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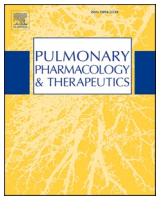

The anti-inflammatory effects of cannabidiol and cannabigerol alone, and in combination

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ABSTRACT

Introduction/background and purpose: Studies with *Cannabis Sativa* plant extracts and endogenous agonists of cannabinoid receptors have demonstrated anti-inflammatory, bronchodilator, and antitussive properties in the airways of allergic and non-allergic animals. However, the potential therapeutic use of cannabis and cannabinoids for the treatment of respiratory diseases has not been widely investigated, in part because of local irritation of airways by needing to smoke the cannabis, poor bioavailability when administered orally due to the lipophilic nature of cannabinoids, and the psychoactive effects of Δ9-Tetrahydrocannabinol (Δ9-THC) found in cannabis. The primary purpose of this study was to investigate the anti-inflammatory effects of two of the non-psychotropic cannabinoids, cannabidiol (CBD) and cannabigerol (CBG) alone and in combination, in a model of pulmonary inflammation induced by bacterial lipopolysaccharide (LPS). The second purpose was to explore the effects of two different cannabinoid formulations administered orally (PO) and intraperitoneally (IP). Medium-chain triglyceride (MCT) oil was used as the sole solvent for one formulation, whereas the second formulation consisted of a Cremophor® EL (polyoxyl 35 castor oil, CrEL)-based micellar solution.

Results: Exposure of guinea pigs to LPS induced a 97 ± 7% and 98 ± 3% increase in neutrophils found in bronchoalveolar lavage fluid (BAL) at 4 h and 24 h, respectively. Administration of CBD and CBG formulated with MCT oil did not show any significant effects on the LPS-induced neutrophilia measured in the BAL fluid when compared with the vehicle-treated groups. Conversely, the administration of either cannabinoid formulated with CrEL induced a significant attenuation of the LPS induced recruitment of neutrophils into the lung following both intraperitoneal (IP) and oral (PO) administration routes, with a 55–65% and 50–55% decrease in neutrophil cell recruitment with the highest doses of CBD and CBG respectively. A combination of CBD and CBG (CBD:CBG = 1:1) formulated in CrEL and administered orally was also tested to determine possible interactions between the cannabinoids. However, a mixture of CBD and CBG did not show a significant change in LPS-induced neutrophilia. Surfactants, such as CrEL, improves the dissolution of lipophilic drugs in an aqueous medium by forming micelles and entrapping the drug molecules within them, consequently increasing the drug dissolution rate. Additionally, surfactants increase permeability and absorption by disrupting the structural organisation of the cellular lipid bilayer.

Conclusion: In conclusion, this study has provided evidence that CBD and CBG formulated appropriately exhibit anti-inflammatory activity. Our observations suggest that these non-psychoactive cannabinoids may have beneficial effects in treating diseases characterised by airway inflammation.

1. Introduction

Cannabis, often referred to as marijuana, is a botanical product derived from the *Cannabis Sativa* L. plant, a dioicous species of the Cannabaceae and broadly distributed all over the world [1]. The use of the cannabis plant for its medicinal properties, source of textile fibre (hemp), and psychoactive/medical effects, stretches back approximately 5000 years. The term 'cannabinoid' or 'phytocannabinoid' (plant-based cannabinoids) refers to a group of lipophilic and pharmacologically active, oxygenated $C_{21\text{-}22}$ aromatic hydrocarbon compounds found in

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the leaves and flowering plants of the *Cannabis Sativa* plant [2]. Since the isolation of Δ^9-tetrahydrocannabinol (Δ^9-THC) [3], more than 144 unique cannabinoid compounds, 100 terpenes, and 20 phenolic compounds synthesised by the cannabis plant have been identified [4]. In addition to the plant-derived cannabinoids, many structurally and biologically associated compounds have been created, which are known as synthetic cannabinoids [5].

The discovery of the endocannabinoid system (ECS) has enabled the growth of scientific evidence supporting the use of cannabis and cannabinoids as therapeutic agents for various diseases. The ECS is a complex lipid cell-signalling system comprised of: the cannabinoid receptors (CBRs; CB1 and CB2); the endogenous cannabinoids (endocannabinoids, ECs), anandamide (N-arachidonoylethanolamide, AEA) and 2-arachidonoylglycerol (2-AG); the AEA transporter protein (TP) and the enzymes responsible for the synthesis and degradation of endocannabinoids (fatty acid amide hydrolase, FAAH, or monoacylglycerol lipase, MGL) [6].

Various studies have suggested the use of cannabinoids as possible treatments for inflammatory diseases in the airways, such as chronic obstructive pulmonary disease (COPD) [7,8]. The phytocannabinoids Δ^9-THC [9], cannabidiol (CBD) [10] and cannabigerol (CBG) [11] are of particular interest due to their important effects on inflammation and the immune system, including inhibiting the activation of pro-inflammatory cells and the synthesis of pro-inflammatory mediators or reducing intracellular and mitochondrial oxidative stress [12]. Additionally, it has been reported that CBD exhibits apoptotic properties in immune cell populations, leading to cannabinoid-induced immunosuppression [13]. CBD and CBG alone, and in combination, have demonstrated apoptotic effects in tumour cells, in addition to their off-target effects essential for effective palliative care such as increased appetite, analgesic and anxiolytic properties [14]. On the other hand, CBD [15] and CBG [16] have been demonstrated to exhibit anti-apoptotic properties in healthy cells under oxidative and inflammatory conditions. The anti-apoptotic effects of cannabinoids are mainly associated with cytokine modulation and antioxidant activity via downregulation of nitric oxide production [17].

COPD is a chronic respiratory disease with considerable unmet medical needs [18]. In 2017, 3.91 million people died from COPD worldwide, and because of its growing prevalence and mortality rate, COPD is expected to become the world's third most common cause of death by 2030 [19]. COPD includes a group of chronic lung conditions characterised by poorly reversible airflow obstruction, abnormal and chronic non-allergic inflammation of the airway, mucous plugging and airway remodelling [20]. This chronic and pathological airway response can result in excessive cough and mucus production (chronic bronchitis), alveolar destruction (emphysema) and/or lesions in the smaller conducting airways (bronchiolitis) [21]. The aberrant inflammatory response in the lungs, particularly in the small airways, is the outcome of the innate and adaptive immune responses to long-term exposure to toxic particles and gases, especially cigarette smoke and other oxidant pollution [20]. Other sources may trigger the development of the disease, such as alpha1-antitrypsin deficiency and telomerase polymorphisms [22]. This response is associated with an increased number of activated macrophages, neutrophils (both part of the innate immune response), T lymphocytes (Tc1, Th1 and ILC3 cells; adaptative immunity) [18] and in some cases, eosinophils [23]. These activated inflammatory cells release inflammatory mediators such as interleukin 8 (IL-8), leukotriene B4 (LTB4), and tumour necrosis factor α (TNF-α), which orchestrate the pathological structural and airway changes in COPD. These changes include tissue remodelling, chronic airways inflammation, oxidative stress, proteinase imbalances and accelerated ageing [24]. As the disease progresses, the degree of inflammation driven primarily by neutrophils also evolves [18].

The second purpose of this study was to investigate the anti-inflammatory effects of cannabinoids formulated in two different formulations. The lipophilic nature of cannabinoids is a significant challenge for developing an effective formulation and bioavailability for optimal therapeutic effect [25]. Due to their lipophilicity, cannabinoids present negligible aqueous solubility. Additionally, they are vulnerable to degradation by auto-oxidation, light and temperature [26]. The first formulation tested in this study was composed of medium-chain triglycerides (MCT). They are lipids with a carbon chain length of 6–12 carbon atoms, making MCTs easier to absorb and metabolise than long-chain fatty acids (LCTs). Due to these characteristics, MCTs have been suggested as a drug vehicle for lipophilic drugs [27]. Our second formulation was a micellar solution composed of ethanol (EtOH), Cremophor® EL (polyoxyl 35 castor oil, CrEL) and sodium chloride 0.9% in purified water (saline). EtOH, a short-chain alcohol, is widely used as a solvent and co-surfactant for lipophilic drugs. CrEL is a non-ionic hydrophilic surfactant used to emulsify and solubilise lipophilic molecules by forming micelles and entrapping the lipophilic molecules within them in aqueous solutions. CrEL can also increase drug absorption by enhancing the dissolution rate of the drug by disrupting the lipid bilayer of cells [28]. Lastly, saline is a water-based solvent included in the formulation to obtain a final isotonic mixture.

Challenge of animals [29] and people [30] with bacterial lipopolysaccharide (LPS) has been extensively used as a model to mimic the neutrophilia characterising COPD and to investigate the actions of novel anti-inflammatory drugs in development for the treatment of this disease [31]. Therefore, we have investigated the effects of highly purified CBD and CBG administered alone or in combination for their impact on LPS-induced neutrophilia.

2. Materials and methods

2.1. Animals

Male adult Dunkin-Hartley guinea pigs were obtained from Marshalls Laboratories (Hull, UK). All guinea pigs weighed 250–350 g at the time of experimentation. All experiments were performed at King's College London according to the ARRIVE (Animal Research: Reporting of *In Vivo* Experiments) guidelines, the UK Animals (Scientific Procedures) Act 1986, and the 2012 amendments, and were approved by the King's College London ethics committee. The animals were housed in rooms under controlled temperature (22 ± 1 °C), humidity (55 ± 10%), and 12-h light/dark cycle. Food and water were available at all times. In total, 171 guinea pigs were used in this study.

2.2. Reagents and cannabinoids

CBD and CBG were provided as a white powder from George Botanicals (certification of analysis provided with 99.9% purity). CBD and CBG were suspended in Cremophor® EL (CrEL)/ethanol/sodium chloride 0.9% (saline) in a ratio of 1:1:18 as previously described [32,33] or medium-chain triglyceride (MCT) oil (provided by Sativa Wellness Group Inc.). Briefly, the weighed CBD or CBG powder was mixed with absolute ethanol by Vortex shaker for 30 s. The resulting mixture was clear and transparent. An equal amount of CrEL was added to the solution and mixed by Vortex shaker for 30 s. This was followed by the addition of saline and mixed by Vortex shaker one further time for 30 s. The solutions were then individually placed in a sonicator for 15 min. The most concentrated mixtures (100 mg/kg for CBD and 90 mg/kg for CBG) were visually white independently of how the drug was dissolved. The solutions with a concentration of 50 mg/kg were cloudy. The 10 mg/kg mixtures were clear. Cannabinoids were injected intraperitoneally (IP) or given orally (PO) via a 1 ml syringe at a dose volume of 1 ml. Both cannabinoids were also administered at the doses of 50 mg/kg and 10 mg/kg in 5 ml/kg injection volumes. Doses were based on the maximal concentration that could be diluted in the solvent [33]. Control animals were administered vehicle solutions, either MCT or a CrEL/ethanol/saline solution as appropriate.

2.3. Lipopolysaccharide-induced inflammatory cell infiltration into the airways

All compounds were administered intraperitoneally (IP) or orally (PO) to the guinea pigs: vehicle (MCT or CrEL), CBD (10, 50 or 100 mg/kg), or CBG (10, 50 or 90 mg/kg). One hour later, the animals were exposed to an aerosolised solution of LPS (100 mg/ml) for 20 min as previously described [7].

Four or 24 h after exposure to LPS, the animals were euthanised with an injection of sodium pentobarbital (1 g/kg; IP). BAL was performed by instilling 5 ml of 0.9% sterile saline into the lungs via the tracheal cannula, and the fluid was immediately aspirated. The same fluid was then re-injected, and the procedure repeated three times. This resulted in a 40–60% recovery of BAL fluid from the lungs of each guinea pig.

2.4. Total and inflammatory cell count in the bronchoalveolar fluid

A 100 μl sample of BAL fluid from the guinea pigs was added to an equal volume of 50% v/v filtered Turk's solution (0.1% methylene blue in 1% acetic acid) for determining the concentration of cells/ml present in the sample by a standard haemocytometer technique (Neubauer haemocytometer, Fisher Scientific) under a ×20 objective. BAL samples (100 μl) were added to filters placed on cytospin slides and centrifuged in a Shandon Cytospin 3 at 1000 rpm for 1 min. The slides were placed at room temperature and allowed to dry for 24 h. Slides were subsequently stained with REASTAIN® Quick-Diff (Reagena, Toivala, Finland) and covered with a DPX mountant. A differential cell count was performed by counting 200 cells from a representative area in each slide. The cells were identified as macrophages, neutrophils, lymphocytes and eosinophils. The number of leukocytes per ml of BAL fluid was then determined by the percentages of each differential cell type present in the total cell count.

2.5. Data analysis

Values are expressed as the total number of cells x 10^4 ml^{-1} ± standard error of the mean (SEM) and *n* denotes the number of animals used per experiment. The two-tailed unpaired Student's t-test was used for comparisons of individual means. Correlations between variables were analysed using Pearson's test, where the derived r^2 value indicated goodness of fit. An *F* test was used to compare variances and test for the likelihood that r^2 values were generated from data with no correlation.

Statistical analysis of means between groups was performed by multiple comparisons single or repeated measures 1-way analysis of variance (ANOVA); 2-way ANOVA or 3-way ANOVA, where appropriate, followed by a Šidák's (for validation studies only) or Tukey's multiple comparison post-tests between different groups. The multiple comparisons post-tests were chosen according to the statistics software recommendations used for data analysis. Differences between mean values were considered significant if $P < 0.05$ between individual groups. All data were processed using GraphPad Prism 8.0 for Windows (GraphPad Software, San Diego, CA).

3. Results

3.1. LPS-induced pulmonary inflammation

Exposure to LPS (100 μg/ml; 20 min) resulted in a significant increase in total leukocytes in BAL fluid obtained 4 h ±30 min post-exposure (saline: 101 ± 7 × 10^4 vs LPS: 348 ± 115 × 10^4 leukocytes/ml, $P < 0.001$; Fig. 1 A; n = 4 for each group). A differential count of the leukocyte cells in the BAL indicated that neutrophils accounted for this

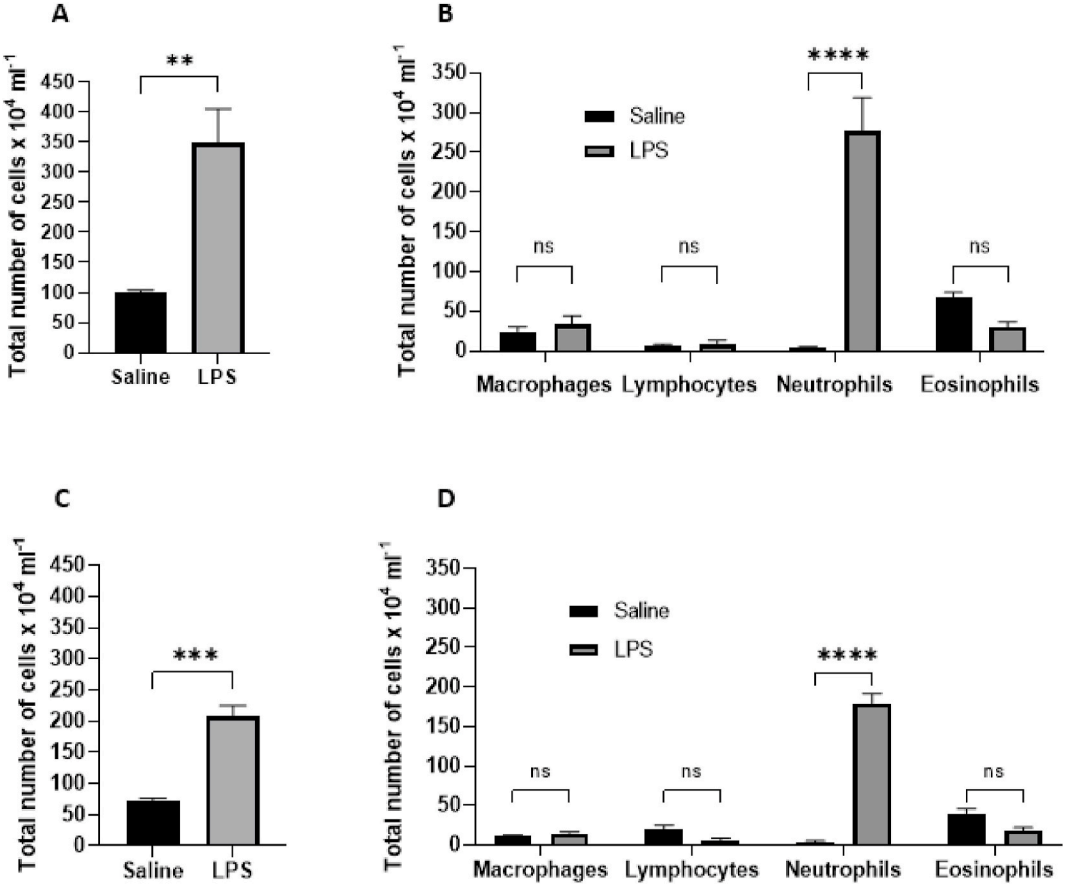

Fig. 1. *Total and differential count of the leukocytes in BAL fluid samples 4 and 24 h post-aerosol administration of LPS for 20 min.*

increase in cell numbers (saline: $7 \pm 7 \times 10^4$ vs LPS: $277 \pm 83 \times 10^4$ neutrophils/ml ($P < 0.0001$, $n = 4$ per group) and represented $97 \pm 7\%$ of the total cell count in LPS-exposed animals (Fig. 1 B).

Similar results were observed in the BAL samples 24 ± 2 h after LPS exposure. A total of $72 \pm 9 \times 10^4$ (saline group) and $208 \pm 33 \times 10^4$ (LPS group) leukocytes/ml ($P < 0.01$, $n = 4$ per group) were observed in the BAL samples obtained from guinea pigs 24 h after exposure to saline or LPS for 20 min (Fig. 1C; $n = 4$ for each group). Further analysis of the BAL samples showed that the increase in the total number of leukocytes following LPS exposure was also primarily due to an increase in the number of neutrophils ($179 \pm 26 \times 10^4$ neutrophils/ml, $P < 0.0001$) and represented 98 ± 3% of the total cell count in LPS-exposed animals (Fig. 1 D). Given these results, there was a substantial inflammatory response recorded at both 4 and 24 h represented by the significant increase of neutrophil infiltration into the lung, which has been previously demonstrated in other studies [7,8,34]. A 4 h time point was used in further experiments investigating the actions of the cannabinoids.

Total (A, C) and differential (B, D) cell count (cells x 10^4/ml) in the bronchoalveolar lavage (BAL) fluid samples of guinea pigs 4 h (A, B) and 24 h (C, D) after exposure to a saline (black bars) or LPS (grey bars) nebulised solutions for 20 min. Data: Mean ± SEM; $n = 4$ for each condition. The Student's unpaired *t*-test was used for comparisons of individual means. Two-way ANOVA with Tukey's multiple comparisons for differential cell count data. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$. Total $n = 16$.

Effect of CBD and CBG on total cell counts in BAL fluid following LPS exposure.

CBD and CBG formulated in CrEL and administered either PO or IP significantly attenuated the total cell count in the BAL fluid samples of experimental guinea pigs in a dose-dependent manner (Fig. 2 C, D; Fig. 3 B, C; $n = 5$ for each group). In contrast, CBD and CBG formulated with MCT oil did not significantly affect the total leukocyte cell count in the BAL samples compared to the vehicle-treated groups (Fig. 2 A, B; Fig. 3 A; $n = 5$ for each group).

Total cell count (cells x 10^4/ml) in the BAL fluid samples of control and CBD-treated guinea pigs, 4 h post-aerosol LPS exposure for 20 min. The drugs were tested individually in two different formulations and two different administration routes. CBD was formulated in Cremophor® EL (CrEL)/ethanol/saline in a ratio of 1:1:18 or medium-chain triglycerides (MCT) oil. Each formulation was administered orally (PO) or intraperitoneally (IP). Animals were pre-treated with increasing concentrations of CBD in MCT PO (A) and IP (B), and CBD in CrEL PO (C) or IP (D). Vehicle animals were treated with the drug vehicle. Data: Mean ± SEM. $n = 5$. One-way ANOVA with Tukey's multiple comparisons. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$. A total of 80 animals were used in this study; $n = 5$ for each group.

CBG in CrEL administered at the doses of 50 and 90 mg/kg PO produced a significant decrease in the total cell count from BAL fluid, when compared to the vehicle group, with an adjusted P-value of <0.01 and <0.001, respectively (Fig. 3 B; $n = 5$ for each condition).

These results suggest that CBD in CrEL administered orally is the most effective formulation at reducing the total number of cells in the BAL fluid samples of LPS-exposed guinea pigs.

Total cell count (cells x 10^4/ml) in the bronchoalveolar lavage (BAL) fluid samples of control and experimental guinea pigs, 4 h post-aerosol LPS exposure for 20 min. The drugs were tested individually in two different formulations and two different administration routes. CBG was formulated in Cremophor® EL (CrEL)/ethanol/saline in a ratio of 1:1:18 or medium-chain triglycerides (MCT) oil. Each formulation was administered orally (PO) or intraperitoneally (IP). Animals were pre-treated with increasing concentrations of CBG in MCT PO (A) and CBG in CrEL PO (B) or IP (C). Vehicle animals were treated with the drug vehicle. Data: Mean ± SEM. n = 5. One-way ANOVA with Tukey's multiple comparisons.

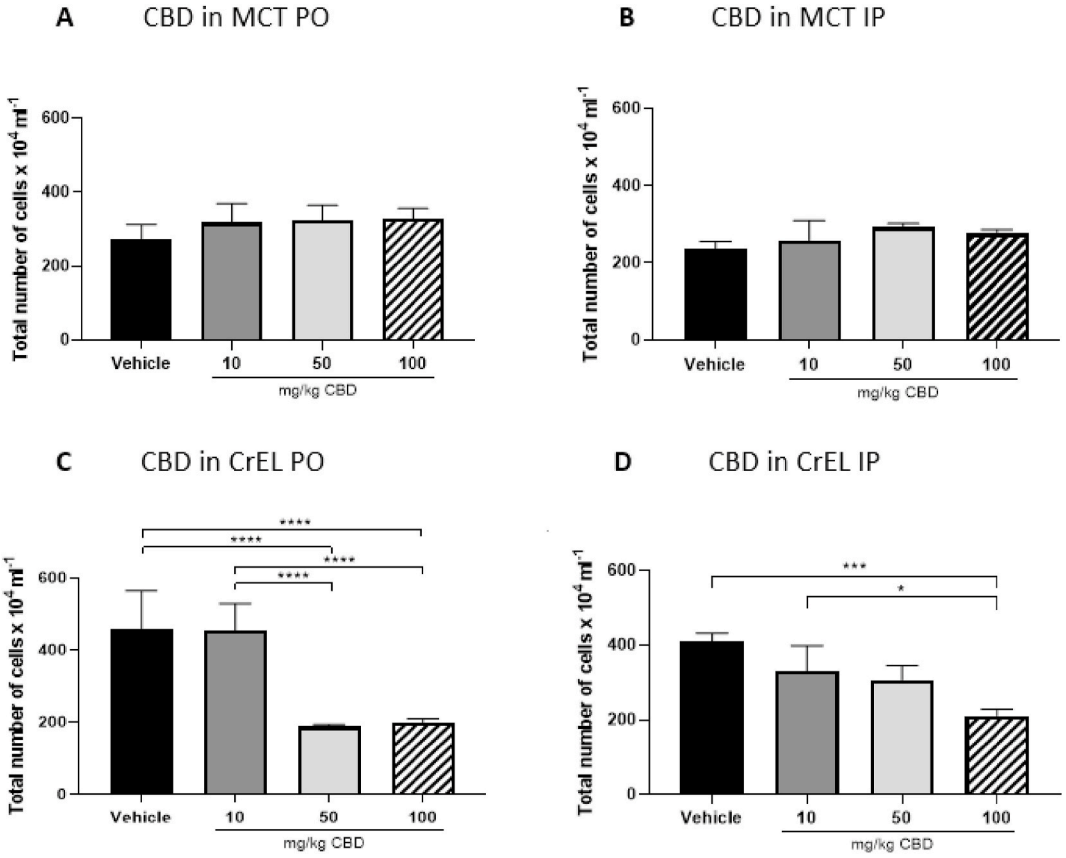

Fig. 2. *The effect of CBD on total cell count in BAL fluid samples* 4hrs *post LPS exposure.*

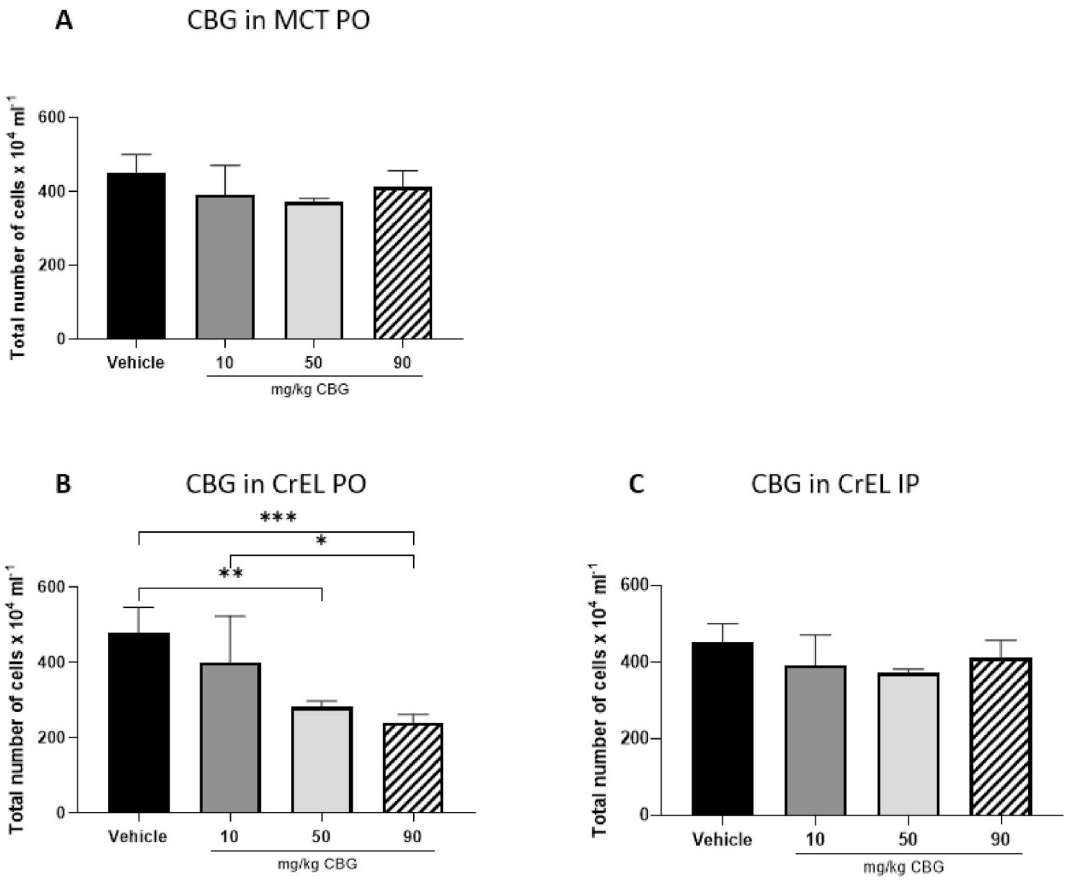

Fig. 3. The effect of CBG on total cell count in BAL fluid samples 4hrs post LPS exposure.

*P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001. A total of 60 animals were used in this study; n = 5 for each group.

3.2. Effect of CBD and CBG on neutrophils in BAL fluid following LPS exposure

A differential cell count of the BAL samples showed that the increase in the total number of leukocytes was primarily due to neutrophils. The number of the other leukocyte cell types (macrophages, lymphocytes, eosinophils) was not affected by any of the doses of cannabinoids administered PO or IP (data not shown). The smallest dose of CBD and CBG (10 mg/kg) did not significantly affect (P > 0.05) the neutrophil cell count when administered PO or IP with either formulation when compared with the vehicle control group (Fig. 4; Fig. 5). The most effective administration route and formulation that showed inhibition of neutrophil recruitment to the lungs was CBD in CrEL PO. The highest doses (50 and 100 mg/kg) accounted for a 55–65% reduction in neutrophil cell count compared to the vehicle and the lowest dose groups (Fig. 4). A similar outcome was observed with the highest doses for CBD (100 mg/kg, IP; Fig. 4) and CBG (90 mg/kg, PO and IP; Fig. 5)

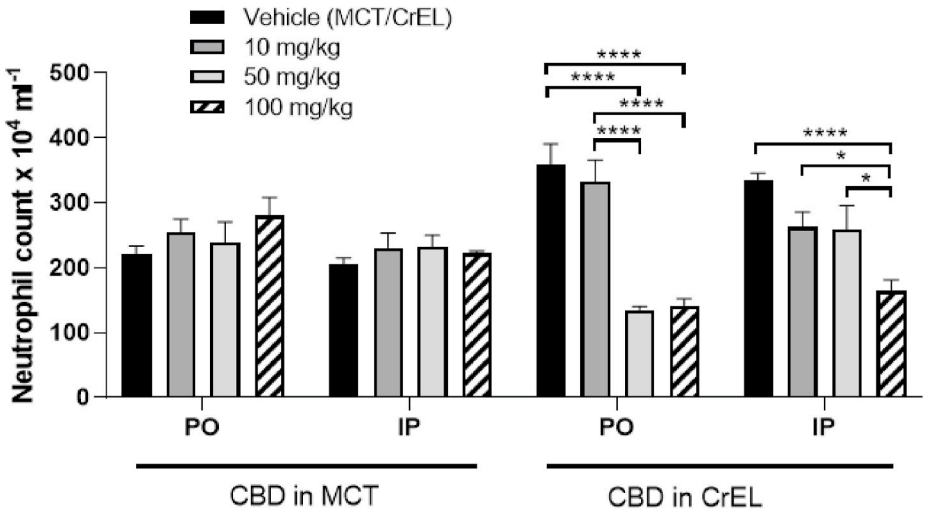

Fig. 4. Neutrophil cell count in the BAL fluid samples of control and experimental guinea pigs pre-treated with CBD in MCT and Cremophor® EL.

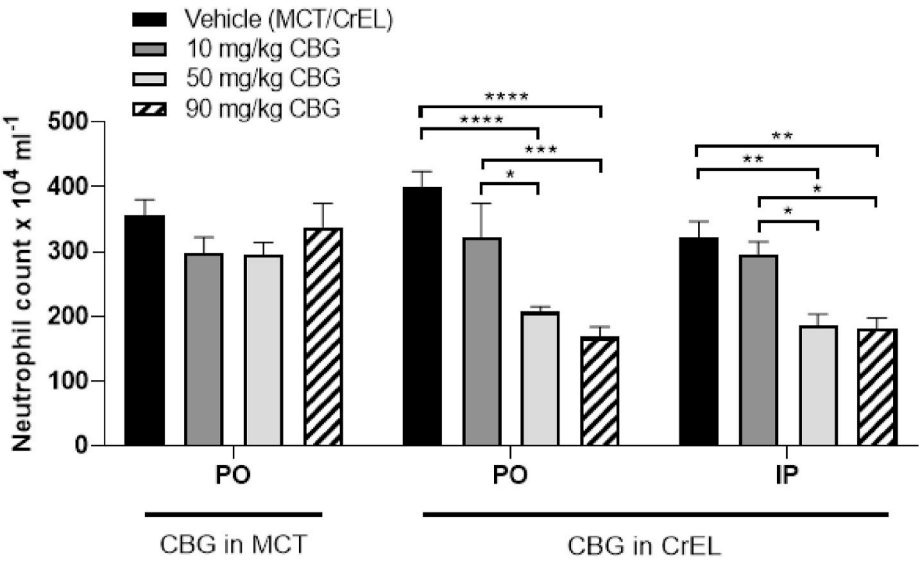

Fig. 5. *Neutrophil cell count in the BAL fluid samples of control and experimental guinea pigs pretreated with CBG in MCT and CrEL.*

for the oral and IP administrations with a 50–55% decrease in cell recruitment.

Neutrophil cell count (cells x 10^4/ml) in the bronchoalveolar lavage (BAL) fluid samples of control and treated guinea pigs, 4 h post-aerosol LPS exposure for 20 min. Animals were pre-treated with increasing concentrations of cannabidiol (CBD) orally (PO) and intraperitoneally (IP). CBD was tested individually in two different formulations; Cremophor® EL (CrEL)/ethanol/saline in a ratio of 1:1:18 or medium-chain triglycerides (MCT) oil. Vehicle animals were treated with the drug vehicle. Data: Mean ± SEM. n = 5. One-way ANOVA with Tukey's multiple comparisons. *P < 0.05; **P < 0.01; ***P < 0.001; ***P < 0.0001; ****P < 0.00001. A total of 80 animals were used in this study; n = 5 for each group.

Neutrophil cell count (cells x 10^4/ml) in the bronchoalveolar lavage (BAL) fluid samples of control and treated guinea pigs, 4 h post-aerosol LPS exposure for 20 min. Animals were pre-treated with increasing concentrations of cannabigerol (CBG) orally (PO) and intraperitoneally (IP). CBG was tested individually in two different formulations; Cremophor® EL (CrEL)/ethanol/saline in a ratio of 1:1:18 or medium-chain triglycerides (MCT) oil. Vehicle animals were treated with the drug vehicle. Data: Mean ± SEM. One-way ANOVA with Tukey's multiple comparisons. *P < 0.05; **P < 0.01; ***P < 0.001; ***P < 0.0001; ****P < 0.00001. A total of 60 animals were used in this study; n = 5 for each group.

The effect of CBD/CBG combinations on cell infiltration in the lung following LPS exposure.

The effect of a combination of CBD and CBD in CrEL administered PO was also investigated in order to determine possible synergistic or antagonistic anti-inflammatory effects. The representative doses were 10 mg/kg and 50 mg/kg, which were determined following our earlier studies with CBD and CBG alone. Each mixture contained a 50% w/w of each cannabinoid. None of the CBD and CBG combinations showed a significant change in the total or differential cell count in the BAL fluid samples when compared with the vehicle control (Fig. 6; n = 5 per group).

Neutrophil cell count (cells x 10^4/ml) in the bronchoalveolar lavage (BAL) fluid samples of control and experimental guinea pigs, 4 h post-aerosol LPS exposure for 20 min. Animals were pre-treated with increasing concentrations of cannabidiol (CBD, A) and cannabigerol (CBG, B) orally (PO) and intraperitoneally (IP). The drugs were tested individually in two different formulations. CBD and CBG were formulated in Cremophor® EL (CrEL)/ethanol/saline in a ratio of 1:1:18 or medium-chain triglycerides (MCT) oil. Vehicle animals were treated with the drug vehicle. Data: Mean ± SEM. One-way ANOVA with Tukey's multiple comparisons. *P < 0.05; **P < 0.01; ***P < 0.001; ***P < 0.0001; ****P < 0.00001. A total of 15 animals were used to study the effects of CBG and CBG combinations in neutrophil infiltration in the lungs; n = 5 for each group.

4. Discussion

Our results have extended the observations that CBD and CBG can demonstrate a clear anti-inflammatory effect in the lung by reducing the ability of LPS to induce neutrophil infiltration. However, more importantly, we have shown the relevance of determining the most appropriate formulation for any drug, as notwithstanding how effective an agent is in vitro, ultimately there is a need to deliver a sufficient amount of drug safely in an in vivo setting, and many factors other than the drug itself are important in this regard. Our results have demonstrated very clearly that the route of drug administration and choice of formulation can critically affect bioavailability and, therefore, efficacy and safety, and our results are consistent with other observations using various preparations of different cannabinoids [33,35]. For example, we have recently reported that just changing the counter ion in a drug salt can dramatically affect pharmacological activity [36], comprehensively reviewed elsewhere [37].

We have evaluated two different formulations for the cannabinoids, CBD and CBG. Using an MCT oil-based formulation, neither CBD nor CBG showed any significant anti-inflammatory effect against LPS-induced inflammation. In response to these negative results, and following earlier studies in this field that used CrEL-based formulations of cannabinoids [33,38], we performed further experiments with CBD and CBG formulated in CrEL. CrEL is a surfactant that reduces the surface tension and improves the dissolution of lipophilic drugs in an aqueous medium by forming micelles, which entrap the drugs within them. Surfactants increase permeability by disrupting the structural organisation of the lipid bilayer leading to permeation enhancement. Surfactants also exert their absorption enhancing effects by increasing the dissolution rate of the drug. On the other hand, MCT is used purely as a solvent/carrier oil, and it does not necessarily enhance absorption [39]. Our results have important implications for the numerous cannabinoid preparations available commercially, as many of these have never been formally tested for bioavailability in humans. It is questionable, therefore, whether they can deliver sufficient cannabinoid to produce the wide variety of beneficial effects that are claimed.

We have also demonstrated that oral administration of CBD and CBG

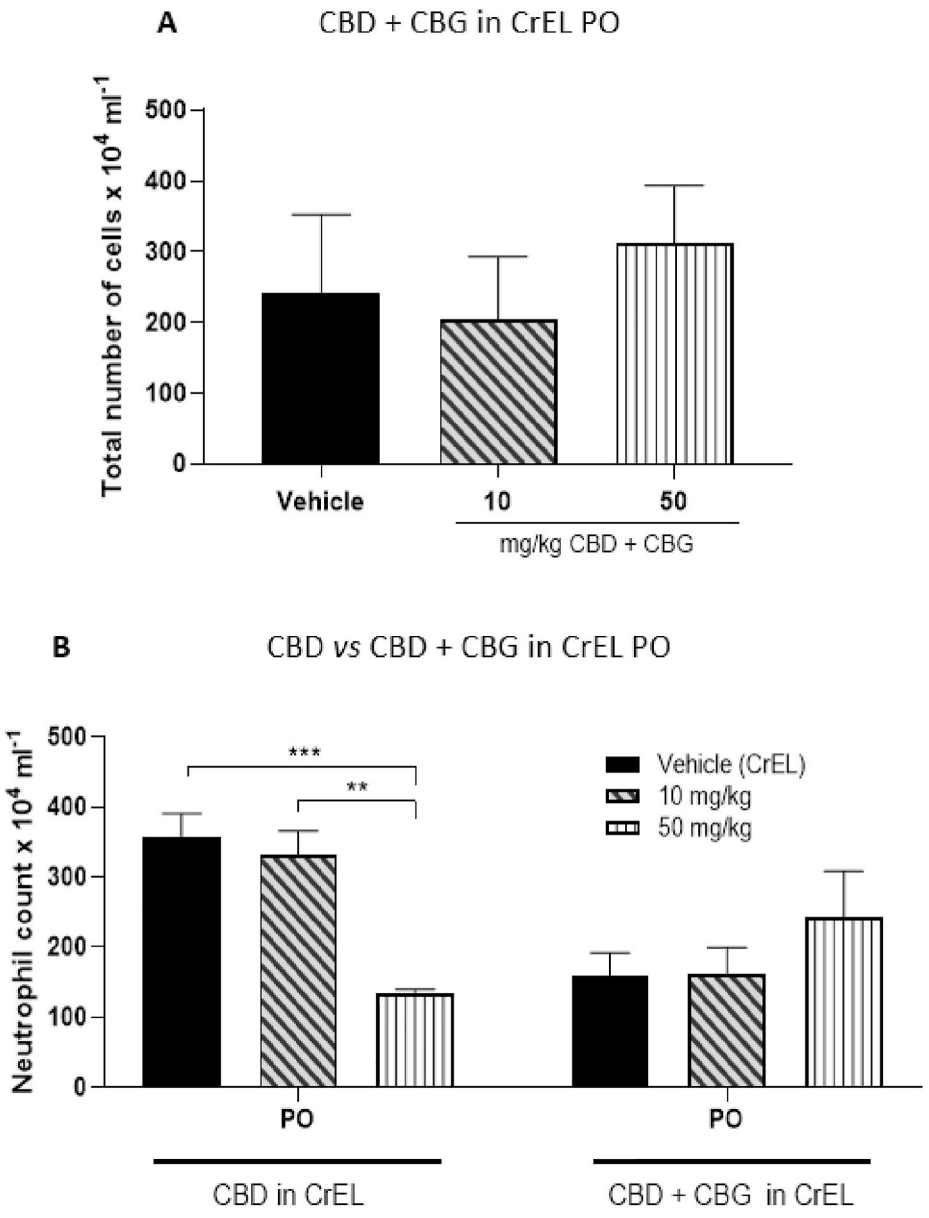

Fig. 6. *Total and neutrophil cell count in the BAL fluid samples of control and experimental guinea pigs pre-treated with CBD and CBG in various combinations.*

in CrEL was more effective than IP administration. The IP administration route is widely used in animal research as it is considered less stressful and has been suggested that theoretically, the bioavailability is similar to that of the IV administration and greater than PO [40]. This discrepancy indicates that factors other than bioavailability play a role in explaining why PO administration of CBD and CBG was more effective than when these cannabinoids were administered IP. It has been suggested that drug absorption in the GI system and metabolism in the liver influence cannabinoid distribution [41]. Furthermore, it is important to state that oral administration for both cannabinoids, was performed in animals fasted for 5–6 h. Various studies have suggested that the absorption and metabolism of cannabinoids are highly dependent on food intake, exercise and weight [41]. Additionally, it has been demonstrated that food intake can influence the PK profile of drugs. Fasting or reducing food intake will decrease liver blood flow and consequently a lower drug clearance by the liver [42]. However, this does not seem to affect IP dosing [43]. Therefore, GI absorption and bioavailability of the cannabinoids may have been increased in fasted animals.

The present findings provide further support for the anti-inflammatory effects of CBD and CBG demonstrated elsewhere [7,8,34,44]. Prompt macrophage and neutrophil recruitment to the inflammatory site is a crucial part of host defence. Additionally, macrophages exhibit tissue remodelling, pro- and anti-inflammatory phenotypes [45]. Macrophages [46] and neutrophils [47] can recruit more neutrophils to the inflammation site in their pro-inflammatory state by the release of pro-inflammatory cytokines, such as TNF-α, IL-6 and IL-12, which could lead to a pathological and overpowering inflammatory response [48]. Based on the available evidence suggesting the degree of CB2R expression in immune cells [49], and considering that macrophages are the first responders against a pathogen invasion, the CBRs likely activated in this study are those found in macrophages, and to a lesser extent, in neutrophils [50].

This study did not explore which cannabinoid receptors are responsible for the observed anti-inflammatory effects of CBD and CBG. Still, various studies have suggested that the immunoregulatory role of cannabinoids is associated with the suppression of the humoral immune response through a decrease in intracellular Ca^{2+} concentration, triggered by the dissociation of the Gi/o-protein β and γ subunits by a pertussis-toxin-sensitive G-protein-coupled mechanism [51]. This, in turn, leads to the inhibition in the release of pro-inflammatory

cytokines, preventing further neutrophil recruitment and, therefore, a disproportionate and pathological inflammatory response [48,52]. Conversely, other studies have demonstrated that CB2 receptor activation by endocannabinoids increases intracellular Ca^{2+} concentrations via the PLC-IP_3 (Phospholipase C-Inositol Triphosphate) signalling pathway [53,54]. Arguably, it is crucial to consider that receptor activation of CB2 receptors by non-endocannabinoid receptor agonists have only shown anti-inflammatory effects on leukocytes by downregulating either reactive oxygen species synthesis [17], pro-inflammatory cytokine release [55] and migration [47]. A large body of research suggests that dampening the overpowering inflammatory processes, for example, during COPD exacerbations or sepsis, would be the desired therapeutic outcome [56–59]. Additionally, the decrease in LPS-induced neutrophil infiltration in the lungs can be associated with the apoptotic properties of CBD in immune cell populations reported elsewhere [13].

While most of the pharmacological effects of CBD and CBG are related to the activation of CB1 and CB2 receptors, there is also evidence suggesting that the anti-inflammatory effects of these cannabinoids are induced by (peroxisome proliferator-activated receptor gamma) PPAR-γ activation [44]. PPAR-γ receptors have been identified in macrophages of various species and associated with their activation during inflammatory processes [60]. PPAR-γ agonists inhibit the synthesis of pro-inflammatory cytokines in monocytes, such as TNF-α, IL-1β, and IL-6 and the activation of macrophages in vitro [32]. The inhibition of macrophage activation prevents the release of MCP-1 and IL-8, which leads to neutrophil stimulation and migration at the inflammation site [61]. This could partially explain the decrease in neutrophil infiltration in the lungs by the two highest CBD and CBG doses administered to the animals.

Other studies have demonstrated that CBD inhibited the agonist activities of various cannabinoids at GPR55 receptors [62]. However, the activation of this receptor by multiple cannabinoids, including CBD, remains controversial [63]. GPR55 receptors are highly expressed in the brain, the gastrointestinal tract and adrenal glands [64] and, of relevance to our current results, are highly expressed on neutrophils [65]. Administration of systemic CBD or CBG could trigger the CB2R-mediated inhibition of oxidative species released by neutrophils, and neutrophil degranulation, leading to a weaker inflammatory response and, therefore, diminished neutrophil infiltration in the lungs following exposure to LPS. Finally, our research group [66] and others [67] have reported that LPS-induced neutrophil infiltration into the lung is platelet dependent. It is of interest that there is some evidence for an effect of cannabinoids on platelet activation [68], suggesting that further work to investigate the effects of CBD and CBG on platelets may be warranted to understand our in vivo observations better.

It has also been reported that endocannabinoids and other agents found in cannabis, such as terpenoids and flavonoids, may interact synergistically or antagonistically [69]. However, we did not find any clear evidence of additional anti-inflammatory effects when combining CBD and CBG when compared with investigating these cannabinoids alone (Fig. 6).

5. Conclusions

Our results suggest that the plant cannabinoids CBD and CBG have significant anti-inflammatory activity in the lung, but that formulation is critical to delivering an effective dose of these agents.

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