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ORIGINAL ARTICLE

Natural cannabinoids suppress the cytokine storm in sepsis-like *in vitro* model

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[a] This author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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ABSTRACT. Natural cannabinoids may have beneficial effects on various tissues and functions including a positive influence on the immune system and the inflammatory process. The purpose of this study was to investigate the effects of natural cannabinoids on the production of pro-inflammatory cytokines by lipopolysaccharide (LPS)-stimulated whole human blood cells. Levels of the pro-inflammatory cytokines interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) were measured before and after exposure of LPS-stimulated whole blood to different concentrations of Cannabidiol (CBD) or a combination of CBD and Tetrahydrocannabinol (THC) extract. LPS stimulated the production of the pro-inflammatory cytokines. Exposure to both CBD and CBD/THC extracts significantly suppressed cytokine production in a dose-dependent manner. Exposure to cannabinoid concentrations of 50 μg/ml or 100 μg/ml resulted in a near-complete inhibition of cytokine production. This study demonstrates that natural cannabinoids significantly suppress pro-inflammatory cytokine production in LPS-stimulated whole blood in a dose-dependent manner. The use of human whole blood, rather than isolated specific cells or tissues, may closely mimic an *in vivo* sepsis environment. These findings highlight the role that natural cannabinoids may play in suppressing inflammation and call for additional studies of their use as possible novel therapeutic agents for acute and chronic inflammation.

Key words: cannabidiol, cannabinoids, inflammation, pro-inflammatory cytokines, sepsis, tetrahydrocannabinol

INTRODUCTION

Endocannabinoid system

The endocannabinoid system includes endogenous lipid mediators or endocannabinoids (arachidonoyl ethanolamide [AEA or anandamide] and 2-arachidonoylglycerol [2-AG]), their enzymes, and G-protein-coupled cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2) [1-4]. At higher concentrations, endocannabinoids can also interact with additional receptors, such as TRPV1, GPR18, GPR119, and GPR55 [5]. Biosynthesis of endocannabinoids occurs "on demand", is mostly calcium-dependent, and can involve multiple pathways [6].

While CB1 are the most abundant G-protein-coupled receptors in the human brain, with low functional levels in most peripheral tissues, including the heart and vasculature, CB2 are normally expressed in immune cells, and can be induced in other tissues under certain pathological conditions [6]. Cannabinoid receptors signal *via* G-protein-dependent pathways to inhibit adenylyl cyclase and modulate ion channels, but also activate mitogen-activated protein kinases and ceramide signaling. They can also involve G-protein-independent pathways *via* β-arrestins [7, 8].

The endocannabinoid system has been linked to various physiological functions, both in the central and peripheral nervous systems and in peripheral tissues. Modulating this system's activity may hold therapeutic potential in a wide range of diseases and pathological conditions, including mood and anxiety disorders, movement disorders, chronic pain, multiple sclerosis, spinal cord injury, cancer, atherosclerosis,

Abbreviations

CBD	Cannabidiol
CNS	Central Nervous System
DMSO	Dimethyl Sulfoxide
EDTA	Ethylenediaminetetraacetic Acid
IL	Interleukin
LPS	Lipopolysaccharide
NSAIDs	Nonsteroidal Anti-Inflammatory Drugs
RPMI	Roswell Park Memorial Institute medium
THC	Tetrahydrocannabinol
TNF-α	Tumor Necrosis Factor-α
WBC	White Blood Cells

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myocardial infarction, stroke, hypertension, obesity, metabolic syndrome, osteoporosis, and glaucoma [9].

Exogenous cannabinoids

Exogenous cannabinoids include dozens of natural phytocannabinoids that are found in the cannabis plant, as well as hundreds of synthetic cannabinoids, all of which may engage CB1 and CB2. Cannabis for medicinal use was introduced into western society during the nineteenth century for analgesia, treating convulsions and relief of spasticity. The use of cannabis as a therapeutic agent was abandoned with the development of other pharmacological agents and due to its psychotropic effects. It became a recreational substance, outlawed in multiple countries. In recent years, it has gained favor and its potential therapeutic effects have been studied in a variety of medical conditions [10].

The *Cannabis sativa* plant contains >700 different chemical compounds, including 104 unique cannabinoids [11, 12]. Since the discovery and isolation of tetrahydrocannabinol (THC) as the primary psychoactive constituent of cannabis in 1964 [13], many other phytocannabinoids have been identified. THC is a partial agonist of both CB1 and CB2. Cannabidiol (CBD), the second most abundant cannabinoid in the plant, has no psychoactive effects and has gained great interest due to its anti-inflammatory properties [14]. CBD has low affinity to CB1 and CB2 receptors, is capable of antagonizing cannabinoid receptor agonists, and has CB1 inverse agonism properties [15]. Combinations of various phytocannabinoids; CBD, THC, other many different cannabinoids and trepenoids derived from the plant, have been suggested to have a synergistic effect [16], and are studied for possible therapeutic uses and effects [17, 18]. Latest approved cannabinoid-based medications include Nabiximols (THC/CBD combination named Sativex), used to treat neuropathic pain and spasticity in multiple sclerosis and as analgesic in adults with advanced cancer [19, 20], and Epidiolex, an oral solution of CBD, which is FDA approved for the treatment of seizures associated with Lennox-Gestaut syndrome or Dravet syndrome [21, 22].

Cannabinoids and inflammation

The anti-inflammatory effects of cannabinoids are attributed to regulation of apoptosis, inhibition of cell propagation, stimulation of regulatory T cells and to inhibitory effects on pro-inflammatory cytokine synthesis and secretion [23]. A study with lipopolysaccharide (LPS)-stimulated human lung macrophages, exposed to smoked cannabis, resulted in decreased tumor necrosis factor- α (TNF-α) and interleukin-6 (IL-6) secretion [24]. However, most studies on the influence of cannabinoids on inflammation and cytokine secretion were conducted in animal tissues and cells. THC treatment of mice and spleen cells, stimulated with LPS or Herpes Simplex Virus type 2, resulted in a decrease in Interferon production [25, 26]. Similar treatment of rat microglial cells exhibited lower mRNA levels of IL-1, IL-6, and TNF-α [27]. In a murine model of acute lung injury by LPS exposure, treatment with CBD resulted in a decrease of TNF-α and IL-6 [28]. CBD treatment of mice which were injected with sublethal doses of LPS resulted in a reduction in serum TNF-α [29], similar to a beneficial effect on capillary permeability in the brains of mice injected with LPS and a reduction of TNF-α secretion [30].

Other models of inflammation, not involving stimulation or challenge with LPS, were also studied in animal models. Along with clinical improvement, CBD treatment of rats with pneumococcal meningitis, mice with arthritis or of mice with ischemic livers, significant reductions of TNF-α levels were observed [29, 31, 32]. Similarly, CBD administration in a mouse colitis model resulted in reduced inflammation in the colon associated with a decrease in IL-1β levels [33]. However, some conflicting reports suggest that cannabinoids may act as pro- rather than anti-inflammatory agents. Injection of THC to mice infected with *Legionella pneumophilia* resulted in increase in TNF-α and IL-6 production [34], and endotoxin-challenged mice treated with THC were found to have a dose-dependent increase in IL-1 levels [35]. Finally, mice with LPS-induced lung injury, followed by oral CBD treatment, showed increased mRNA of IL-5, IL-23, and TNF-α [36].

Given the key role of pro-inflammatory cytokines in mediating the immune response, it is possible that some or most of the suppressive effects of cannabinoids may be associated with their suppressive effects on the pro-inflammatory cytokine machinery. Therefore, we aim to study the role of cannabinoids on pro-inflammatory cytokine (IL-1β, IL-6, and TNF-α) production and secretion by LPS-stimulated human whole blood, thus mimicking in vivo conditions.

MATERIALS AND METHODS

Blood was drawn from 10 healthy, nonpregnant, nonsmoking, adult volunteers who did not use cannabis or any anti-inflammatory drugs during the month prior to the study. The blood was drawn from the cubital vein and collected into 2 lithium heparinized tubes (Vacuette, Greiner Bio-One, Austria), 4 mL each. One Ethylenediaminetetraacetic acid (EDTA) tube was sent for a complete blood count.

The study was conducted in 1 ml Eppendorf tubes containing 650 µl of Roswell Park Memorial Institute medium (RPMI, Life Technologies, Grand Island, New York, USA) containing Penicillin (100 U/ml) and Streptomycin (100 µg/ml) as well as 10 ng of LPS (Sigma-Aldrich, St. Louis, MO, USA). We then added 250 µl of whole blood diluted in RPMI (1:2 to 1:8). One sample from each volunteer was left untreated and used as a baseline for measuring cytokine production in response to LPS exposure. Other samples were treated with CBD or CBD/THC 18:1 (named "Avidekel") extracts in pure olive oil at concentrations of 1, 10, 50, or 100 µg/ml (all provided by Tikun Olam, Israel), or with pure olive oil controls without cannabinoids. Dimethyl sulfoxide (DMSO, Sigma-Aldrich, St. Louis, MO, USA) at a final concentration of 0.05% was used to dissolve the lipophilic extracts.

The pure olive oil controls contained DMSO in the same concentration used in the cannabinoid intervention groups, so we consider this group as a control for both olive oil and DMSO use. The tubes were incubated for 24 hours at 37^0C followed by the collection of the supernatants and lysis of the sediments with Triton x-100 (Bio-Rad Laboratories, Richmond, CA, USA) at a final concentration of 0.5%. Cell viability was assessed using Trypan Blue after adding the cannabinoids to the whole blood and after the 24-hour incubation. The IL-1β, IL-6, and TNF-α concentrations were determined by ELISA (R&D systems, MN, USA) according to the manufacturer's instructions.

All the cytokine measurements were determined in the lysate fraction of whole blood. Results were expressed as mean and standard deviations of cytokine levels in pg/1,000 white blood cells (WBC). Differences in cytokine levels from pre to post cannabinoids exposures were compared by the use of a paired T-test. All of the analyses were considered significant at a two-tailed p-value of <0.05. Statistical analyses were performed with the SPSS statistical package (SPSS, Chicago, IL).

The study was approved by the Institutional Review Board Committee of our medical center and a signed informed consent was obtained from all volunteers.

RESULTS

Ten healthy adult volunteers (6 males and 4 females) were included in this study. Viability of WBC, assessed before and after incubation, was >99%. There was no difference in viability of cells in the samples treated with either cannabinoid preparation as compared with controls of LPS only or LPS with pure olive oil, at the same concentrations used to dissolve the cannabinoid preparations (data not shown).

IL-6

Detailed results are shown in *table 1*. Whole blood stimulated with LPS alone resulted in IL-6 concentration of 12.7 ± 6.5 pg/1,000 WBC (*figure 1*). Treatment with olive oil at the same concentrations used to dissolve the cannabinoid preparations did not significantly affect IL-6 concentration as compared with LPS-stimulated whole blood with no olive oil (10.4-12.2 vs. 12.7 pg/1,000 WBC, p = NS) (*figure 1A*). Treatment with CBD at increasing doses (1, 10, 50, and 100 μg/ml) resulted in a dose-dependent decrease in IL-6 concentration (12.6, 7.1, 1.9, and 1.4 pg/1,000 WBC, respectively) as compared with no treatment (*figure 1B*). Treatment with CBD/THC 18:1 (Avidekel) at the same doses resulted in a similar dose-dependent decrease in IL-6 concentration (11.3, 9.7, 1.4, and 1.4 pg/1,000 WBC, respectively), as compared with no treatment (*figure 1C*). Doses of 10, 50, and 100 μg/ml of CBD and doses of 50 and 100 μg/ml of CBD/THC 18:1 (Avidekel) significantly reduced IL-6 concentration ($p < 0.001$ for all except CBD 10 μg/ml, for which $p = 0.047$).

*IL-1*β

Detailed results are shown in *table 2*. Whole blood stimulated with LPS alone resulted in IL-1β concentration of 5.8 ± 3.1 pg/1,000 WBC (*figure 2*). Treatment with olive oil at the same concentrations used to dissolve the cannabinoid preparations did not significantly affect IL-1β concentration as compared with LPS-stimulated whole blood with no olive oil (4.1-5.6 vs. 5.8 pg/1,000 WBC, p = NS) (*figure 2A*). Treatment with CBD at increasing doses (1, 10, 50, and 100 μg/ml) resulted in a dose-dependent decrease in IL-1β concentration (5, 3.6, 0.9, and 0.5 pg/1,000 WBC, respectively) as compared with no treatment (*figure 2B*). Treatment with CBD/THC 18:1 (Avidekel) at the

Table 1
IL-6 concentrations in LPS-stimulated whole blood (pg/1,000 WBC).

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Mean ± SD
LPS only	11.00	28.48	13.93	11.04	5.81	6.11	13.21	10.54	7.46	18.98	12.66 ± 6.47
OLIVE OIL 1 μg/ml	11.17	36.30	11.94	8.37	7.48	7.42	13.73	6.82	6.43	12.82	12.25 ± 8.41
OLIVE OIL 10 μg/ml	11.24	16.54	15.15	11.52	7.01	10.84	12.32	10.51	9.74	8.98	11.39 ± 2.65
OLIVE OIL 50 μg/ml	9.29	16.45	14.82	9.22	8.02	7.96	9.34	7.00	11.31	13.36	10.68 ± 3.03
OLIVE OIL 100 μg/ml	21.62	20.44	29.14	2.44	3.69	2.56	1.69	3.11	11.46	7.89	10.40 ± 9.40
CBD 1 μg/ml	16.26	14.62	10.79	9.05	5.29	18.01	19.80	11.89	7.46	13.14	12.63 ± 4.42
CBD 10 μg/ml	12.28	4.73	7.58	8.12	3.03	8.75	4.50	4.68	8.09	9.42	7.12 ± 2.68
CBD 50 μg/ml	1.27	0.82	2.19	1.48	1.28	2.09	4.16	1.74	1.68	2.61	1.93 ± 0.89
CBD 100 μg/ml	1.27	0.82	1.16	1.28	1.28	1.22	1.44	1.74	1.68	1.77	1.37 ± 0.28
AVIDEKEL 1 μg/ml	7.97	13.25	16.42	8.12	7.05	8.72	11.59	5.53	28.76	5.93	11.33 ± 6.66
AVIDEKEL 10 μg/ml	5.48	14.74	8.19	16.20	9.39	5.77	16.21	4.12	10.38	6.08	9.66 ± 4.36
AVIDEKEL 50 μg/ml	1.27	0.82	1.16	1.28	1.28	1.22	1.44	1.74	1.68	1.77	1.37 ± 0.28
AVIDEKEL 100 μg/ml	1.27	0.82	1.16	1.28	1.28	1.22	1.44	1.74	1.68	1.77	1.37 ± 0.28

AVIDEKEL, CBD:THC 18:1; CBD: cannabidiol; IL-6: interleukin-6; LPS: lipopolysaccharide; THC: tetrahydrocannabinol

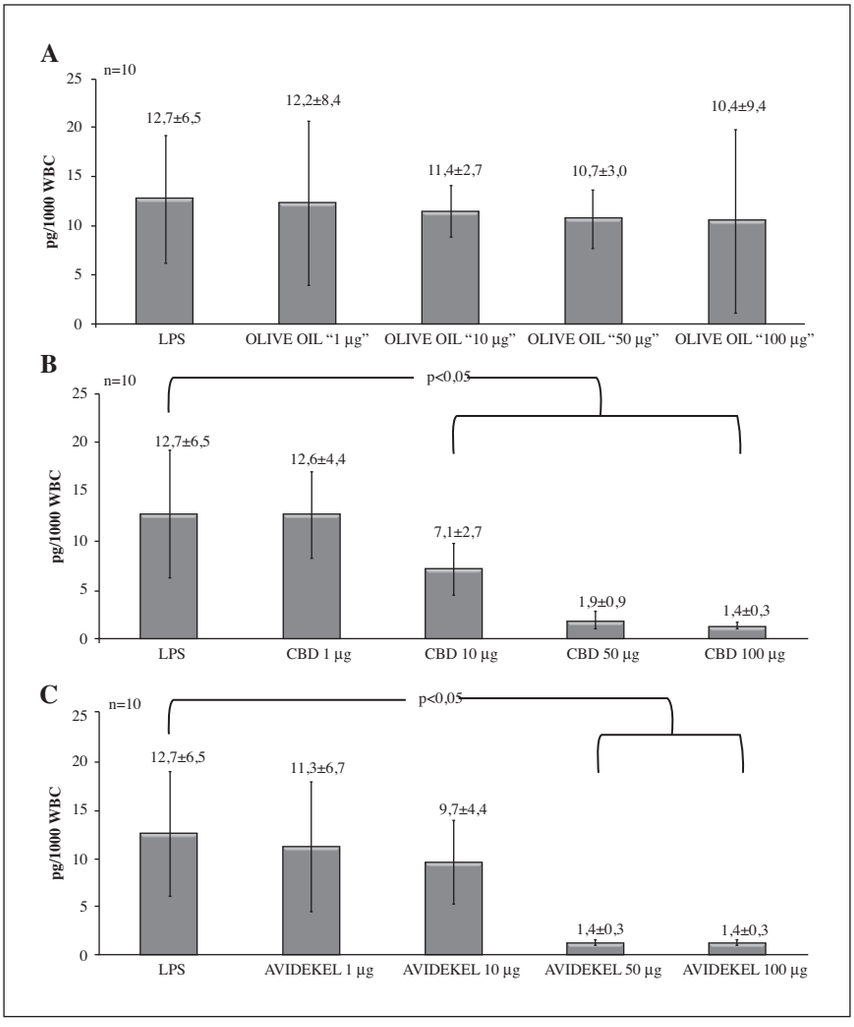

Figure 1
IL-6 concentrations in LPS-stimulated whole blood (pg/1,000 WBC). **A.** Treatment with olive oil and DMSO at the same concentrations used to dissolve the cannabinoids. **B.** Treatment with CBD at 1-100 µg/ml. **C.** Treatment with CBD/THC 18:1 (Avidekel) at 1-100 µg/ml.

Table 2
IL-1β concentrations in LPS-stimulated whole blood (pg/1,000 WBC).

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Mean ± SD
LPS only	5.35	6.04	13.03	5.23	3.62	2.51	2.83	3.93	5.35	9.95	5.78 ± 3.13
OLIVE OIL 1 µg/ml	4.37	6.30	9.91	5.50	2.38	2.71	3.04	6.21	2.56	5.26	4.82 ± 2.23
OLIVE OIL 10 µg/ml	6.05	6.36	8.78	4.67	3.09	3.49	2.87	4.08	2.78	5.18	4.74 ± 1.81
OLIVE OIL 50 µg/ml	4.97	7.73	15.13	4.97	3.19	2.21	3.92	3.27	3.00	7.21	5.56 ± 3.62
OLIVE OIL 100 µg/ml	7.67	7.59	11.91	5.88	1.32	0.95	1.16	0.80	0.91	2.56	4.08 ± 3.73
CBD 1 µg/ml	7.10	6.40	9.13	3.15	3.10	2.06	4.71	4.93	4.00	5.87	5.05 ± 2.02
CBD 10 µg/ml	10.88	6.40	3.29	3.89	2.16	1.26	1.96	1.98	1.23	3.27	3.63 ± 2.82
CBD 50 µg/ml	0.65	1.39	0.58	1.11	0.65	0.48	0.93	1.79	0.71	0.99	0.93 ± 0.39
CBD 100 µg/ml	0.48	0.32	0.34	0.48	0.54	0.54	0.51	0.60	0.72	0.74	0.53 ± 0.13
AVIDEKEL 1 µg/ml	4.18	7.62	5.85	3.13	1.95	5.17	1.79	2.74	1.42	3.97	3.78 ± 1.89
AVIDEKEL 10 µg/ml	3.80	8.44	11.4	5.00	6.13	4.46	2.63	4.43	1.90	3.96	5.22 ± 2.68
AVIDEKEL 50 µg/ml	0.6	0.36	0.41	0.21	0.17	0.54	0.04	0.60	0.72	0.23	0.39 ± 0.21
AVIDEKEL 100 µg/ml	0.57	0.12	0.09	0.03	0	0.54	0.51	0.11	0.72	0.16	0.29 ± 0.25

AVIDEKEL, CBD:THC 18:1; CBD: cannabidiol; IL-1β: interleukin-1β; LPS: lipopolysaccharide; THC: tetrahydrocannabinol

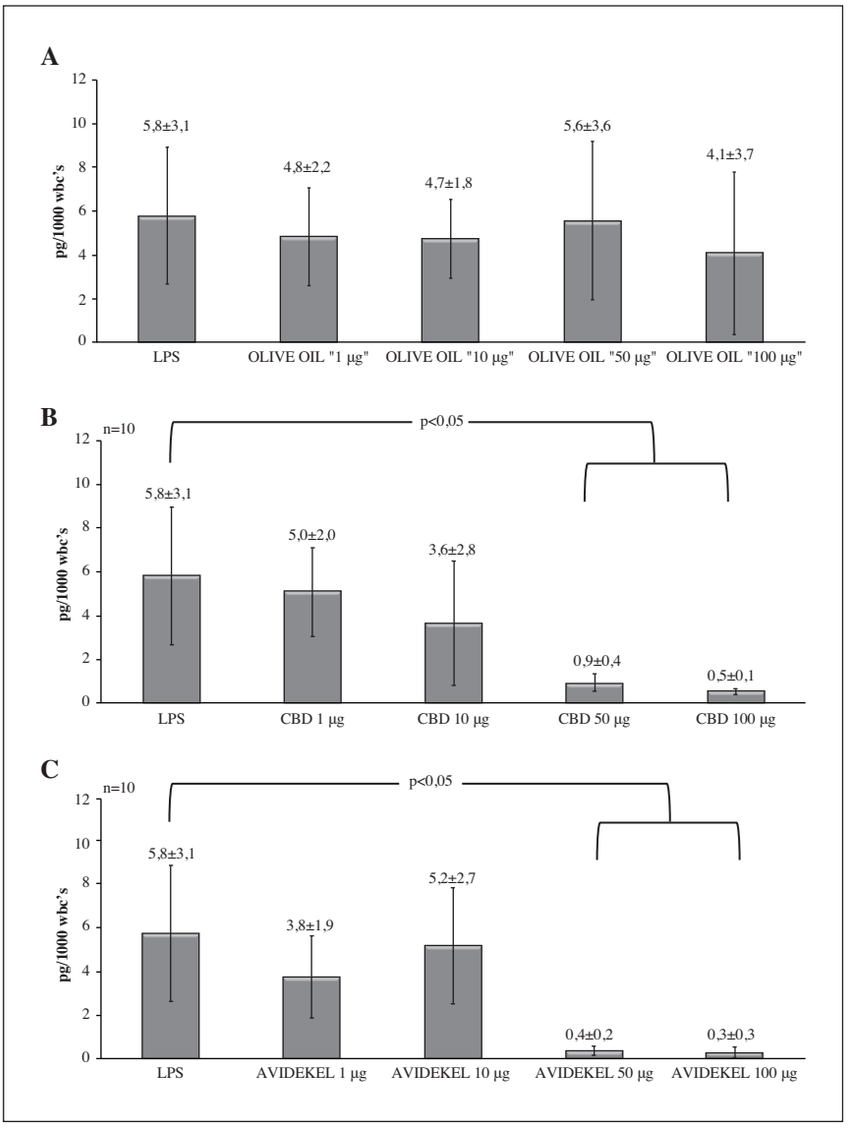

Figure 2
IL-1β concentrations in LPS-stimulated whole blood (pg/1,000 WBC). **A.** Treatment with olive oil and DMSO at the same concentrations used to dissolve the cannabinoids. **B.** Treatment with CBD at 1-100 µg/ml. **C.** Treatment with CBD/THC 18:1 (Avidekel) at 1-100 µg/ml.

same doses, also resulted in a decrease in IL-1β concentration (3.8, 5.2, 0.4, and 0.3 pg/1,000 WBC, respectively), as compared with no treatment (*figure 2C*). Doses of 50 and 100 µg/ml of either CBD or CBD/THC 18:1 (Avidekel) significantly reduced IL-1β concentration ($p < 0.001$ for all except CBD 50 µg/ml, for which $p = 0.001$).

TNF-α

Detailed results are shown in *table 3*. Whole blood stimulated with LPS alone resulted in TNF-α concentration of 9.2 ± 4.1 pg/1,000 WBC (*figure 3*). Treatment with olive oil at the same concentrations used to dissolve the cannabinoid preparations did not significantly affect TNF-α concentration as compared to LPS-stimulated whole blood with no olive oil (7.6-8.7 vs. 9.2 pg/1,000 WBC, p = NS) (*figure 3A*). Treatment with CBD at increasing doses (1, 10, 50, and 100 µg/ml) resulted in a dose-dependent decrease in TNF-α concentration (8.8, 5.2, 3.4, and 2.3 pg/1,000 WBC, respectively), as compared with no treatment (*figure 3B*). Treatment with CBD/THC 18:1 (Avidekel) at the same doses also resulted in a decrease in TNF-α concentration (8.2, 7.1, 2.2, and 2.4 pg/1,000 WBC, respectively), as compared with no treatment (*figure 3C*). Doses of 10, 50, and 100 µg/ml of CBD and doses of 50 and 100 µg/ml of CBD/THC 18:1 (Avidekel), significantly reduced TNF-α concentration ($p < 0.001$ for all except CBD 10 µg/ml, for which $p = 0.039$ and CBD 50 µg/ml, for which $p = 0.001$).

DISCUSSION

The purpose of the present study was to evaluate the effects of natural cannabinoid components on the production of pro-inflammatory cytokines in lipopolysaccharide (LPS)-stimulated whole human blood.
This is the first study that uses a model of whole blood stimulated by LPS and treated with cannabinoids. In contrast to other studies in which LPS stimulation was limited to specific subsets of leukocytes, such as

Table 3
TNF-α Concentrations in LPS-stimulated whole blood (pg/1,000 WBC).

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Mean ± SD
LPS only	6.51	12.16	10.25	9.22	3.94	5.37	11.4	8.14	6.36	19.00	9.24 ± 4.12
OLIVE OIL 1 µg/ml	11.02	12.53	11.24	5.22	3.86	4.06	7.52	6.26	6.16	13.70	8.12 ± 3.46
OLIVE OIL 10 µg/ml	13.61	10.03	11.4	7.30	4.00	4.23	7.76	8.82	7.98	11.85	8.70 ± 2.98
OLIVE OIL 50 µg/ml	14.83	0	13.2	7.55	4.49	4.91	7.72	7.7	7.63	16.99	8.50 ± 4.88
OLIVE OIL 100 µg/ml	13.65	15.61	10.6	2.96	2.94	3.27	6.00	4.35	9.10	7.31	7.58 ± 4.33
CBD 1 µg/ml	12.47	12.92	6.16	5.06	3.27	7.58	14.15	7.43	7.38	11.58	8.80 ± 3.52
CBD 10 µg/ml	11.64	3.3	5.49	2.84	2.77	3.12	4.91	4.99	7.55	5.16	5.18 ± 2.58
CBD 50 µg/ml	4.10	2.38	3.21	2.58	2.32	2.74	5.10	3.68	3.51	4.12	3.37 ± 0.86
CBD 100 µg/ml	3.02	1.83	2.11	2.00	1.83	1.72	3.05	2.79	2.55	2.30	2.32 ± 0.48
AVIDEKEL 1 µg/ml	12.51	4.77	6.01	3.83	3.06	4.01	7.68	4.92	24.2	10.66	8.17 ± 6.09
AVIDEKEL 10 µg/ml	13.86	8.89	7.21	5.22	3.29	4.39	8.69	4.04	8.55	6.93	7.11 ± 2.97
AVIDEKEL 50 µg/ml	2.52	1.79	2.99	1.61	2.00	1.85	2.71	2.36	2.13	2.52	2.25 ± 0.42
AVIDEKEL 100 µg/ml	3.43	2.03	2.81	2.30	1.50	2.06	2.95	2.31	2.60	2.11	2.41 ± 0.52

AVIDEKEL, CBD:THC 18:1; CBD: cannabidiol; TNF-α: tumor necrosis factor alpha; LPS: lipopolysaccharide; THC: tetrahydrocannabinol

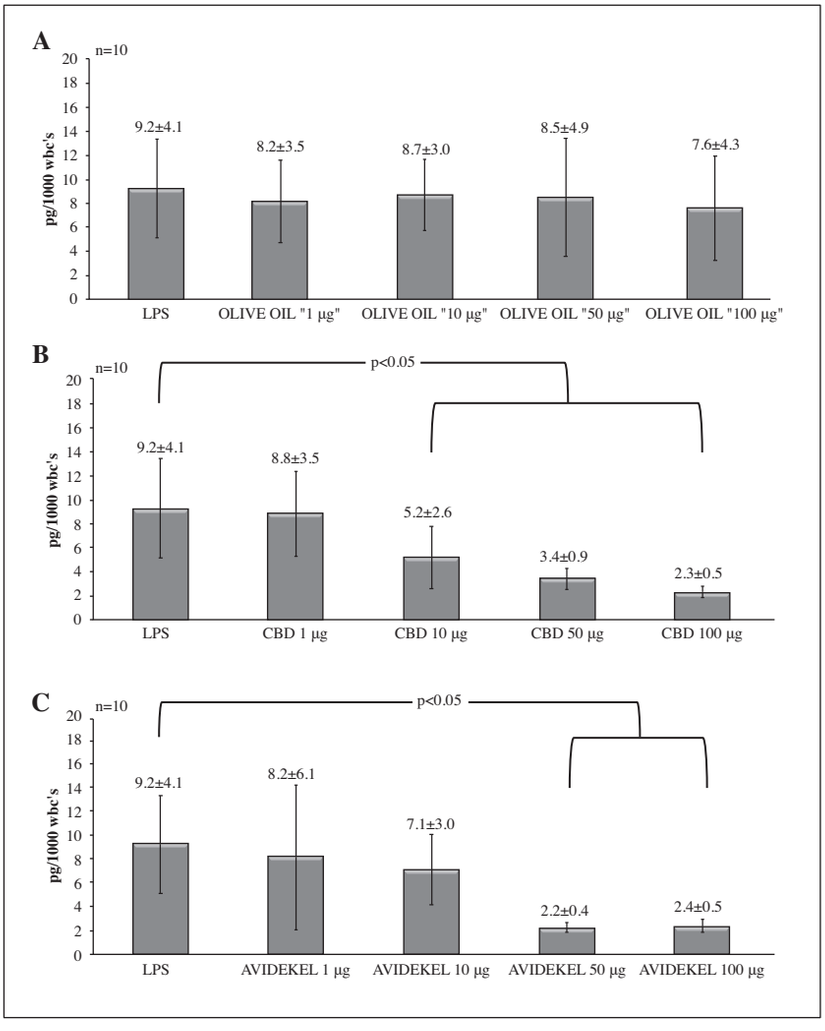

Figure 3
TNF-α Concentrations in LPS-stimulated whole blood (pg/1,000 WBC). **A.** Treatment with olive oil and DMSO at the same concentrations used to dissolve the cannabinoids. **B.** Treatment with CBD at 1-100 µg/ml. **C.** Treatment with CBD/THC 18:1 (Avidekel) at 1-100 µg/ml.

monocytes or macrophages, our model involved stimulation of all cellular components. Use of whole blood could have allowed for interactions between different cell types and plasma protein components, closely mimicking an in vivo environment of gram-negative sepsis.

The natural cannabinoids that were tested, and not the olive oil controls, had a significant dose-dependent suppressive effect on the production of the pro-inflammatory cytokines IL-6, IL-1β, and TNF-α from LPS-stimulated whole blood. The production of cytokines was suppressed both by pure CBD and by the CBD/THC 18:1 (Avidekel) extracts. Suppression of IL-1β and IL-6 production was more significant than that of TNF-α. At cannabinoid concentrations of 50 and 100 μg/ml, the production of IL-1β and IL-6 was almost completely suppressed in all subjects. TNF-α production was also suppressed in these doses, albeit more moderately.

It is important to note that in vitro cell death was not observed as a result of exposure to cannabinoids in all doses, so that the minimal concentrations of cytokines express true suppression of their production, rather than cell death as a result of exposure to the cannabinoids themselves.

A study examining the safety of CBD use found that it is probably safe and well tolerated in humans, including in chronic use and in doses of up to 1,500 mg/day (a higher dose was not tested) [37]. These doses are roughly equivalent to the maximal doses tested in our study, which can help in planning follow-up studies in vivo.

For many years, innovative anti-inflammatory treatments have been the center of intense research. Steroid drugs, nonsteroidal anti-inflammatory drugs (NSAIDs), and others are widely used in various inflammatory conditions and have been shown to be effective in reducing inflammation, in part by suppressing the inflammatory machinery. For the past 20 years, biological treatments targeting pro-inflammatory cytokines have been used as an effective treatment for a variety of inflammatory diseases such as Rheumatoid Arthritis [38], Inflammatory Bowel Disease [39], Psoriasis [40], and more. Despite the proven efficacy, factors such as tolerance, adverse reactions to the biological agents, and host antibodies raised against the different agents have become significant issues, thus reducing the effectiveness of these therapies [41]. In addition, the use of some anticytokine biological agents is associated with an increased risk of developing Tuberculosis and Lymphoma [42]. Various studies have examined the effect of immunomodulatory therapy on infection and sepsis, although without impressive results. Attempts to treat the "cytokine storm," a massive cytokine activation in cases of sepsis, have failed [43, 44]. Cannabis and cannabinoid-based drugs are slowly being incorporated into routine clinical practice, mainly for pain control in chronic and malignant conditions, alongside other, less frequent, indications [19, 20, 45]. They are even being used in inflammatory bowel disease, but there is still a lack of large-scale clinical studies to prove their efficacy [46]. Cannabis may in fact be a readily, inexpensive and available treatment for many conditions in which suppression of inflammation is needed. In the field of immunomodulation during sepsis, where there is a true unmet clinical need, our findings suggest that cannabis might be a relevant therapeutic agent, as inflammatory cytokines were suppressed even after exposure to large concentrations of LPS, mimicking an already advanced stage of sepsis.

One of the known limitations of therapeutic use of cannabinoids, namely confusion and psychoactive effects, are more relevant to THC than CBD. In our study, THC was used only in part of the samples, and in a very low-dose, always as a secondary adjunct to CBD.

Limitations

We acknowledge several limitations of this study. The study includes a relatively small number of samples. Using whole blood samples, rather than specific immune system cells, helps minimize the intrinsic limitation of an in-vitro study, in which samples are treated outside their natural environment, but this limitation cannot be ignored altogether. Our investigations were limited to cannabinoids that were comprised mostly or entirely of CBD. Therefore, we did not directly study the anti-inflammatory effects of THC. We chose to focus on CBD, rather than THC-based cannabinoids because CBD is devoid of psychoactive effects that may prevent administration in high doses and because the ability to obtain compounds with verified high CBD cannabinoid concentrations and CBD/THC ratios.

We also acknowledge the fact that our study did not approach the mechanistic aspect of the anti-inflammatory properties of the cannabinoids used. We studied a limited panel of the major inflammatory pyrogenic cytokines, not including anti-inflammatory cytokines such as IL-1 receptor antagonist (IL-1RA) and IL-10. Adding such an analysis to future studies might shed more light on the promising anti-inflammatory mechanisms of cannabinoids.

Conclusion

The results of our study, which demonstrated a suppressive effect of phytocannabinoids on the production of pro-inflammatory cytokines, suggest that cannabinoid components may have a therapeutic role in suppressing inflammation. This justifies further investigations of mechanisms responsible for this observation as well as for research regarding the use of cannabinoids as part of the arsenal for suppressing acute or chronic inflammation.

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