections were deparaffinized with xylene, stained with haematoxylin–eosin, and observed in a DM 4000 B Leica microscope (Leica Microsystems, Milan, Italy). For microscopic scoring we used a modified version of the scoring system reported by D'Argenio and colleagues [27]. Briefly, colon was scored considering (1) the submucosal infiltration (0, none; 1, mild; 2–3, moderate; 4–5 severe), (2) the crypt abscesses (0, none, 1–2 rare; 3–5, diffuse) and (3) the mucosal erosion (0, absent; 1, focus; 2–3, extended until the middle of the visible surface; 4–5, extended until the entire visible surface).

For immunohistochemical detection of Ki-67, paraffin-embedded slides were immersed in a Tris/ethylenediaminetetraacetic acid buffer (pH 9.0), heated in a decloaking chamber at 125 °C for

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