

Cannabidiol and periodontal inflammatory disease: A critical assessment

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Cannabidiol (CBD), a non-psychoactive cannabinoid produced by the genus *Cannabis*, is a phytochemical that activates the endocannabinoid system (ECS) through binding to CB1 and CB2 receptors. The ECS is involved in cellular homeostasis and regulates metabolic processes in virtually all mammalian tissues. Published studies on CBD focus, *inter alia*, on its use in prophylaxis and as an anti-inflammatory agent. Here the authors present a critical assessment of the effects of CBD on inflammatory periodontal diseases caused by bacterial virulence factors, and evaluate critically the possible benefits and drawbacks of CBD use in dentistry. Particular attention is paid to the interaction of CBD with microbially colonized oral tissues, the inflammatory response in relation to the immune response, and the destruction/regeneration of hard and soft tissues of the periodontium.

Key words: cannabidiol, endocannabinoid system, CB1/2 receptors, microbial colonisation, bacterial virulence, immune system, gingivitis, periodontitis

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INTRODUCTION

Inflammatory periodontal diseases such as gingivitis and periodontitis are health issues affecting a large percentage of the world's population¹. The oral cavity is the entry to the gastrointestinal tract not only for micro- and macronutrients, but also environmental toxins and microorganisms. The health of the oral hard and soft tissues is impacted by the genetic diversity of bacteria in the supra- and subgingival plaque, the mucosal immune system, protective salivary factors, genetic predisposition, and socioeconomic status². In individuals with impaired immunity, genetic predisposition, mental health problems, or poor oral hygiene, the oral ecology is perturbed and the composition of the oral microflora altered in favor of periodontal colonization by pathogenic Gram-negative anaerobes at the expense of commensal bacteria. Periopathogens include *Aggregatibacter actinomycetemcomitans*, *Filifactor alocis*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythia*, *Treponema denticola*, *Selenomonas artemidis*, and *Veillonella dispar*, as well as several species of streptococci, most notably *Streptococcus oralis*, *S. reptococcus*, *S. mitis*, *S. sanguis*, and *S. mutans*. Bacterial virulence factors activate cells of the innate immune system in periodontal tissues: monocytes, polymorphonuclear leukocytes (neutrophils), and macrophages to produce proinflammatory cytokines and chemokines³. Proinflammatory cytokines produced by the cells of the immune system initiate the destruction of both pathogenic bacteria and periodontal

tissue, especially periodontal ligament fibroblasts, odontoblasts, and alveolar bone osteoblasts⁴.

Sodium fluoride, zinc chloride and zinc sulphate, stannous fluoride, and aluminum lactate are used as active components of medicinal and cosmetic products for the chemoprophylaxis of the oral cavity. Synthetic organic compounds including amine fluoride, cetylpyridinium chloride, hexetidine, octenidine, triclosan, and chlorhexidine are the most common. Phytochemicals are complex extracts or individual secondary metabolites of medicinal plants based on essential oils, terpenes, polyphenols and quaternary isoquinoline alkaloids⁵. These are applied topically and have predominantly bacteriostatic and bactericidal effects⁶⁻⁹.

Cannabidiol (CBD) belongs to the group of non-psychoactive cannabinoids (Fig. 1). Limited knowledge is available about the effects of CBD on inflamed gingiva

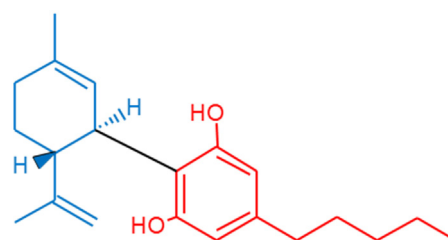


Fig. 1. Molecular structure of cannabidiol. The molecule is composed of two structural motifs, 4-isopropyl-1-methylcyclohexene (blue) and 5-pentylresorcinol (red).

and periodontia. CBD acts pleiotropically and biphasically in a dose-dependent manner. When administered as a complex extract, its effect may be influenced (synergistically or antagonistically) by the presence of other components. The pleiotropic properties of CBD may be due to its being composed of structural motifs of two natural substances, limonene (a cyclic monoterpene) and olivetol (an alkylphenol) (ref.^{10,11}). Most studies of CBD focus on its neuroprotective, anti-epileptic, anxiolytic, anti-psychotic and anti-inflammatory effects^{12,13}. However, the biological activity and potential application in dentistry of CBD and other secondary metabolites of the genus *Cannabis* have been recently discussed by Lowe et al.¹⁴. This brief review offers a critical assessment of the chemoprophylactic effects of CBD on inflammatory periodontal diseases caused by bacterial virulence factors. The interaction of CBD with microbially colonized oral tissues and the effect of CBD on the inflammatory response, or the destruction and/or regeneration of hard and soft tissue cells, are also covered.

CBD AND THE ENDOCANNABINOID SYSTEM RECEPTORS

CBD and psychotropic Δ^9 -tetrahydrocannabinol (THC) are the most abundant cannabinoids of the genus *Cannabis*¹⁵. In mammals, the biological activity of phytocannabinoids is linked to their interaction with the two subtypes of cannabinoid G-protein-coupled receptors, CB1 and CB2 (ref.¹⁶). These receptors are located on the surface of the cell membrane structures of all mammalian tissues¹⁷. CBD is a partial agonist that binds to the orthosteric site of the CB2 receptor, whereas it is a direct antagonist of the binding site of the CB1 receptor. It is a negative allosteric modulator of both receptors¹⁸.

CB1 receptors occur primarily in brain tissue and regulate neuronal activities. CB2 receptors are located in cells of the innate immune system and peripheral nervous tissue, and are involved in the regulation of anti-inflammatory and immunomodulatory activity. The interaction of exogenous ligands (phytocannabinoids, synthetic cannabinoids) and endogenous ligands (endocannabinoids such as arachidonic acid amides and esters) with CB1 and CB2 receptors activates the endocannabinoid system (ECS) (ref.^{19,20}). The ECS consists of cooperating receptors, their ligands, enzymes active in the synthesis and/or degradation of ligands, and some other signaling molecules²¹. The ECS is primarily responsible for cellular homeostasis. In brain tissue, it has a direct effect on the physiological processes regulating pain perception, cognitive, affective, and motor functions.

As for periodontal health, Konermann et al. demonstrated the presence of CB1 and CB2 receptors and changes in expression in human periodontal ligament fibroblasts (HPLFs) *in vitro* and *in vivo*²². *In vivo*, CB1 expression was significantly higher in healthy periodontal ligament (PDL) cells compared to CB2. Bacterial inflammation produced a decrease in CB1 and an increase in

CB2, whereas sterile inflammation caused extensive accumulation of both CB1 and CB2. Under *in vitro* conditions, CB2 was expressed at the gene and protein levels, while CB1 was undetectable at the protein level²². After the above study, mechanically stressed HPLFs were incubated with the endocannabinoids anandamide (AEA) and palmitoylethanolamide (PEA) for 6 and 10 h. AEA significantly downregulated the transcription of inflammatory cytokines IL-1 β , IL-6 and TNF α , whereas PEA accessorially upregulated these cytokines. Thus the ECS can play a regulatory function in periodontal cells under stress conditions²³.

CBD interacts with peroxisome proliferator-activated receptors (PPARs) (ref.²⁴). Inflammatory processes in periodontal tissue increase the expression of the PPAR- γ isoform²⁵. CBD can bind to PPAR- γ and increase its transcriptional activity, and produce effects that are inhibited by selective antagonists of PPAR- γ (ref.²⁴). PPAR- γ modulates the course of inflammation *via* the decreased production of COX-2 and the proinflammatory cytokines IL-1 β , IL-6 and TNF- α (ref.²⁶). CBD-activated PPAR- γ also cooperates with the transcription factor Nrf2, a regulator of the expression of endogenous antioxidants²⁷, which affects redox homeostasis in tissues^{28,29}. The antioxidant capacity of cells is crucial for protecting tissues from destruction by free radicals, particularly reactive oxygen and nitrogen species such as may be produced during inflammation.

INFLUENCE OF CBD ON THE IMMUNE SYSTEM OF THE PERIODONTIUM

CBD is an effective modulator of the immune response. It suppresses immunoglobulin secretion and proinflammatory cytokine production in autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus³⁰. Gingivitis and chronic periodontitis are caused by alterations in the proportions of commensal and pathogenic bacteria (dysbiosis) in the oral cavity. The virulence factors of oral pathogens, *e.g.* lipopolysaccharide (LPS) produced by *P. gingivalis* (Table 1), interact with Toll-like receptors (TLR) to initiate the TLR-PI3K-GSK3 β inflammatory signaling axis in host macrophages and neutrophils^{31,32}. Experiments using mice with knocked-out CB2 receptor or PI3K genes demonstrated that CBD is a potent proinflammatory suppressor

Table 1. Virulence factors of *P. gingivalis* act at different stages of bacterial infection³.

Colonization and attachment:
Fimbriae, hemagglutinins, outer membrane proteins and vesicles, Cys-gingipain
Evasion (modulation) of host immune responses:
LPS, fimbriae, capsule, complement proteases, other anti-phagocytic products
Damage to host tissues and spread:
Proteinases (Arg- and Lys-gingipains), collagenase, fibrinolytic, keratinolytic, and other hydrolytic enzymes

and IL-10 inducer, its mode of action involves CB2/PI3K signaling that does not amplify the canonical GSK3 β anti-inflammatory pathway^{31,32}. Inflammation was induced by the repeated inoculation of *P. gingivalis* into the maxillary gingiva. Negative long-term regulation of the production of proinflammatory cytokines by CBD can lead to the persistence of periodontal bacteria, enhancing the risk of chronic periodontitis.

CBD AND THE ECOLOGY OF ORAL MICROFLORA

The physiological function of saliva and the diversity of the bacterial biofilm on the surface of soft and hard tissues are important for the symbiosis of the commensal bacteria underlying homeostasis of the oral cavity microbial ecosystem^{33,34}. The antibacterial effects of phytocannabinoids have been known for centuries, but experimental validation of their antibacterial activity only dates back to the first decade of the new millennium³⁵. The activity of CBD towards clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) strains was published in 2008 (ref.³⁶). Martinenghi et al.³⁷ confirmed a significant antibacterial effect of CBD on Gram-positive antibiotic-resistant strains of staphylococci (MIC range: 1-2 μ g/mL), with no antimicrobial effect towards the Gram-negative species examined. The authors attributed this difference to the different structures of the membranes of Gram-negative vs. Gram-positive bacteria, which led to different membrane permeability/disruption susceptibilities for CBD.

Blaskovich et al. evaluated minimal inhibitory concentrations (MICs) of CBD and clinically-used antibiotics on selected isolates of Gram-positive and Gram-negative bacteria³⁸. The antibacterial activity of CBD was comparable to those of antibiotics used to treat infections caused by resistant strains of Gram-positive bacteria, though against Gram-negative bacteria, CBD was primarily active against *Neisseria* species. Amongst periopathogens, growth suppression by CBD has been observed in *P. gingivalis* (Gram-negative) and *F. alocis* (Gram-positive), but not in *Treponema denticola* (a Gram-negative spirochete) (ref.³¹).

CBD inhibits the release of membrane vesicles (MVs) from both Gram-positive and Gram-negative bacteria. MVs participate in interbacterial communication, including the transfer of cargo molecules, and help shield bacteria from antibiotic adsorption. CBD increases the efficacy of some antibiotics by modulating the release of MVs (ref.³⁹), and the application of CBD changes the protein profile of MVs.

INFLUENCE OF CBD ON PRODUCTION OF PROINFLAMMATORY CYTOKINES

The oral microbiome is unique to each individual, and likewise the spectrum of virulence factors of specific bacterial strains is not uniform. The most commonly used virulence factor in mechanistic studies with periopathogens is the lipopolysaccharide from *P. gingivalis*. Studies of the effects of CBD on periodontal tissues have been predominantly performed using cell cultures of human

Table 2. The effect of CBD on LPS induced cytokine production in experimental cellular models.

Cell line (cultivation)	CBD and LPS	Evaluated markers	Application form of CBD	Ref.
GF* (PBK)	0.01-30 μ M CBD; 24-144 h	$\uparrow$ TGF- β , $\uparrow$ fibronectin, $\downarrow$ MMP-1, $\downarrow$ MMP-2 (higher concentration)	1 mg/mL, MeOH	40
GMSC* (PBK)	5 μ M CBD; 24 h	$\downarrow$ expression of proinflammatory genes	0.1%, DMSO	52
TIGK* (BL)	1.0 μ g/mL CBD + 0.1 μ g/mL LPS; 20 h	$\downarrow$ IL-6, $\downarrow$ IL-8, $\downarrow$ IL-12, $\downarrow$ TNF- α	1.0 mg/mL, MeOH	31
PBMC* (PBK)	30 mg oral doses of CBD + 1.0 μ g/mL LPS; 48 h	$\downarrow$ TNF- α	30 mg soluble CBD <i>per os</i> . After 90 min PBMC were separated	41
MMF (BL RAW 264.7)	Preincubated with 10, 30, 100 and 300 μ M CBD; 0.5 h + 1.0 μ g/mL LPS; 4 h	$\downarrow$ TNF- α	Cremophor/EtOH/ culture medium (1:1:18)	46
BM* (PBK)	0.5, 1, 5 and 10 μ M CBD + 10 ng/mL LPS; 22 h	$\downarrow$ IL-1 β , $\downarrow$ IL-6, $\downarrow$ IL-10, $\downarrow$ TGF- β 1	Not specified	45
HMSC* (PBK)	3 μ M CBD + 10 μ g/mL LPS; 48 h	Unchanged IL-2, IL-5, IL-6, IL-18, TNF, VEGF, IGF, TGF- β	0.01%, EtOH	44
MMF (BL RAW 264.7)	Preincubated with 2.5 and 5 μ M CBD; 2 h + 10 μ g/mL LPS; 24 h	$\uparrow$ IL-10, $\downarrow$ Nrf2, $\downarrow$ iNOS, $\downarrow$ TLR4, $\downarrow$ NF- κ B	0.1%, DMSO	43
MONO (BL U937)	10.6 μ M CBD + 1.0 μ g/mL LPS; 24 h	$\downarrow$ IL-8, $\downarrow$ IL-6, $\downarrow$ TNF- α	Not specified	42

*Cells were isolated from healthy volunteers; PBK, primary culture; BL, cell line; GF, human gingival fibroblasts; GMSC, human gingival mesenchymal stem cells; TIGK, human gingival keratinocytes; PBMC, peripheral blood mononuclear cells; BM, blood monocytes; MMF (RAW 264.7), murine macrophage cells; HMSC, human mesenchymal stem cells; MONO (U937), monocyte cell line

gingival fibroblasts, keratinocytes, periodontal ligament fibroblasts, mesenchymal stem cells, and LPS-intoxicated monocytes. The effects of CBD concentration and incubation time on cell viability and the suppression of pro-inflammatory factor production after LPS stimulation were investigated. The results of several recent studies are summarized in Table 2. After 1-6 days of incubation of gingival fibroblasts with CBD alone, fibronectin and TGF- β production increased⁴⁰. On the other hand, a biphasic effect of CBD on MMP-1 and MMP-2 production was observed. It was increased after incubation with lower concentrations (0.1-0.5 μ M) of CBD, and decreased after incubation with a higher concentration⁴⁰. The data show that the application of CBD may positively promote the regeneration of the periodontal connective tissue. Besides this, the combination of CBD with LPS has an immunosuppressive effect^{31,41-46}.

Various methods and approaches are used to induce periodontitis in laboratory animals⁴⁷. Napimoga et al. tested markers of periodontitis induced by ligature placement around both mandible first molars of rats⁴⁸. Rats were given 5 mg/kg *i.p.* CBD dissolved in a vehicle (2% Tween 80 in saline), or pure vehicle as placebo, for 30 days. In contrast to the placebo, rats in the CBD group exhibited diminished alveolar bone loss and lower expression of the activator of nuclear factor- κ B ligand RANKL/RANK. In addition, neutrophil migration and the production of pro-inflammatory cytokines IL-1 β and TNF- α were reduced⁴⁸.

PILOT CLINICAL STUDIES ON THE EFFECTS OF CBD ON ORAL BACTERIA

The development of oral hygiene products (toothpastes, mouthrinses, dental gels) containing CBD is based on its analgesic, healing, and anti-inflammatory properties as well the popularity of *Cannabis* plants in traditional/complementary medicine. The effects of CBD on gingival and periodontal inflammatory diseases have been mostly studied using primary cell cultures or cell lines, with relatively few experiments on laboratory animals⁴⁹.

Only two pilot studies have been conducted with human patients. The aim of the first study was to verify the *in vitro* antimicrobial effects of cannabinoids, CBD, cannabichromene (CBC), cannabinol (CBN), cannabigerol (CBG) and cannabigerolic acid (CBGA), compared to Oral B and Colgate toothpastes⁵⁰. Patients (aged 18-45 years, n=60) were divided according to the Dutch Periodontal Screening Index (DPSI) into 6 groups (n=10). After culturing bacteria from plaque collected from individual subjects for 24 h on agar plates coated with 12.5% cannabinoid solution or undiluted toothpaste, it was observed that the antibacterial effect of the cannabinoids was significantly better than that of the commercial toothpastes, depending on the colony count examined. The same authors published a study comparing the bactericidal effect of mouthrinses containing 1% CBD or CBG with 0.2% chlorhexidine gluconate, undiluted mouthrinses containing essential oils and ethanol, and an alcohol-free mouthrinse that contains fluoride⁵¹. Patients aged 18-83

years (n=72) were divided into three groups according to DPSI. Evaluation of mouthrinses according to the number of colonies after 24 h of culture showed a comparable bactericidal effect of CBD or CBG with chlorhexidine. Specifically, it was observed that the CBD preparation was significantly ($P<0.05$) better than the chlorhexidine one. The CBG preparation was also slightly better than chlorhexidine with a similarly significant difference ($P<0.06$). The authors observed no significant difference ($P>0.3$) between CBD and CBG mouthrinses. However, the authors of the two studies did not evaluate which species of bacteria survived in the colonies, or whether the ecology of the oral microflora was perturbed by pathogenic bacteria.

CONCLUSIONS AND CRITICAL ASSESSMENT

The anti-inflammatory activity of CBD in the oral cavity will depend not only its effect on periodontal tissues, but also its interaction with oral microflora, especially periopathogens (Fig. 2). Interactions of CBD with the interplay between Gram-positive and Gram-negative bacteria comprising the oral microflora and periodontal cells (including immunocompetent cells) can lead to anti-inflammatory or cytoprotective effects. However, in inter-

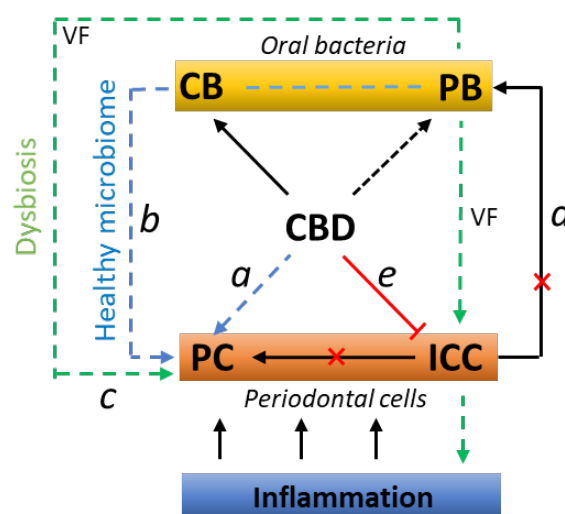


Fig. 2. The role of cannabidiol (CBD) in the interplay of oral bacteria and periodontal cells in the immune-inflammatory response. **a.** CBD has anti-inflammatory and cytoprotective effects on periodontal cells (PC). **b.** CBD affects growth of Gram-positive (and sometimes Gram-negative) bacteria. Development of Gram-negative pathogenic bacteria (PB) can be suppressed by CBD, e.g. *P. gingivalis*. If the ecology of the commensal bacteria (CB) is not disturbed in favor of PB, the oral microbiome will maintain oral homeostasis and good oral health (dotted blue). **c.** When there is dysbiosis, the periodontium is adversely affected (green). Bacterial inflammation is exacerbated by bacterial virulence factors (VF, see Table 1). **d.** Bacterial infection is detected and eliminated by immunocompetent periodontal cells (ICC). **e.** CBD has an immunosuppressive effect, which prevents destruction of the periodontium by the immune system, but does not eradicate bacterial infection (red).

preting studies describing the anti-inflammatory effects of CBD, its immunosuppressive effects have to be considered, since CBD can suppress the periodontal immune system and prevent periopathogen clearance (Fig. 2). Immunosuppression may be of clinical relevance with the long-term (chronic) application of CBD. Moreover, CBD is an exogenous ligand of the CB receptors; ECS activation not only initiates an anti-inflammatory cascade *via* the CB2 receptor, but also interferes with the highly interindividual ECS of the periodontium and associated tissues. In particular, CBD modulates the hydrolysis of endogenous cannabinoids. The positives and/or negatives of this intervention in the ECS from a holistic perspective have not been sufficiently explored.

There is no knowledge about the interaction of CBD with salivary components, so this avenue remains to be explored. CBD has a pleiotropic effect, and its impact on individual signaling pathways and periodontal/salivary processes will be strongly influenced by whether it is administered prophylactically or applied to a pre-existent inflammation. The applied dose will also play an important role, with regard to the composition of the oral microflora. CBD is a substance that exhibits biphasic behaviour. The concentrations of CBD utilized in the above-described experiments may not correspond to the concentrations normally applied topically in clinical practice or as oral hygiene products. The design and implementation of fully-fledged clinical trials will be crucial to further understanding the role CBD may be able to play in controlling periodontal inflammation. No interventional placebo-controlled randomized clinical observations have been published to date, which presents a major challenge in terms of a realistic assessment of the utility of CBD in the prophylaxis and treatment of periodontal inflammation. For a clinical trial, it is important that pure synthetic CBD be administered, rather than botanically sourced CBD, which carries a risk of contamination with other substances, or complex plant extracts in which CBD constitutes only one of the ingredients. We currently have minimal knowledge about the anti-inflammatory action and interaction of other (non-CBD) non-psychoactive phytocannabinoids with the periodontium. There is also a lack of information on the analgesic effect of CBD on oral tissues.

ABBREVIATIONS

AEA, Anandamide; CB, Commensal bacteria; CBC, Cannabichromene; CBD, Cannabidiol; CBG, Cannabigerol; CBGA, Cannabigerolic acid; CBN, Cannabinol; DPSI, Dutch periodontal screening index; ECS, Endocannabinoid system; GSK3 β , Glycogen synthase kinase-3 β ; HPLF, Human periodontal ligament fibroblast; ICC, Immunocompetent cell; LPS, Lipopolysaccharide; MIC, Minimal inhibitory concentration; MRSA, Methicillin-resistant *Staphylococcus aureus*; MV, Membrane vesicle; PB, Periopathogenic bacteria; PC, Periodontal cell; PDL, Periodontal ligament; PEA,

Palmitoylethanolamide; PI3K, Phosphatidylinositol-3-kinase; PPAR, Peroxisome proliferator-activated receptor; THC, Δ^9 -tetrahydrocannabinol; TLR, Toll-like receptor; VF, virulence factor.

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Author contributions: VS, JS, JV: conceptualization; VS: critical review; JV: literature search; JF, JV: graphics and tables; PJ, AJ, JF: manuscript writing – original draft preparation; VS, JV: manuscript writing – review and editing.

Conflict of interest statement: JS is CEO and has financial interest in CB21 Pharma Ltd., CBDepot Ltd. and PharmaCan Ltd. JV and VS are involved in the scientific board of CB21 Pharma Ltd. JV is engaged in scientific collaboration and has financial support from CB21 Pharma Ltd., CBDepot Ltd. and 4MP Technologies Ltd. All other authors declare no conflicts of interest with the contents of this article.

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