



Effects of Cannabidiol on Cytokines from Cigarette Smoke Condensate Treated-Human Gingival Fibroblasts.

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Abstract

Introduction: Cannabis sativa, commonly known as marijuana, is the most frequently used illicit drug for recreational purposes. Cannabidiol (CBD) is the major non-psychoactive constituent of Cannabis, composing as much as 45-50% of Cannabis extracts. It has been suggested that the use of CBD might reduce inflammation as it occurs in Periodontitis. Tobacco is a significant risk factor for the development and progression of Periodontitis. Aim: To evaluate the effects of CBD on cytokine expression from cigarette smoke condensate (CSC) treated human gingival fibroblasts (HGFs) in vitro.

Methods: To determine the highest non-toxic level of CBD that does not affect cell proliferation, the effects of CBD on HGFs cell proliferation and cytotoxicity were assessed using WST-1 and LDH assays at different CBD concentrations, respectively. HGFs were then incubated for 72 hours in serum-free media with CBD (31.4 µg/mL: 1µm), with CSC (100 µg/mL), with CBD and CSC, or with neither. Conditioned media were collected and examined for changes in cytokine expression using cytokine protein arrays.

Results: It was determined that 31.4 µg/mL of CBD was not toxic to the cells and did not affect cell growth, while all the higher concentrations examined did. The effects of CSC+CBD combined on IL-10 was determined to be synergistic (CSC+CBD was significantly greater than the sum of the individual effects of CSC and CBD, P-value 0.045). The effects of CSC+CBD combined were not significantly different from an additive effect for GRO a/b/g, GRO alpha (CXCL1), IL-3, IL-6, IL-8 (CXCL8), or MCP-1 (CCL2), all P-values greater than 0.05.

Conclusions: CBD at 31.4 µg/mL or lower does not affect cell proliferation and was not toxic to HGFs. There was a synergistic effect on HGFs with the combination of CSC+CBD on IL-10 protein expression, an anti-inflammatory cytokine. It has been suggested that the use of CBD might reduce inflammation as it occurs in Periodontitis and Implant failure. Tobacco is a significant risk factor for the development and progression of Periodontitis, as well as implant failure. This effect suggests that CBD could be of therapeutic value for tobacco users that developed Periodontitis or have Implants.

Background

Periodontal disease is an infectious and chronic inflammatory condition which affects the tooth-supporting tissues, leading to bone loss, pocket formation, attachment loss and eventually tooth loss. Tobacco use has been recognized as a significant risk factor for the development and progression of periodontal disease. The relative risk for periodontal disease in tobacco users is considerably higher than in non-tobacco users. Cigarette smoke condensate (CSC), the particulate matter of cigarette smoke is a complex mixture of up to 6000 components that include nitrosamines, benzenes, aldehydes, carbon monoxide and nicotine. Nicotine is a major component and the most pharmacologically active component of tobacco that exerts an addictive effect and may also affect the host immune responses. The purpose of this study was to investigate the effects of CBD on cigarette smoke condensate-treated human gingival fibroblasts. Experiments will determine if CBD will alter the expression of inflammatory cytokines increased by CSC treated human gingival fibroblasts.

Methods

Previously it was determined that 100 µg/mL of CSC was not toxic or affected cell proliferation of human gingival fibroblasts.⁴ HGFs were treated with CSC at a concentration of 100 µg/mL to determine if the HGFs currently being grown in the lab responded to CSC. Media was collected from CSC treated and untreated HGFs and gelatin zymography performed.

A CBD dose response on human gingival fibroblast cell proliferation and cell cytotoxicity were determined using WST-1 and LDH assays, respectively. Human gingival fibroblasts were then incubated for 72 hours in serum -free media with CBD (31.4 µg/mL: 1µm), with CSC (100 µg/mL), with CBD and CSC, or with neither. Conditioned media were collected and stored at -20°C until cytokine protein arrays (Ray Biotech, Norcross, GA) were performed.

Array C5 detects for 23 human cytokines and growth factors. The membranes from each cytokine antibody array kit were blocked for 30 minutes, incubated for 3 hours with conditioned media at room temperature, washed, and incubated for an additional 2 hours with horseradish peroxidases-conjugated streptavidin. Array kits contain detection agents that were mixed and applied to each membrane for approximately 2 minutes. Cytokines on the membranes were then visualized by autoradiography on X-ray film. Relative densities of visible spots were determined with an imaging software for semi-quantification. Each array contains duplicates of the same cytokines, yielding us a total of 6 determinations measured for their density for semi-quantification analyses. The density values obtained from positive controls of their density for semi-quantification analyses. The density values obtained from positive controls of each membrane were averaged and used to normalize the density of results from each array membrane. The experiments were repeated in triplicate and the averages was calculated.

Results

Table 1. WST-1 Assay

Concentration(µm)	Proliferation(%)	SD	Sig.
0	100.00	0.00	
15.9	2.72	0.64	.000 *
10.3	2.70	1.38	.000*
5.2	22.92	2.74	.000*
2.6	105.95	11.63	.966
1.3	109.14	14.77	.801
MeOH	100.24	9.71	1.000

*p values<0.05 statistically significant.

Table 2. LDH Assay

Concentration(µm)	Cytotoxicity(%)	SD	Sig.
0	0.00	0.00	
15.9	100.74	7.07	.000*
10.3	95.73	10.17	.000*
5.2	58.26	1.80	.000*
2.6	-5.20	2.22	.843
1.3	-1.46	0.62	1.000
MeOH	-3.48	2.68	.972

* p values<0.05 statistically significant.

Table 3. Ray Biotech human cytokine antibody Array C5 ((Ray Biotech, Norcross, GA) detects for 23 human cytokines.

Each antibody is spotted in duplicate vertically		A	B	C	D	E	F	G	H
	1	POS	POS	NEG	NEG	GCSF	GM-CSF	GRO a/b/g	GRO alpha (CXCL1)
	2								
	3	IL-1 alpha (IL-1 F1)	IL-2	IL-3	IL-5	IL-6	IL-7	IL-8 (CXCL8)	IL-10
	4								
	5	IL-13	IL-15	IFN-gamma	MCP-1 (CCL2)	MCP-2 (CCL8)	MCP-3 (CCL7)	MIG (CXCL9)	RANTES (CCL5)
	6								
	7	TGF beta 1	TNF alpha	TNF beta	BLANK	BLANK	BLANK	BLANK	POS
	8								

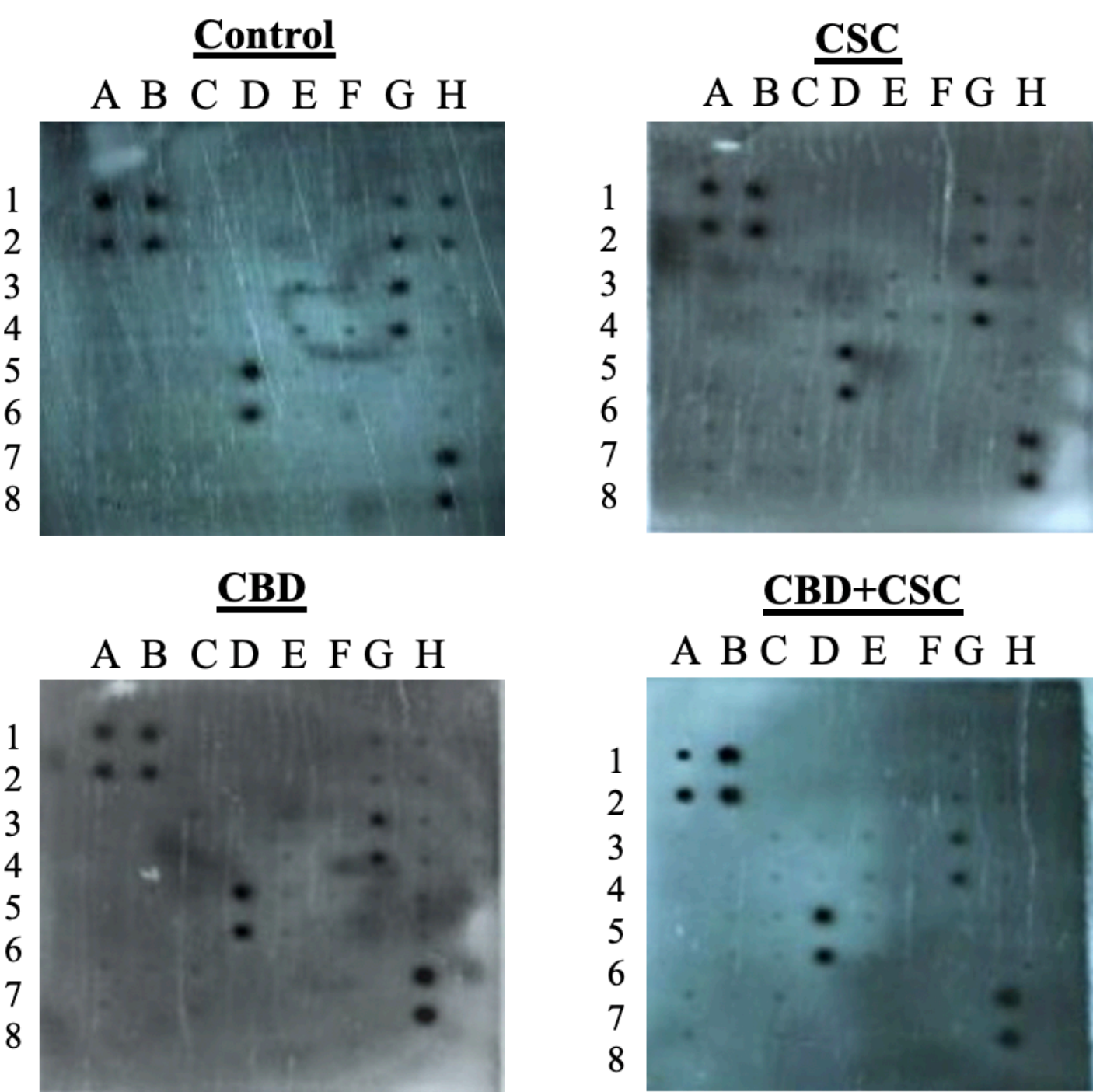


Figure 1. Cytokines expression on x-ray film.

Table 4. Results of pair-wise comparisons between groups

	CBD vs control		CBD vs CSC		CBD vs CSC+CBD		CSC vs control		CSC vs CSC+CBD		CSC+CBD vs control		CSC+CBD vs sCSCCBD	
Cytokine	Result	p	Result	p	Result	p	Result	p	Result	p	Result	p	Result	p
GRO a/b/g	n.s	0.336	n.s	0.399	CBD<CSC+CB D	0.042	n.s	0.164	n.s	0.365	CSC+CBD>c	0.028	n.s	0.383
GRO alpha (CXCL1)	n.s	0.133	n.s	0.234	CBD<CSC+CB D	0.014	CSC>c	0.044	n.s	0.123	CSC+CBD>c	0.006	n.s	0.901
IL-3	CBD>c	0.030	n.s	0.434	n.s	0.126	CSC>c	0.047	n.s	0.207	n.s	0.899	n.s	0.123
IL-5											n.s	0.323		
IL-6	n.s	0.181	n.s	0.752	n.s	0.752	n.s	0.231	n.s	0.585	n.s	0.153	n.s	0.379
IL-8 (CXCL8)	n.s	0.135	n.s	0.970	n.s	0.149	n.s	0.146	n.s	0.149	CSC+CBD>c	0.032	n.s	0.228
IL- 10	n.s	0.133	n.s	0.416	CBD<CSC+CB D	0.018	n.s	0.116	CSC<CSC+CB D	0.006	n.s	0.060	CSC+CBD>sCSCCBD	0.045
MCP- 1 (CCL2)	n.s	0.336	n.s	0.604	n.s	0.520	n.s	0.400	n.s	0.720	n.s	0.521	n.s	0.140
MCP- 2 (CCL8)							n.s	0.442	n.s	0.171	n.s	0.154		
MCP-3 (CCL7)							CSC>c	0.039	CSC<CSC+CB D	0.017	CSC+CBD>c	0.005		
RANTES (CCL5)			n.s	0.526										
TGF beta 1							n.s	0.569	n.s	0.425	n.s	0.331		
TNF alpha					n.s	0.269								
TNF beta									n.s	0.823				

p=P-value, sCSCCBD=sum of the individual effects of CSC and CBD, n.s.= not significant, P-values greater than 0.05 and yellow highlights= P-values less than 0.05.

Results Summary

- ❖ CBD was significantly higher than control for IL-3.
- ❖ CSC was significantly higher than control for GRO alpha (CXCL1), IL-3, and MCP-3 (CCL7).
- ❖ CSC+CBD was significantly higher than control for GRO a/b/g, GRO alpha (CXCL1), IL-8 (CXCL8), and MCP-3 (CCL7).
- ❖ CSC+CBD was significantly higher than CBD for GRO a/b/g, GRO alpha (CXCL1), and IL-10.
- ❖ CSC+CBD was significantly higher than CSC for IL-10 and MCP-3 (CCL7).
- ❖ The effect of CSC+CBD combined on IL-10 (an anti-inflammatory cytokine) was determined to be synergistic on HGFs (CSC+CBD was significantly greater than the sum of the individual effects of CSC and CBD). The effect of CSC+CBD combined was not significantly different from an additive effect for GRO a/b/g, GRO alpha (CXCL1), IL-3, IL-6, IL-8 (CXCL8), or MCP-1 (CCL2).

Conclusions

- ❖ CBD at 31.4 µg/mL or lower did not affect HGF proliferation and was not toxic. Individually CBD or CSC turned on the expression of IL-10. CBD+CSC was synergistic and increased IL-10 expression 2.54-fold compared to CSC alone and 9.33-fold compared to CBD alone. CBD or CSC individually turned-on IL-3 expression compared to the control and CBD+CSC turned off the expression of pro-inflammatory cytokine IL-3. These results appear to be mechanisms by which cells reestablishes homeostasis. These effects suggest that CBD could be of therapeutic value for tobacco users that developed Periodontitis.