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Cannabidiol-Driven Alterations to Inflammatory Protein Landscape of Lipopolysaccharide-Activated Macrophages *In Vitro* May Be Mediated by Autophagy and Oxidative Stress

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Abstract

Background: The nonpsychotropic phytocannabinoid cannabidiol (CBD) presents itself as a potentially safe and effective anti-inflammatory treatment relative to clinical standards. In this present study, we compare the capacity of CBD to the corticosteroid dexamethasone (Dex) in altering the secreted protein landscape of activated macrophages and speculate upon the mechanism underpinning these alterations.

Materials and Methods: Human THP-1 monocytes were differentiated into macrophages (THP-1 derived macrophages [tMACs]), activated with lipopolysaccharide (LPS), and then treated with 5, 10, 25, 50, or 100 μM CBD or 10 μM Dex for 24 h. Following treatment, cytotoxicity of CBD and protein expression levels from culture supernatants and from whole cell lysates were assessed for secreted and intracellular proteins, respectively.

Results: High concentration (50 and 100 μM) CBD treatments exhibit a cytotoxic effect on LPS-activated tMACs following the 24-h treatment. Relative to the LPS-activated and untreated control (M[LPS]), both 25 μM CBD and 10 μM Dex reduced expression of pro-inflammatory markers—tumor necrosis factor alpha, interleukin 1 beta, and regulated on activation, normal T cell expressed and secreted (RANTES)—as well as the pleiotropic marker interleukin-6 (IL-6). A similar trend was observed for anti-inflammatory markers interleukin-10 and vascular endothelial growth factor (VEGF). Dex further reduced secreted levels of monocyte chemoattractant protein-1 in addition to suppressing IL-6 and VEGF beyond treatments with CBD. The anti-inflammatory capacity of 25 μM CBD was concurrent with reduction in levels of phosphorylated mammalian target of rapamycin Ser 2448, endothelial nitric oxide synthase, and induction of cyclooxygenase 2 relative to M(LPS). This could suggest that the observed effects on macrophage immune profile may be conferred through inhibition of mammalian target of rapamycin complex 1 and ensuing induction of autophagy.

Conclusion: Cumulatively, these data demonstrate cytotoxicity of high concentration CBD treatment. The data reported herein largely agree with other literature demonstrating the anti-inflammatory effects of CBD. However, there is discrepancy within literature surrounding efficacious concentrations and effects of CBD on specific secreted proteins. These data expand upon previous work investigating the effects of CBD on inflammatory protein expression in macrophages, as well as provide insight into the mechanism by which these effects are conferred.

Keywords: Cannabidiol; macrophages; dexamethasone; inflammation; cytotoxicity

Introduction

Acute inflammatory conditions within local tissues, resulting from insults, injuries, or disease, are critical components of the body's ability to heal or stave off infections. Inappropriate immune responses, however, contribute to the pathology of many conditions—such as type II diabetes, atherosclerosis, osteoarthritis, and osteoporosis[1–5]—and worsen the body's ability to recover by interfering with wound healing and tissue regeneration.[6–10] Macrophages are a primary constituent

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of the body's immune system as they are first responders to sites of insult or infection and major communicators and perpetuators of inflammatory responses. Macrophage functions encompass not only these pro-inflammatory duties but also play further roles in inflammation resolution and wound healing.[6,9] With this in mind, developing treatment modalities which target macrophages to modify immune responses is highly attractive for various conditions and pathologies.

Generally, many inflammation-associated pathologies lack safe, efficacious, and broadly available treatments.[11–14] For instance, the most typically prescribed immunosuppressants, corticosteroids, have limited efficacy on top of a multitude of deleterious side effects ranging from increased risk of infection and thrombosis to weight gain and psychological perturbances.[15–18] Dexamethasone (Dex), a commonly prescribed synthetic corticosteroid used to treat a broad range of inflammation driven pathologies,[19–21] poses additional risk to adolescents in terms of adverse neuropsychiatric events and stunted growth.[18,22] Due to these negative externalities, corticosteroids are regarded as poor candidates for long-term treatment of chronic inflammatory pathologies, despite their widespread usage. Therefore, there has been a considerable effort to expand, diversify, and develop anti-inflammatory therapeutic strategies which mitigate adverse side effects.[23]

Cannabidiol (CBD), a nonpsychotropic cannabinoid and a primary constituent of *Cannabis sativa*, presents itself as a promising candidate as a simple, safe, and potentially efficacious treatment of inflammatory pathologies.[24–31] CBD is one of many phytocannabinoids (pCBs): a class of plant-derived lipids which interact with the body's endocannabinoid system (eCBS). The eCBS is a complex and ubiquitous lipid signaling pathway that is extant in nearly all bodily systems and has a wide range of physiological functions, such as regulation of synaptic sensitivity or alterations to proliferation and differentiation of progenitor and stem cell populations.[32,33] Most pertinent is the eCBS's role as a mediator between cells in local tissues and immune responders, including macrophages, whereby native cells can recruit, inform, and modify the behavior of immune cell responses through short-range, "on-demand," lipid cues.[34–38] This makes the eCBS a highly attractive pharmacological target of pCBs for immunoregulation.

The work reported herein aims to further characterize specific concentration-dependent effects of CBD on macrophages. Macrophages express high levels of cannabinoid receptors, such as cannabinoid receptors 1 and 2 (CB1/CB2), which have been reported to attenuate macrophage polarization and behavior.[39–41] Furthermore, CBD has been previously explored as an immunomodulator, with research utilizing it toward treatment of pathologies like bowel inflammation, idiopathic autism spectrum disorder, Dravet syndrome, and Parkinson's disease.[27,42–48] Specifically in macrophages, reports by Silva et al. and Rajan et al. have explored the anti-inflammatory effects of CBD through assessing tumor necrosis factor alpha (TNFα) suppression, while others like Muthumalage and Rahman have investigated a larger panel of immune markers following exposure to CBD-containing e-liquids.[25,26,28] Subtle differences in experimental designs elicited substantial differences in their results pertaining to TNFα expression. Indeed, the disparity pertaining to CBD's macrophage-specific effects—arising from differences in treatment duration, inflammatory environment, and utilized delivery vehicles—incurred contrasting results with respect to impact in secreted inflammatory proteins and cytotoxicity. Our work seeks to expand upon macrophage inflammatory protein landscape explored in previous investigations, as well as provide additional insight into CBD's mechanism of action.

Currently, there lacks definitive mechanistic characterization within literature as to how CBD's effects are conferred. Reports have shown CBD's anti-inflammatory effects to be related to inhibition of nuclear factor kappa-light-chain-enhancer of activated B cells[25,28]; however, there have been no studies conducted exploring the effects CBD has on autophagy, which is known to impart immunomodulatory effects.[49,50] CBD has been shown to influence phosphoinositide 3-kinase (PI3K) and protein kinase B (Akt) signaling pathways which modulate autophagy through mammalian target of rapamycin complex 1 (mTORC1)—a main negative regulator of autophagy.[51–58] In addition, oxidative stress and autophagy are intrinsically linked[59,60]; however, their relationship and the effects CBD has on oxidative stress and autophagy are in conflict within literature.[61–64] Herein, we hypothesize that CBD acts, in part, through mTORC1 to achieve its anti-inflammatory effects and investigates markers of cellular oxidative response to provide insight into the role oxidative stress may be playing. Briefly, we examined cytotoxicity and expanded upon alterations to cytokine, chemokine, and growth factor secretion from lipopolysaccharide (LPS) activated macrophages following treatment with varying CBD concentrations. The concentration range,

5 to 25 μM, was selected to remain consistent with literature and, 50 to 100 μM, to investigate effects of high concentrations of CBD.[25,26,28,65] Finally, we begin to explore potential mechanistic underpinnings of CBD on LPS-activated macrophages specifically within the context of autophagy.

Materials and Methods

Cell culture

THP-1 (ATCC® TIB202™) human monocytes were thawed and maintained, in suspension, at 37°C/5% CO_2 in maintenance media containing RPMI 1640 with L-glutamine (Gibco), supplemented with 10% heat-inactivated fetal bovine serum (Gibco). THP-1s were then seeded into 24-well plates at 2.63×10^5 cells/cm^2 and cultured in maintenance media supplemented with 10 ng/mL phorbol 12-myristate 13-acetate (PMA; Sigma) for 72 h to differentiate them into macrophages (THP-1 derived macrophages [tMACs]). Following differentiation, wells were washed with Dulbecco's phosphate buffered saline (Corning) to remove undifferentiated cells,[66] and media was replaced with maintenance media containing 100 ng/mL LPS (Sigma) and varying concentrations of CBD (Cerilliant)—5, 10, 25, 50, and 100 μM (Fig. 1).[25,67,68] Ten micromolar Dex (Sigma) was added as an additional group to serve as a clinical standard anti-inflammatory treatment consistent with literature.[25,68] CBD was prepared by evaporation of methanol from original carrier before reconstitution in dimethyl sulfoxide (DMSO) and was stored protected from light at −80°C. DMSO was used as the vehicle for all treatments. Final DMSO concentration was kept constant among groups at 0.16% (v/v), well below levels which impact macrophage behavior.[69] Protein level data from tMAC vehicle controls exposed to this concentration of DMSO are included in Supplementary Table S1 along with secreted protein levels from all other treatment groups. After 24 h, culture supernatants and whole cell lysates (200 μL/well lysis buffer; 100 mM Tris, 500 mM lithium chloride, 10 mM ethylenediaminetetraacetic acid, 1% lithium dodecyl sulfate, 5 mM dithiothreitol, 1% phosphatase and protease inhibitor [Thermo Scientific], pH ~7.8) were collected for proteomic analysis.

Viability and cytotoxicity assessments

Total DNA content was measured using Quant-iT™ PicoGreen dsDNA Assay as per the manufacturer's instructions (Invitrogen). DNA content was used as a measure of net cell proliferation and loss, as previously reported.[70–72] Cytotoxicity due to CBD exposure was assessed through measuring the secretion of lactate dehydrogenase (LDH) using a commercial kit (Roche). Briefly, cell culture supernatants were collected and centrifuged at 600 *g* for 5 min. The centrifuged supernatants were reacted with the kit working solution for

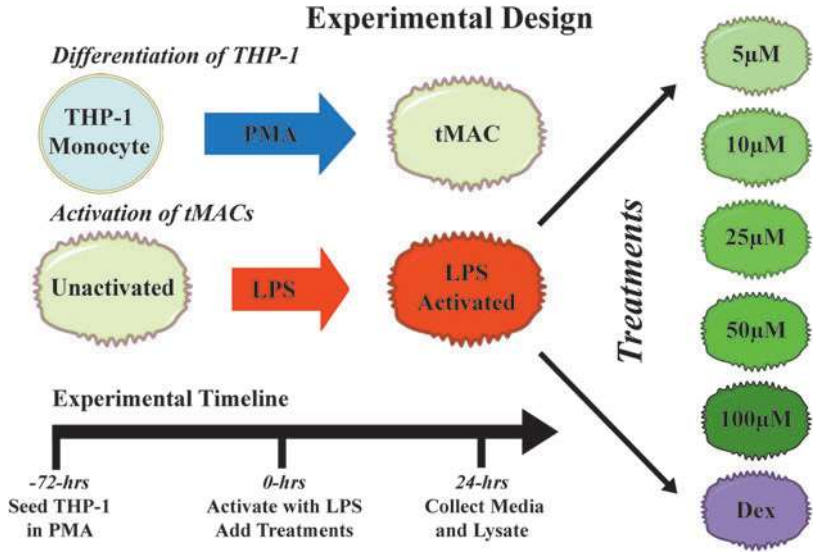

FIG. 1. Experimental design schematic. Parts of the figure are adapted from Servier Medical Art and are licensed under a Creative Commons Attribution 3.0 Unported License. Color images are available online.

25 min, followed by addition of reaction stop solution and measurement of absorbance at 490 nm (BioTek Synergy HTX multi-mode reader). To assess the number of dead cells contributing to the signal, LDH secretion was assessed against a standard curve prepared from lysed tMACs at a concentration ranging from 250,000 cells/mL to 0 cells/mL. DNA content and cytotoxicity were presented relative to the LPS-activated and untreated control group M(LPS). LDH data indicating the lack of cytotoxicity of treatments with LPS and Dex are presented in Supplementary Figure S1 and are presented relative to the vehicle control (CTL).

MAGPIX immunoassay multiplexing

Expression of proteins secreted into culture supernatants was assessed using a Human Magnetic Bead Analyte Kit (R&D Systems) as per manufacturer's instructions. A MAGPIX system (Luminex) was used to evaluate median fluorescence intensity (MFI) of each analyte. Concentrations of assessed markers were calculated comparing the MFI to generated standard curves of each analyte, as previously described.[73,74] Assessed analytes were as follows: TNFα; interleukins -1beta, -6, and -10 (IL-1β, IL-6, and IL-10, respectively); monocyte chemoattractant protein-1 (MCP-1); regulated on activation, normal T cell expressed and secreted (RANTES); and vascular endothelial growth factor (VEGF). Levels of secreted proteins were normalized to M(LPS). Unnormalized data, as well as data from the CTL group, are reported in Supplementary Table S1.

Western blot analysis

Western blot analyses were performed on cell lysates as previously described with minor modifications.[74] Briefly, concentrated denatured protein samples containing 1000 ng DNA were loaded into 6–15% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) Tris-glycine gels. Levels of expressed proteins were quantified through integrated band densitometry using Adobe Photoshop CS2 (version 9.0). Determined band intensities were normalized to loaded DNA content. Antibodies used are as follows: polyclonal mouse anti-phosphorylated mTOR Ser 2448 (p-mTOR S2448; 1:500; 59.ser2448; Santa Cruz Biotechnology), polyclonal rabbit anti-endothelial nitric oxide synthase (NOS-3; 1:500; C-20; Santa Cruz Biotechnology), polyclonal mouse anti-cyclooxygenase 2 (COX-2; 1:500; D-12; Santa Cruz Biotechnology), and polyclonal rabbit anti-beta actin (1:8000; ab8227; Abcam). Representative blot images are shown in Supplementary Figure S2.

Statistical analyses

Data are reported as mean $\pm$ standard error of the mean. Levene's test was conducted to verify homogeneity of variance. Intergroup differences in experimental means were assessed using one-way analysis of variance (ANOVA), followed by Tukey honestly significant difference (HSD) *post hoc* test for means assuming homogenous variance, otherwise Games-Howell *post hoc* test was performed. Experiments that reported with an $n=8$ were performed twice each with four independent wells, and experiments reporting $n=4$ were from one experiment with four independent wells. This is consistent with our previous work[75] and work by Silva et al.[28] Sufficient n was ensured by performing power analysis for one-way ANOVA. Analyses were conducted using SPSS software (Version 26.0). Statistical significance was determined at p-value <0.05.

Results

Concentration-dependent effects of CBD on tMAC DNA content and cytotoxicity

We initially assessed potential cytotoxic effects of CBD on M(LPS) using total DNA content and LDH secretion. Relative to M(LPS), treatments with 50 and 100 μM CBD substantially decreased DNA content (>5.30-fold; $p<0.001$; Fig. 2A), while treatment of 25 μM displayed a more modest decrease in DNA content (~ 1.27-fold; $p<0.035$). To clarify the extent to which these differences in DNA levels were due to differences in proliferation versus cytotoxicity, lactose dehydrogenase (LDH) was also measured. As shown in Figure 2B, there was no significant difference in cytotoxicity, relative to M(LPS), for treatments of 5, 10, or 25 μM CBD. CBD treatment at 100 μM, however, displayed a substantial increase (~ 3.48-fold; $p<0.020$) relative to M(LPS). Despite a ~ 2.98-fold increase in cytotoxicity, 50 μM CBD treatment did not yield a difference relative to M(LPS) ($p=0.090$). However, due to the combined DNA and cytotoxicity results for 50 and 100 μM CBD relative to M(LPS), the remainder of our work focused on concentrations of CBD—5 to 25 μM.

Concentration-dependent effects of CBD on proteins secreted from LPS activated tMACs

We next conducted proteomic-level analysis of secreted cytokines, chemokines, and growth factors 24 h after LPS activation and treatment with CBD or Dex (Fig. 3). Relative to M(LPS), treatments of 10 and 25 μM CBD and 10 μM Dex showed a significant reduction in expression of pro-inflammatory cytokines

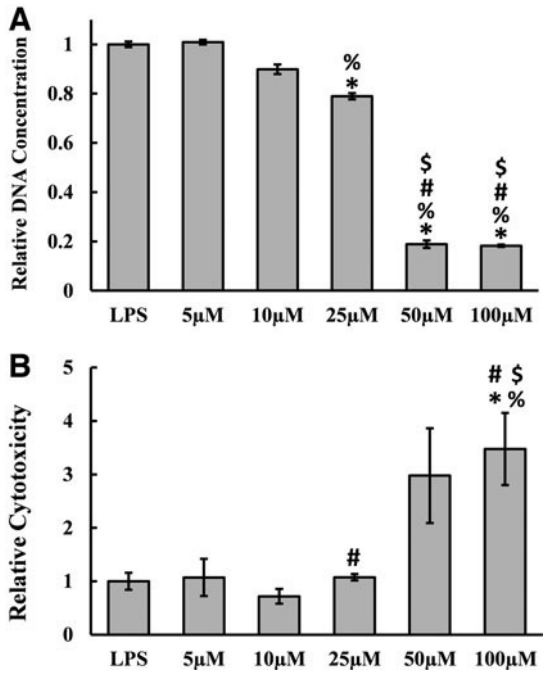

FIG. 2. **(A)** Relative DNA measures from sample lysates following 24-h CBD treatment with varying concentrations. All groups are normalized to M(LPS). Error bars correspond to the standard error of the mean. Statistical significance was determined using Tukey *post hoc* test ($n=4$, $p<0.05$). **(B)** Cytotoxicity, normalized to M(LPS), following 24-h CBD treatment (5–100 μM) as determined by LDH assay. Error bars correspond to the standard error of the mean. Statistical significance was determined using Games-Howell *post hoc* test ($n=4$, $p<0.05$). The asterisk (*) denotes significant difference relative to M(LPS). The percent (%) denotes significant difference relative to 5 μM CBD. The pound (#) denotes significant difference to 10 μM CBD. The dollar sign ($) denotes significant difference to 25 μM CBD. CBD, cannabidiol; LDH, lactate dehydrogenase; M(LPS), LPS-activated and untreated control.

TNFα ($\sim$1.43-fold; $p<0.019$, $\sim$2.13-fold; $p<0.001$, and $\sim$4.55-fold; $p<0.001$, respectively), IL-1β ($\sim$2.17-fold; $p<0.001$, $\sim$5.88-fold; $p<0.001$, and $\sim$10.0-fold; $p<0.001$, respectively) (Fig. 3A), and the chemokine RANTES ($\sim$1.45-fold; $p<0.001$, $\sim$3.85-fold; $p<0.001$, and $\sim$2.22-fold; $p<0.001$, respectively) (Fig. 3B). Furthermore, treatments of 25 μM CBD and 10 μM Dex decreased levels of TNFα, IL-1β, and RANTES beyond treatment with 10 μM CBD (greater than $\sim$1.49-fold; $p<0.001$, greater than $\sim$2.71-fold; $p<0.004$, and greater than $\sim$1.53-fold; $p<0.024$, respectively). Relative levels of MCP-1 were only reduced by treatment with Dex ($\sim$68.5-fold; $p<0.001$).

Interestingly, relative levels of assessed pleiotropic and anti-inflammatory markers demonstrated similar levels of reduction following treatment of CBD (Fig. 3). Relative to M(LPS), treatment with 10 μM CBD elicited a modest reduction in both the cytokine IL-10 ($\sim$1.45-fold; $p<0.004$) (Fig. 3A) and growth factor VEGF ($\sim$1.52-fold; $p<0.006$) (Fig. 3B) secretion, but did not alter the levels of the cytokine IL-6. However, 25 μM CBD and 10 μM Dex treatments greatly reduced expression levels of IL-6 relative to M(LPS) ($>$4.17-fold; $p<0.001$), IL-10 ($>$4.35-fold; $p<0.001$), and VEGF ($>$2.27-fold; $p<0.001$). Furthermore, 10 μM Dex decreased levels of IL-6 and VEGF compared to 25 μM CBD treatment ($\sim$14.6-fold; $p<0.001$ and $\sim$3.38-fold, respectively; $p<0.001$). CBD at 5 μM had no significant effects on any assessed secreted proteins, relative to M(LPS). Cumulatively, these data suggest the following: that (1) CBD's potency in altering expression of assessed markers is concurrent with increasing concentrations; (2) treatments with 25 μM CBD act in an inhibitory manner for the secretion of markers associated with both pro- and anti-inflammatory states; and (3) while treatments with 25 μM CBD and 10 μM Dex display similar suppression of key inflammatory proteins, the immunological profiles induced by CBD may be more indicative of an altered polarization state compared to the more quiescent profile by Dex.

CBD driven alterations to markers of autophagy and cellular oxidative stress in tMACs

To explore a potential mechanism through which CBD may confer the observed effects, we then assessed expression levels of proteins linked to autophagy and oxidative stress in CBD-treated tMACs (Fig. 4). p-mTOR S2448, a phosphorylation site correlated with mTORC1 activation,[54] was assessed as a proxy for suppression of autophagic activity. Compared to the vehicle control CTL, M(LPS) displayed a strong increase in p-mTOR S2448 expression ($\sim$3.03-fold; $p<0.004$) suggestive of suppressed autophagy. CBD at 5 and 10 μM did not reduce p-mTOR S2448 levels compared to M(LPS). However, 25 μM CBD resulted

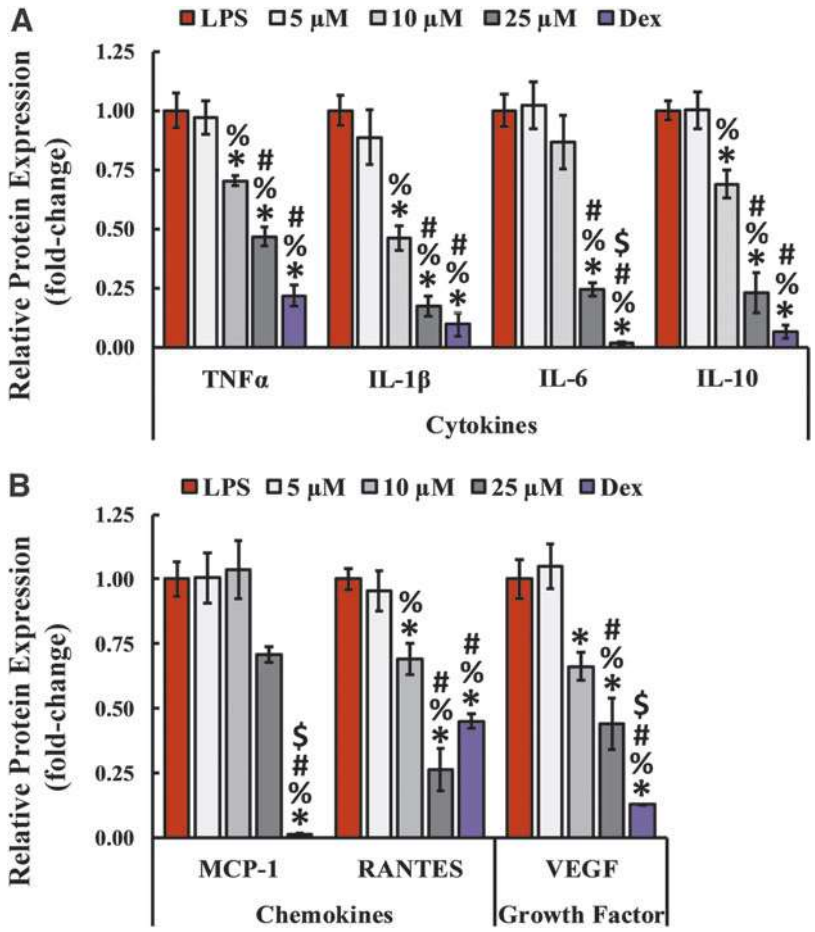

FIG. 3. Relative expression levels of **(A)** cytokines **(B)** chemokines and growth factor assessed from LPS-activated, tMAC culture supernatants following 24-h CBD (5–25 μM) or Dex (10 μM) treatment. All groups are shown relative to M(LPS) and in terms of fold-change (pg/pg). Results are from independent experiments ($n = 8$) for LPS, 5, 10, and 25 μM CBD, and ($n = 4$) for 10 μM Dex. Error bars correspond to the standard error of the mean. The asterisk (*) denotes significant difference relative to M(LPS). The percent (%) denotes significant difference relative to 5 μM CBD. The pound (#) denotes significant difference relative to 10 μM CBD. The dollar sign ($) denotes significant difference relative to 25 μM CBD. Statistical significance was determined using Tukey (RANTES) and Games-Howell (TNFα, MCP-1, IL-1β, IL-6, IL-10, and VEGF) *post hoc* tests ($p < 0.05$). Dex, dexamethasone; IL-1β, interleukin-1beta; IL-6, interleukin-6; IL-10, interleukin-10; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein-1; RANTES, regulated on activation, normal T cell expressed and secreted; TNFα, tumor necrosis factor alpha; VEGF, vascular endothelial growth factor. Color images are available online.

in a substantive reduction in p-mTOR S2448 expression relative to M(LPS) ($\sim$3.12-fold; $p < 0.004$) to comparable levels to that in CTL. NOS-3, an inducer of nitric oxide species (NO),[76] was also assessed. M(LPS) demonstrated a strong increase compared to CTL ($\sim$2.02-fold; $p < 0.027$) suggesting increased NO generation in the M(LPS) group. A concentration of 25 μM CBD showed a significant reduction in NOS-3 compared to M(LPS) ($\sim$2.34-fold; $p < 0.011$). Although concentrations of 5 and 10 μM CBD also displayed a concentration dependent suppressive effect on NOS-3, this was not statistically significant. Surprisingly, treatment with 25 μM CBD elicited a dramatic increase in COX-2—a responder to oxidative stress—compared to

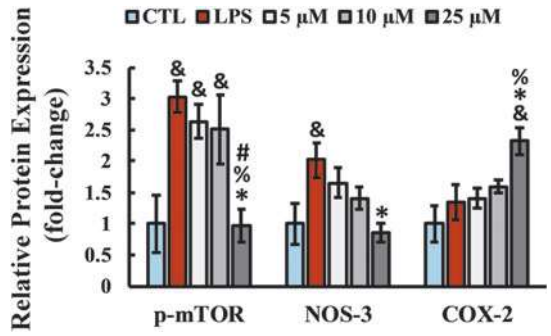

FIG. 4. Relative expression levels of autophagy and cellular oxidative stress related proteins p-mTOR S2448, NOS-3, and COX-2 were assessed from LPS-activated tMAC whole cell lysates following 24-h treatment with CBD (5–25 μM). All groups are presented relative to CTL. Results are from independent experiments ($n=4$). Error bars correspond to standard error of the mean. The ampersand (&) denotes significant differences relative to CTL. The asterisk (*) denotes significant difference relative to M(LPS). The percent (%) denotes significant difference relative to 5 μM CBD. The pound (#) denotes significant difference relative to 10 μM CBD. Statistical significance was determined using one-way ANOVA and Tukey *post hoc* tests ($p<0.05$). ANOVA, analysis of variance; COX-2, cyclooxygenase 2; p-mTOR, phosphorylated mammalian target of rapamycin; NOS-3, endothelial nitric oxide synthase. Color images are available online.

CTL, M(LPS), and 5 μM CBD (greater than $\sim$1.65-fold; $p<0.031$). Cumulatively, these data suggest that treatment with 25 μM CBD may reverse LPS-induced mTORC1 activation thereby restoring basal autophagic activity, but these effects may be mediated by non-NO driven oxidative stress as indicated by the reduction in NOS-3 accompanied by COX-2 elevation.

Discussion

The present work sought to expand the understanding on the specific effects of a nonpsychotropic cannabinoid—CBD—on alterations to secreted cytokines, chemokines, and growth factors by LPS-activated macrophages and to explore potential mechanisms which may be responsible for these alterations. Briefly, human THP-1 monocytes were differentiated to macrophages, simultaneously activated with the pro-inflammatory LPS and treated with a broad range of concentrations of CBD for 24 h. Following this, culture supernatants were collected and analyzed for secreted proteins and relative cytotoxicity, and whole cell lysates were collected and analyzed for autophagy and oxidative stress-related proteins.

Our DNA and LDH results suggest that high concentrations of CBD—50 and 100 μM—exhibit a strong cytotoxic effect on tMACs. While reports of CBD cytotoxicity in literature appear to be conflicting across different cell types,[27,77,78] immune cells appear to have heightened sensitivity to the cytotoxic effects of cannabinoids.[25,28,79] For instance, evaluation of CBD-containing e-liquid by Muthumalage and Rahman demonstrated concentrations up to 42.4 μM to be noncytotoxic to lung epithelial cells and fibroblasts but noted a slight cytotoxic effect in RAW264.7 macrophages.[25] Similarly, Silva et al. found a slight toxic response at 58 μM CBD with regards to viability of RAW264.7 macrophages.[28] Furthermore, recent reports documented that immune responders display variable sensitivity to cannabinoids associated with a dynamic cannabinoid receptor presentation in different polarization states.[39,44,80–82] For example, Carlisle et al. found that in murine macrophages, CB2 was not present in their inactivated state, whereas it was highly expressed when they are activated with interferon gamma but, to a lesser extent, following activation with LPS.[39] Altogether, this suggests that immune cell-specific cytotoxicity to CBD is affected by multiple factors, including polarization state at time of CBD exposure and presentation of CBD-sensitive receptors. Immediate future work will explore macrophage-specific response to high concentration CBD in alternative polarization states to deconvolute the influence of alterations to cannabinoid receptor profiles and activation signals.

In this study we found that 10 and 25 μM CBD treatment had a significant effect on decreasing secreted levels of inflammatory proteins. The reduction in expression of cytokines associated with strong inflammatory response, such as TNFα and IL-1β, is indicative of a potentially strong immunosuppressive effect. With respect to TNFα, our results with tMACs are largely consistent with other reports which utilized murine macrophages.[24,26–28] Specifically, Rajan et al. and Silva et al. reported a reduction in TNFα production in RAW264.7 murine macrophages for CBD concentrations of 2.5 and 5 μM and of 30 and 100 μM, respectively.[26,28] In contrast, Muthumalage and Rahman

reported that 21.2 μM CBD treatment increased levels of TNFα. This discrepancy may be due to their use of CBD-containing e-liquids containing propylene glycol or vegetable glycerin, which have been previously implicated in disrupting macrophage function.[83] Another potential explanation for the differences between our results compared to other works is different levels of CBD metabolites in our experimental conditions. The effects of these metabolites on macrophages specifically remain unexplored but are regarded to be potentially significant.[84,85] As to the reduction in IL-1β expression, no previous studies demonstrated this in macrophages following CBD treatment. However, suppressive effects of CBD on IL-1β production have been previously observed in other immune cell types, such as BV-2 microglia, following similar CBD concentrations.[86,87] Overall, these data allude to CBD having a significant suppressive effect on typical inflammatory markers that are major constituents and perpetuators of immune responses.

In terms of chemokine production, our data demonstrate reduction to RANTES, but not MCP-1, following CBD treatment. Both MCP-1 and RANTES are chemoattractants, and their elevation suggests a recruitment of immune cells *in vivo*.[88–90] Despite MCP-1 being generally described as a pro-inflammatory marker, several studies have also reported it to have a robust role in wound healing.[91–93] In contrast, RANTES, unlike MCP-1, is generally considered deleterious to the alternative noninflammatory roles of macrophages.[94,95] Therefore, its suppression appears to be desirable toward altering inflammatory polarization states.

A notable finding in our work was the comparable suppression of pro-inflammatory markers by CBD, relative to Dex, without as strong of a reduction in pleiotropic and anti-inflammatory protein expression. Specifically, we observed that CBD does not have as stringent effect on the expression of IL-6, VEGF, and IL-10 as Dex, although not significantly for IL-10. The impact of Dex treatment is contextualized in reports, which demonstrate corticosteroid impedance of macrophage-facilitated processes, such as wound healing,[6,15,16] especially given the significance of VEGF and IL-6 in macrophage-facilitated wound healing.[96,97] Future work comparing the impact of CBD and Dex on macrophage function in wound healing models, and exploring chronic application of CBD, will be necessary for supporting CBD's potential as an alternative therapeutic strategy.

A limitation of this work is that it was restricted to exploring the effects of acute inflammation and coincident LPS-CBD administration due to the limited life span of tMACs following PMA withdrawal.[98] Furthermore, it is important to note that the concentrations explored within this study are substantially higher than blood concentrations seen during typical ingestion of CBD or other cannabinoids.[99] The significant immunosuppressive effects seen at these high concentrations may help inform development of new acute anti-inflammatory treatment modalities. However, chronic administration of more typical concentrations should be explored within future work toward development of habitual anti-inflammatory treatments. Finally, while CBD's interactions with glucocorticoid receptors appear negligible,[100] it is known that CBD and Dex have opposing effects on cytochrome P450 and could be harmful if taken in conjunction,[101,102] indicating that combinatorial treatments with CBD and Dex may be potentially inadvisable.

Concurrent to its effects on inflammatory proteins, CBD treatment was observed to alter the expression of p-mTOR S2448—an indicator of mTORC1 activation and subsequent autophagic suppression[54]—and the expression of NOS-3 and COX-2—a generator of NO and responder to oxidative stress,[103,104] respectively. Our observed CBD results suggested induction of autophagy—through decrease in p-mTOR S2448 expression—in agreement with literature. For example, 10 μM CBD treatment of breast cancer cell lines—MDA-MB-231, MCF-7, SK-BR-3, ZR-75-1—and human umbilical vein endothelial cells was, respectively, reported by Shrivastava et al. and Böckman and Hinz to induce autophagy,[61,63] specifically with coincident suppression in p-mTOR S2448 expression in the report by Shrivastava et al.[61] Both these reports also demonstrate a CBD-induced upregulation of oxidative stress response. The discrepancy between these reports and our observations with respect to NOS-3 reduction may be explained by the contribution of non-NO-derived cellular oxidative stress response. This is further supported by our observed increase in COX-2 expression by CBD given the role COX-2 plays as a responder to oxidative stress. This warrants further investigation given previous reports of CBD being an antioxidant.[105] Altogether, these data provide preliminary evidence of a link between CBD-driven alterations to inflammatory proteins and oxidative stress and autophagic induction. Overall, our data are in support of CBD's potential as an anti-inflammatory treatment modality, corroborating a growing body of literature exploring CBD treatments.[30,106,107]

Conclusions

Our results compared the efficacy of CBD to the commonly prescribed corticosteroid, Dex, in altering the secreted protein landscape of LPS-activated macrophages. Our initial data indicated increased cytotoxicity and decreased macrophage viability following treatment with CBD concentrations greater than 25 μM. As observed, CBD concentrations—10 to 25 μM—attenuated the expression of pro-inflammatory proteins similarly to treatments with Dex. In contrast, CBD suppression of pleiotropic and anti-inflammatory markers was less severe than what was observed following treatment with Dex. Future studies will be needed to expand on CBD's ability to promote macrophage inflammatory responses conducive to improved wound healing and inflammation resolution. Finally, we provide some initial evidence suggesting that these anti-inflammatory effects are potentially driven by CBD suppressing mTORC1 concomitantly inducing autophagy. Future work holistically characterizing the effects CBD has on the PI3K/Akt/mTOR pathways, cellular oxidative stress response, and how this relates to inflammation machinery is necessary to understand the mechanisms through which CBD acts.

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Author Disclosure Statement

D.J.Y. conceived and designed the experiments. D.J.Y. and A.S.A. performed experiments and collected, analyzed, and interpreted data. D.J.Y. and A.S.A. wrote the article. M.S.H. revised and edited the document. All authors have reviewed and approved the article before submission. The authors declare that the article has not been published, in press, or submitted elsewhere. The authors have no conflicts of interest to declare.

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Supplementary Material

Supplementary Table S1
Supplementary Figure S1
Supplementary Figure S2

References

1. Khanna S, Biswas S, Shang Y, et al. Macrophage dysfunction impairs resolution of inflammation in the wounds of diabetic mice. PLoS One. 2010;5:e9539.
2. Cochain C, Zernecke A. Macrophages in vascular inflammation and atherosclerosis. Eur J Physiol. 2017;469:485–499.
3. Ni Z, Kuang L, Chen H, et al. The exosome-like vesicles from osteoarthritic chondrocyte enhanced mature IL-1β production of macrophages and aggravated synovitis in osteoarthritis. Cell Death Dis. 2019;10:522.
4. Michalski MN, McCauley LK. Macrophages and skeletal health. Pharmacol Ther. 2017;174:43–54.
5. Yang DH, Yang MY. The role of macrophage in the pathogenesis of osteoporosis. Int J Mol Sci. 2019;20:2093.
6. Guo S, DiPietro LA. Factors affecting wound healing. J Dent Res. 2010;89:219–229.
7. Serra MB, Barroso WA, da Silva NN, et al. From inflammation to current and alternative therapies involved in wound healing. Int J Inflam. 2017;2017:3406215.
8. Goodman SB, Pajarinen J, Yao Z, et al. Inflammation and bone repair: from particle disease to tissue regeneration. Front Bioeng Biotechnol. 2019;7:1–11.
9. Sindrilaru A, Scharffetter-Kochanek K. Disclosure of the culprits: macrophages—versatile regulators of wound healing. Adv Wound Care. 2013;2:357–368.
10. Sindrilaru A, Peters T, Wieschalka S, et al. An unrestrained proinflammatory M1 macrophage population induced by iron impairs wound healing in humans and mice. J Clin Invest. 2011;121:985–997.
11. Na SY, Moon W. Perspectives on current and novel treatments for inflammatory bowel disease. Gut Liver. 2019;13:604–616.
12. Petta I, Peene I, Elewaut D, et al. Risks and benefits of corticosteroids in arthritic diseases in the clinic. Biochem Pharmacol. 2019;165:112–125.
13. Garza-Mayers AC, McClurkin M, Smith GP. Review of treatment for discoid lupus erythematosus. Dermatol Ther. 2016;29:274–283.
14. Zhang W, Ouyang H, Dass CR, et al. Current research on pharmacologic and regenerative therapies for osteoarthritis. Bone Res. 2016;4:15040.
15. Wang AS, Armstrong EJ, Armstrong AW. Corticosteroids and wound healing: clinical considerations in the perioperative period. Am J Surg. 2013;206:410–417.
16. Liu YZ, Akhter MP, Gao X, et al. Glucocorticoid-induced delayed fracture healing and impaired bone biomechanical properties in mice. Clin Interv Aging. 2018;13:1465–1474.
17. Chikanza IC, Kozaci DL. Corticosteroid resistance in rheumatoid arthritis: molecular and cellular perspectives. Rheumatology. 2004;43:1337–1345.
18. Yu SH, Drucker AM, Lebwohl M, et al. A systematic review of the safety and efficacy of systemic corticosteroids in atopic dermatitis. J Am Acad Dermatol. 2018;78:733–740.e11.
19. Grodzinsky AJ, Wang Y, Kakar S, et al. Development of post-traumatic osteoarthritis. J Orthop Res. 2017;35:406–411.
20. Keeney GE, Gray MP, Morrison AK, et al. Dexamethasone for acute asthma exacerbations in children: a meta-analysis. Pediatrics. 2014;133:493–499.
21. Magro G. COVID-19: review on latest available drugs and therapies against SARS- CoV-2. Coagulation and inflammation cross-talking. Virus Res. 2020;286:1–9.
22. Kenna HA, Poon AW, de los Angeles CP, et al. Psychiatric complications of treatment with corticosteroids: review with case report. Psychiatry Clin Neurosci. 2011;65:549–560.
23. Dinarello CA. Anti-inflammatory agents: present and future. Cell. 2010;140:935–950.
24. Ben-Shabat S, Hanuš LO, Katzavian G, et al. New cannabidiol derivatives: synthesis, binding to cannabinoid receptor, and evaluation of their antiinflammatory activity. J Med Chem. 2006;49:1113–1117.
25. Muthumalage T, Rahman I. Cannabidiol differentially regulates basal and LPS-induced inflammatory responses in macrophages, lung epithelial cells, and fibroblasts. Toxicol Appl Pharmacol. 2019;382:114713.

26. Rajan TS, Giacoppo S, Iori R, et al. Anti-inflammatory and antioxidant effects of a combination of cannabidiol and moringin in LPS-stimulated macrophages. Fitoterapia. 2016;112:104–115.
27. Mammana S, Cavalli E, Gugliandolo A, et al. Could the combination of two non-psychotropic cannabinoids counteract neuroinflammation? Effectiveness of cannabidiol associated with cannabigerol. Medicina (Kaunas) 2019;55:747.
28. Silva RL, Silveira GT, Wanderlei CW, et al. DMH-CBD, a cannabidiol analog with reduced cytotoxicity, inhibits TNF production by targeting NF-kB activity dependent on A 2A receptor. Toxicol Appl Pharmacol. 2019;368:63–71.
29. Sacerdote P, Martucci C, Vaccani A, et al. The nonpsychoactive component of marijuana cannabidiol modulates chemotaxis and IL-10 and IL-12 production of murine macrophages both *in vivo* and *in vitro*. J Neuroimmunol. 2005;159:97–105.
30. Ribeiro A, Ferraz-de-paula V, Pinheiro ML, et al. Cannabidiol, a non-psychotropic plant-derived cannabinoid, decreases inflammation in a murine model of acute lung injury: role for the adenosine A 2A receptor. Eur J Pharmacol. 2012;678:78–85.
31. Liu D, Hu C, Huang C, et al. Cannabidiol attenuates delayed-type hypersensitivity reactions via suppressing T-cell and macrophage reactivity. Acta Pharmacol Sin. 2010;31:1611–1617.
32. Brown SP, Brenowitz SD, Regehr WG. Brief presynaptic bursts evoke synapse-specific retrograde inhibition mediated by endogenous cannabinoids. Nat Neurosci. 2003;6:1048–1057.
33. Galve-Roperh I, Chiurchiù V, Díaz-Alonso J, et al. Cannabinoid receptor signaling in progenitor/stem cell proliferation and differentiation. Prog Lipid Res. 2013;52:633–650.
34. Maccarrone M, Bab I, Cabral GA, et al. Endocannabinoid signaling at the periphery: 50 years after THC. Trends Pharmacol Sci. 2016;36:277–296.
35. Tanasescu R, Constantinescu CS. Cannabinoids and the immune system: an overview. Immunobiology. 2010;215:588–597.
36. Sugiura T, Oka S, Gokoh M, et al. New perspectives in the studies on endocannabinoid and cannabis: 2-arachidonoylglycerol as a possible novel mediator of inflammation. J Pharmacol Sci. 2004;375:367–375.
37. Cabral GA, Ferreira GA, Jamerson MJ. Endocannabinoids and the immune system in health and disease. Handb Exp Pharmacol. 2015;231:185–211.
38. Acharya N, Penukonda S, Shcheglova T, et al. Endocannabinoid system acts as a regulator of immune homeostasis in the gut. Proc Natl Acad Sci U S A. 2017;114:5005–5010.
39. Carlisle SJ, Staab A, Ludwick C, et al. Differential expression of the CB 2 cannabinoid receptor by rodent macrophages and macrophage-like cells in relation to cell activation. Int Immunopharmacol. 2002;2:69–82.
40. O'Sullivan SE. An update on PPAR activation by cannabinoids. Br J Pharmacol. 2016;173:1899–1910.
41. Romano B, Borrelli F, Fasolino I, et al. The cannabinoid TRPA1 agonist cannabichromene inhibits nitric oxide production in macrophages and ameliorates murine colitis. Br J Pharmacol. 2013;169:213–229.
42. Zgair A, Lee JB, Wong JCM, et al. Oral administration of cannabis with lipids leads to high levels of cannabinoids in the intestinal lymphatic system and prominent immunomodulation. Sci Rep. 2017;7:14542.
43. Coffey RG, Yamamoto Y, Snella E, et al. Tetrahydrocannabinol inhibition of macrophage nitric oxide production. Biochem Pharmacol. 1996;52:743–751.
44. Cabral GA, Raborn ES, Griffin L, et al. CB 2 receptors in the brain: role in central immune function. Br J Pharmacol. 2008;153:240–251.
45. Celorrio M, Rojo-Bustamante E, Fernández-Suárez D, et al. GPR55: a therapeutic target for Parkinson's disease? Neuropharmacology. 2017;125:319–332.
46. Gallily R, Yekhtin Z, Hanus LO. The anti-inflammatory properties of terpenoids from cannabis. Cannabis Cannabinoid Res. 2018;3:282–290.
47. Devinsky O, Cross JH, Laux L, et al. Trial of cannabidiol for drug-resistant seizures in the Dravet syndrome. N Engl J Med. 2017;376:2011–2020.
48. Barchel D, Stolar O, De-Haan T, et al. Oral cannabidiol use in children with autism spectrum disorder to treat related symptoms and Co-morbidities. Front Pharmacol. 2019;9:1–5.
49. Zhong Z, Sanchez-Lopez E, Karin M. Autophagy, NLRP3 inflammasome and auto-inflammatory/immune diseases. Clin Exp Rheumatol. 2016;34:12–16.
50. Qian M, Fang X, Wang X. Autophagy and inflammation. Clin Transl Med. 2017;6:24.
51. Dibble CC, Cantley LC. Regulation of mTORC1 by PI3K signaling Christian. Trends Cell Biol. 2015;25:545–555.
52. Weinberg MA. RES-529: A PI3K/AKT/mTOR pathway inhibitor that dissociates the mTORC1 and mTORC2 complexes. Anticancer Drugs. 2016;27:475–487.
53. Zhou M, Xu W, Wang J, et al. Boosting mTOR-dependent autophagy via upstream TLR4-MyD88-MAPK signalling and downstream NF-κB pathway quenches intestinal inflammation and oxidative stress injury. EBioMedicine. 2018;35:345–360.
54. Rosner M, Siegel N, Valli A, et al. MTOR phosphorylated at S2448 binds to raptor and rictor. Amino Acids. 2010;38:223–228.
55. Lanza Cariccio V, Scionti D, Raffa A, et al. Treatment of periodontal ligament stem cells with MOR and CBD promotes cell survival and neuronal differentiation via the PI3K/Akt/mTOR pathway. Int J Mol Sci. 2018;19:2341.
56. Lin B, Gao Y, Li Z, et al. Cannabidiol alleviates hemorrhagic shock-induced neural apoptosis in rats by inducing autophagy through activation of the PI3K/AKT pathway. Fundam Clin Pharmacol. 2020;34:640–649.
57. Giacoppo S, Pollastro F, Grassi G, et al. Target regulation of PI3K/Akt/mTOR pathway by cannabidiol in treatment of experimental multiple sclerosis. Fitoterapia. 2017;116:77–84.
58. Rabanal-Ruiz Y, Otten EG, Korolchuk VI. MTORC1 as the main gateway to autophagy. Essays Biochem. 2017;61:565–584.
59. Li L, Tan J, Miao Y, et al. ROS and autophagy: interactions and molecular regulatory mechanisms. Cell Mol Neurobiol. 2015;35:615–621.
60. Sarkar S, Korolchuk VI, Renna M, et al. Complex inhibitory effects of nitric oxide on autophagy. Mol Cell. 2011;43:19–32.
61. Shrivastava A, Kuzontkoski PM, Groopman JE, et al. Cannabidiol induces programmed cell death in breast cancer cells by coordinating the cross-talk between apoptosis and autophagy. Mol Cancer Ther. 2011;10:1161–1172.
62. Jeong S, Kim BG, Kim DY, et al. Cannabidiol overcomes oxaliplatin resistance by enhancing NOS3- and SOD2-Induced autophagy in human colorectal cancer cells. Cancers (Basel). 2019;11:1–18.
63. Böckmann S, Hinz B. Cannabidiol promotes endothelial cell survival by heme oxygenase-1-mediated autophagy. Cells. 2020;9:1703.
64. Yang L, Rozenfeld R, Wu D, et al. Cannabidiol protects liver from binge alcohol-induced steatosis by mechanisms including inhibition of oxidative stress and increase in autophagy. Free Radic Biol Med. 2014;68:260–267.
65. Fitzpatrick J-M, Minogue E, Curham L, et al. MyD88-dependent and -independent signalling via TLR3 and TLR4 are differentially modulated by Δ9-tetrahydrocannabinol and cannabidiol in human macrophages. J Neuroimmunol. 2020;343:577217.
66. Tsuchiya S, Gota Y, Okumura H, et al. Induction of maturation in cultured human monocytic leukemia cells by a phorbol diester. Cancer Res. 1982;42:1530–1536.
67. Vogel DYS, Glim JE, Stavenuiter AWD, et al. Human macrophage polarization *in vitro*: maturation and activation methods compared. Immunobiology. 2014;219:695–703.
68. Hosono H, Homma M, Ogasawara Y, et al. Expression of phosphatidylserine-specific phospholipase A1 mRNA in human THP-1-derived macrophages. Cell Transplant. 2010;19:759–764.
69. De Abreu Costa L, Ottoni MHF, Dos Santos MG, et al. Dimethyl sulfoxide (DMSO) decreases cell proliferation and TNF-α, IFN-, and IL-2 cytokines production in cultures of peripheral blood lymphocytes. Molecules. 2017;22:1–10.
70. Jones LJ, Gray M, Yue ST, et al. Sensitive determination of cell number using the CyQUANT® cell proliferation assay. J Immunol Methods. 2001;254:85–98.
71. Blaheta RA, Kronenberger B, Woitaschek D, et al. Development of an ultrasensitive *in vitro* assay to monitor growth of primary cell cultures with reduced mitotic activity. J Immunol Methods. 1998;211:159–169.
72. Quent VMC, Loessner D, Friis T, et al. Discrepancies between metabolic activity and DNA content as tool to assess cell proliferation in cancer research. J Cell Mol Med. 2010;14:1003–1013.
73. Diaz-Rodriguez P, Chen H, Erndt-Marino JD, et al. Impact of select sophorolipid derivatives on macrophage polarization and viability. ACS Appl Bio Mater. 2019;2:601–612.
74. Arabiyat AS, Diaz-Rodriguez P, Erndt-Marino JD, et al. Effect of poly(sophorolipid) functionalization on human mesenchymal stem cell osteogenesis and immunomodulation. ACS Appl Bio Mater. 2019;2:118–126.
75. Erndt-Marino J, Yeisley DJ, Chen H, et al. Interferon-gamma stimulated murine macrophages *in vitro*: impact of ionic composition and osmolarity and therapeutic implications. Bioelectricity. 2020;2:48–58.

76. Vicente AM, Guillén MI, Alcaraz MJ. Heme oxygenase-1 induction and regulation in unstimulated mouse peritoneal macrophages. Biochem Pharmacol. 2003;65:905–909.
77. Urasaki Y, Beaumont C, Workman M, et al. Potency assessment of CBD oils by their effects on cell signaling pathways Yasuyo. Nutrients. 2020; 12:1–17.
78. Rosenthaler S, Pöhn B, Kolmanz C, et al. Differences in receptor binding affinity of several phytocannabinoids do not explain their effects on neural cell cultures. Neurotoxicol Teratol. 2014;46:49–56.
79. Schultze N, Wanka H, Zwicker P, et al. Mitochondrial functions of THP-1 monocytes following the exposure to selected natural compounds. Toxicology. 2017;377:57–63.
80. Garofano F, Schmidt-Wolf IGH. High expression of cannabinoid receptor 2 on cytokine-induced killer cells and multiple myeloma cells. Int J Mol Sci. 2020;21:1–11.
81. Miller LK, Devi LA. The highs and lows of cannabinoid receptor expression in disease: mechanisms and their therapeutic implications. Pharmacol Rev. 2011;63:461–470.
82. Lee SF, Newton C, Widen R, et al. Differential expression of cannabinoid CB 2 receptor mRNA in mouse immune cell subpopulations and following B cell stimulation. Eur J Pharmacol. 2001;423:235–241.
83. Ween MP, Whittall JJ, Hamon R, et al. Phagocytosis and inflammation: exploring the effects of the components of E-cigarette vapor on macrophages. Physiol Rep. 2017;5:1–12.
84. Ujváry I, Hanuš L. Human metabolites of cannabidiol: a review on their formation, biological activity, and relevance in therapy. Cannabis Cannabinoid Res. 2016;1:90–101.
85. Gonçalves J, Rosado T, Soares S, et al. Cannabis and its secondary metabolites: their use as therapeutic drugs, toxicological aspects, and analytical determination. Medicines. 2019;6:31.
86. Kozela E, Pietr M, Juknat A, et al. Cannabinoids Δ9-tetrahydrocannabinol and cannabidiol differentially inhibit the lipopolysaccharide-activated NF-κB and interferon-β/STAT proinflammatory pathways in BV-2 microglial cells. J Biol Chem. 2010;285:1616–1626.
87. Juknat A, Pietr M, Kozela E, et al. Differential transcriptional profiles mediated by exposure to the cannabinoids cannabidiol and Δ 9-tetrahydrocannabinol in BV-2 microglial cells. Br J Pharmacol. 2012;165: 2512–2528.
88. Deshmane SL, Kremlev S, Amini S, et al. Monocyte chemoattractant protein-1 (MCP-1): an overview. J Interferon Cytokine Res. 2009;29: 313–326.
89. Bianconi V, Sahebkar A, Atkin SL. The regulation and importance of monocyte chemoattractant protein-1. Curr Opin Hematol. 2018;25:44–51.
90. Li J, Ley K. Lymphocyte migration into atherosclerotic plaque. Arter Thromb Vasc Biol. 2015;35:40–49.
91. Edholm E, Rhoo KH, Robert J. Macrophage differentiation in normal and accelerated wound healing. Results Probl Cell Differ. 2017;62:353–364.
92. Ishida Y, Kuninaka Y, Nosaka M, et al. CCL2-Mediated reversal of impaired skin wound healing in diabetic mice by normalization of neovascularization and collagen accumulation. J Invest Dermatol. 2019;139: 2517–2527.e5.
93. Hoh BL, Fazal HZ, Hourani S, et al. Temporal cascade of inflammatory cytokines and cell-type populations in monocyte chemotactic protein-1 (MCP-1)-mediated aneurysm healing brian. J Neurointerv Surg. 2018;10: 301–305.
94. Mikolajczyk TP, Nosalski R, Szczepaniak P, et al. Role of chemokine RANTES in the regulation of perivascular inflammation, T-cell accumulation, and vascular dysfunction in hypertension. FASEB J. 2016;30:1987–1999.
95. Ridiandries A, Bursill C, Tan J. Broad-spectrum inhibition of the CC-chemokine class improves wound healing and wound angiogenesis. Int J Mol Sci. 2017;18:1–15.
96. Vannella KM, Wynn TA. Mechanisms of organ injury and repair by macrophages. Annu Rev Physiol. 2017;79:593–617.
97. McFarland-Mancini MM, Funk HM, Paluch AM, et al. Differences in wound healing in mice with deficiency of IL-6 versus IL-6 receptor. J Immunol. 2010;184:7219–7228.
98. Spano A, Barni S, Sciola L. PMA withdrawal in PMA-treated monocytic THP-1 cells and subsequent retinoic acid stimulation, modulate induction of apoptosis and appearance of dendritic cells. Cell Prolif. 2013;46:328–347.
99. Grotenhermen F. The pharmacokinetics and the pharmacodynamics of cannabinoids. Clin Pharmacokinet. 2003;42:328–359.
100. Charles Eldridge J, Landfield PW. Cannabinoid interactions with glucocorticoid receptors in rat hippocampus. Brain Res. 1990;534:135–141.
101. Yamaori S, Ebisawa J, Okushima Y, et al. Potent inhibition of human cytochrome P450 3A isoforms by cannabidiol: role of phenolic hydroxyl groups in the resorcinol moiety. Life Sci. 2011;88:730–736.
102. Villikka K, Kivistö KT, Neuvonen PJ. The effect of dexamethasone on the pharmacokinetics of triazolam. Pharmacol Toxicol. 1998;83:135–138.
103. De la Monte SM, Chiche JD, von dem Bussche A, et al. Nitric oxide synthase-3 overexpression causes apoptosis and impairs neuronal mitochondrial function: relevance to Alzheimer's-type neurodegeneration. Lab Invest. 2003;83:287–298.
104. Onodera Y, Teramura T, Takehara T, et al. Reactive oxygen species induce Cox-2 expression via TAK1 activation in synovial fibroblast cells. FEBS Open Bio. 2015;5:492–501.
105. Atalay S, Jarocka-karpowicz I, Skrzydlewskas E. Antioxidative and anti-inflammatory properties of cannabidiol. Antioxidants. 2020;9:1–20.
106. Mukhopadhyay P, Rajesh M, Horváth B, et al. Cannabidiol protects against hepatic ischemia/reperfusion injury by attenuating inflammatory signaling and response, oxidative/nitrative stress, and cell death. Free Radic Biol Med. 2011;50:1368–1381.
107. Naftali T, Mechulam R, Lev LB, et al. Cannabis for inflammatory bowel disease. Dig Dis. 2014;32:468–474.

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Abbreviations Used

Akt = protein kinase B
ANOVA = analysis of variance
CB1 = cannabinoid receptor 1
CB2 = cannabinoid receptor 2
CBD = cannabidiol
COX-2 = cyclooxygenase 2
Dex = dexamethasone
DMSO = dimethyl sulfoxide
eCBS = endocannabinoid system
HSD = honestly significant difference
IL-1β = interleukin-1beta
IL-6 = interleukin-6
IL-10 = interleukin-10
LDH = lactate dehydrogenase
LPS = lipopolysaccharide
MCP-1 = monocyte chemoattractant protein-1
MFI = median fluorescence intensity
M(LPS) = LPS-activated and untreated control
mTORC1 = mammalian target of rapamycin complex 1
NOS-3 = endothelial nitric oxide synthase
pCB = phytocannabinoid
p-mTOR S2448 = phosphorylated mammalian target of rapamycin Ser2448
PI3K = phosphoinositide 3-kinase
PMA = phorbol 12-myristate 13-acetate
RANTES = regulated on activation, normal T cell expressed and secreted
ROS = reactive oxygen species
SDS-PAGE = sodium dodecyl sulfate–polyacrylamide gel electrophoresis
tMACs = THP-1 derived macrophages
TNFα = tumor necrosis factor alpha
VEGF = vascular endothelial growth factor