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# Can Cannabinoids Modulate Fibrotic Progression in Systemic Sclerosis?

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Since ancient times, plants have been used for therapeutic purposes. *Cannabis sativa* has been widely used as a medicinal herb by Ayurveda and traditional Chinese medicine for centuries [1]. In the 1990s the first active compound of *Cannabis sativa*, $\Delta^9$-tetraidrocannabinol ($\Delta^9$-TCH), was isolated, leading to the discovery of the 'cannabinoid world' and its biological activity [2]. Indeed, the discovery of $\Delta^9$-TCH was followed by the identification of its receptors, two specific G-coupled proteins known as cannabinoid type 1 (CB1) and cannabinoid type 2 (CB2), and their endogenous ligands (anandamide, 2-aciglicerol) [3-5]. Together, cannabinoid receptors, endo-cannabinoids, and the enzymes involved in their synthesis and degradation constitute the endocannabinoid system.

Today, more than 60 active terpenophelic compounds called phytocannabinoids have been identified within the resin of *Cannabis sativa*. In addition, several endogenous fatty acid derivatives have been described as endocannabinoids and a wide collection of synthetic cannabinoids has been developed. The common feature of these three groups of cannabinoids is their ability to bind and modulate cannabinoid receptors. CB1 receptors are primarily localized in the central nervous system and mediate the well-known psychoactive effects of cannabinoids [3]. On the other hand, CB2 receptors are primarily distributed in the periphery and modulate immunological and inflammation responses [4,6].

In recent years there has been an increased demand for cannabinoid-based medicines, particularly for the symptomatic amelioration of several chronic diseases. For this purpose herbal cannabis is available under special access conditions as an unapproved drug in many countries [7,8].

## CANNABINOIDS AND FIBROSIS

A wide range of biological effects, including cell turnover, inflammation and immunity modulation, has been attributed to cannabinoids. However, not all of the biological activities of cannabinoids can be fully explained by CB1 or CB2-mediated mechanisms. Additional receptor pathways, including the transient receptor potential vannilloid type-1 (TRPV1), the peroxisome proliferator-activated receptors (PPARs), G-protein receptor 55 (GPR55) as well as nicotine, 5-HT3 and adenosine A2A receptors, have been involved in cannabinoid signal transduction [9,10].

In the last few years, fibrosis modulation has emerged as one of the more fascinating biological activities of cannabinoids. Initial data referred to liver fibrosis, focusing on the antifibrotic effect by CB1 receptor antagonism [11]. Subsequent studies showed that cannabinoids may play a pro- or antifibrogenic role depending on their interaction with CB1 or CB2 receptors [12].

## CANNABINOIDS AND SYSTEMIC SCLEROSIS

Based on the above observations, we aimed to investigate the role of cannabinoids in systemic sclerosis (SSc), an autoimmune disease characterized by diffuse fibrosis [13,14]. In SSc, autoimmunity, small vessel vasculopathy and inflammation represent the main features that precede fibrosis by an inappropriate fibroblast activation and trans-differentiation into myofibroblasts [15,16]. This leads to excessive extracellular matrix (ECM) synthesis and deposition, with consequent damage of the affected tissues.

## PRELIMINARY IN VITRO EXPERIMENTS

We first evaluated the expression of cannabinoid receptors on skin fibroblasts from patients with diffuse SSc (dSSc). Protein expression experiments showed that both CB1 and CB2 receptors were overexpressed in dSSc skin fibroblasts compared to healthy ones. In addition, the incubation of dSSc fibroblasts with a synthetic cannabinoid receptor agonist, WIN55,212-2, decreased their expression in a dose-dependent manner together with a parallel reduction in collagen production as well as in pro-fibrotic cytokines, including transforming growth factor-beta (TGFβ), connective tissue growth factor and interleukin-6 (IL-6). Interestingly, this inhibitory effect was not abrogated by selective cannabinoid receptor antagonism, suggesting that it was not mediated by classical CB1 nor CB2 receptors [17]. As mentioned earlier, dSSc fibroblasts are

inappropriately activated and characterized by the inability to undergo apoptosis. Since activated fibroblasts are characterized by expression of the cytoskeletal protein α-smooth muscle actin (αSMA), we evaluated whether cannabinoids could influence dSSc fibroblast over-activation. The experiments showed a significantly lower rate of apoptotic cells in dSSc fibroblasts as compared with healthy ones. Cannabinoid exposure of dSSc fibroblasts increased the number of apoptotic cells with a parallel decrease in α-MA mRNA and protein expression. Again, these effects were not reverted by selective cannabinoid receptor antagonists [17].

The results of this first study suggested that cannabinoid receptor agonism might modulate fibrogenesis in dSSc fibroblasts by a non-CB1 or CB2-mediated mechanism, in contrast to the specific role played by these two classical cannabinoid receptors suggested by other authors [18,19].

## FROM BENCH TO MURINE MODELS

Based on these premises, in vivo experiments on a bleomycin mouse model were conducted. Bleomycin-induced dermal fibrosis is considered a reliable experimental model for scleroderma as it primarily reflects early stages of SSc with increased release of collagen and other ECM components, migration of inflammatory cells into the skin, and substitution of the adipose layer by fibrotic tissue [20].

Our study protocol comprised three groups of animals. Each group was treated with saline solution (control group), bleomycin, or bleomycin plus synthetic cannabinoid receptor agonist (WIN55,212-2) for 3 weeks. Skin specimens showed normal skin appearance in the control group, whereas bleomycin-treated animals showed a significant increase in skin thickness and collagen content, inflammatory infiltrates and the typical loss of the fat layer. The co-treatment with the synthetic cannabinoid showed a significant reduction in skin thickness and collagen content as well as a significantly lower degree of inflammation [21]. However, since our in vitro data failed to demonstrate a classical cannabinoid receptor pathway for the anti-fibrotic activity of cannabinoids, we performed further in vivo experiments using ajulemic acid (AjA), a non-psychoactive synthetic analogue of tetrahydrocannabinol able to bind the peroxisome proliferator-activated receptor-γ (PPARγ), which was recently shown to play critical role in connective tissue homeostasis [22-24]. In fact, aberrant PPARγ function seems to be implicated in pathological fibrosis, including SSc [25]. Following the same study protocol as before, oral administration of AjA prevented the development of skin fibrosis, and reduced skin thickness almost to control levels. In addition, we evaluated the effect of AjA on established fibrosis, less dependent on inflammatory mechanisms, by using a modified model of bleomycin-induced dermal fibrosis. Mice were challenged with bleomycin for 6 weeks and AjA treatment was begun during the last 3 weeks of bleomycin injections when fibrosis was already established. Results showed that AjA administration in the last 3 weeks of bleomycin challenge arrested further progression of fibrosis, but did not alter preexisting ECM accumulation. These results were also confirmed in another model of dermal fibrosis, independent of autoimmune and inflammatory phenomena, the constitutively active TGFβ receptor type I mouse model, characterized by generalized dermal fibrosis and fibroblast-specific activation of TGFβ signaling [26].

Although the synthetic tetrahydrocannabinol analogue AjA is a weak ligand of the classical cannabinoids receptors CB1 and CB2, and since our previous studies with high affinity cannabinoid receptor agonist WIN55,212-2 showed that the cannabinoid anti-fibrotic effects were not mediated by the classic cannabinoid receptors, we suggested that AjA might act to reduce fibrosis directly through a PPARγ-mediated mechanism. Therefore, parallel to the in vivo study, we performed in vitro experiments by incubating dSSc fibroblasts with AjA at different concentrations. Consistent with the literature, we observed a reduced expression of PPARγ in dSSc fibroblasts compared to healthy ones. Results showed that AjA promoted a dose-dependent reduction of procollagen and TGFβ, mirrored by a concomitant increase of PPARγ expression and its endogenous ligand PGJ2. In addition, pre-incubated with the highly selective PPARγ antagonist GW9662 completely reverted the inhibitory effect of AjA on collagen production, suggesting a PPARγ pathway dependency [26].

## CONCLUSIONS

According to our in vitro and in vivo experimental models, cannabinoids are able to modulate fibrosis. The exact mechanism underlying this effect requires further investigation, but it seems to go beyond their anti-inflammatory and immunomodulatory properties. In addition, besides CB1 and CB2 cannabinoid receptors, PPARγ might play a key role in the modulation of fibrosis by cannabinoids.

Since preclinical data on cannabinoids show their capability to modulate fibrosis, inflammation and vasodilatation, these molecules could be ideal drugs for targeting SSc. In accordance, a phase II double-blind, randomized, placebo-controlled trial was recently initiated to evaluate the safety, tolerability and efficacy of a synthetic oral endocannabinoid-mimetic drug in patients affected by diffuse cutaneous systemic sclerosis.

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