CANNABIS SATIVA L. EXTRACT AND CANNABIDIOL EXERT ANTI-INFLAMMATORY EFFECTS IN HUMAN FIBROBLASTS AND KERATINOCYTES

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Introduction: Skin inflammatory diseases, such as dermatitis and psoriasis, are the results of a series of complex events which include dysregulation and abnormal expression of inflammatory mediators or their receptors. Fibroblasts, which strictly interact with keratinocytes, are deeply involved in skin wound healing, stimulating keratinocyte proliferation, differentiation, and migration. Keratinocytes, the most abundant cells in the epidermis, play a key role in the release of numerous pro-inflammatory mediators (e.g. IL-8, MMP9, and VEGF). IL-8is involved in neutrophil recruitment, VEGF regulates the angiogenesis process, while MMP9contributes to extracellular matrix degradation. These pro-inflammatory mediators are regulated by different transcription factors, including NF-κB. *Cannabis sativa* L. (hemp) is an annual herbaceous plant belonging to the Cannabaceae Family. The flowered tops contain the highest concentration of cannabinoids which include delta-9tetrahydrocannabinol (Δ9-THC), and cannabidiol (CBD). CBD is the second major cannabinoid, without psychotropic activity, occurring in C. sativa and its anti-inflammatory activity on skin has been demonstrated in mice; however, no studies on human keratinocytes inflammation have been reported so far. The aim of the present study was to investigate the potential effect of a *Cannabis sativa* L. ethanolic extract (CSE) standardized in CBD as anti-inflammatory agent in the skin, unraveling the molecular mechanisms in human keratinocytes and fibroblasts.

Materials and methods: CSE was prepared from *Cannabis sativa* L. flowers by ethanolic extraction by LINNEA SA (Riazzino, Switzerland). Cannabidiol (CBD, 99.5% HPLC purity) was isolated and purified; material was subjected to prolonged decarboxylation to allow conversion of the acidic form to CBD. CSE and CBD were assayed in both fibroblast (HDF) and keratinocytes (HaCaT) induced by TNFα or UVB. IL-8, MMP-9and VEGF release from cells were measured by ELISA assays, NF-κB driven transcription by a reporter plasmid, while gene expression by real-time PCR.

Results: In HaCaT cells, CSE was able to inhibit TNFα-induced IL-8secretion only at the highest concentration tested (50 mg/ml), whereas in HDF showed a more pronounced inhibitory effect, with low IC_{50} (15.13mg/ml). CSE also reduced MMP-9secretion in both cell lines (IC_{50} 18.0 and 7.21, for HaCaT and HDF respectively) and VEGF from HaCaT cells (IC_{50} 26.8mg/ml). CBD showed low or no inhibitory effect on IL-8, VEGF and MMP-9, with the exception of TNFα-induced MMP-9secretion in HaCaT cells, however CBD reduced NF-κB driven transcription in both cell lines. CSE (25μg/mL) and CBD (4μM) were tested on the expression of 84genes related to inflammation and wound healing, in HaCaT and HDF respectively. CSE decreased the mRNA levels of the TNFα-up-regulated genes, whereas CBD was not able to fully explain the activity elicited by the extract in both cell lines.

Conclusions: These results suggest that CSE is able to inhibit the release of inflammatory mediators in fibroblasts and keratinocytes, the most abundant cells occurring in the skin. The mechanism of action seems to involve NF-kB impairment, confirmed by the reduction of IL-8, MMP-9, and VEGF, whose gene expressions are highly dependent by NF-kB. The down-regulation of genes involved in wound healing and skin inflammation were not strictly associated to the presence of CBD, suggesting that other unknown compounds occurring in the extract my exert anti-inflammatory effects.