Can Cannabinoids Modulate Fibrotic Progression in Systemic Sclerosis?

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ince 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, Δ9-tetrahydrocannabinol (Δ9-TCH), was isolated, leading to the discovery of the 'cannabinoid world' and its biological activity [2]. Indeed, the discovery of Δ9-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 vanilloid 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 anti-fibrotic effect by CB1 receptor antagonism [11]. Subsequent studies showed that cannabinoids may play a pro- or anti-fibrogenic 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 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.

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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References

Out-RANKing osteosarcoma

Osteosarcoma, the most common primary bone cancer, can be difficult to treat, especially in patients with metastatic disease. Chen et al. developed genetically engineered mouse models of osteosarcoma and used them to demonstrate that receptor activator of nuclear factor-κB ligand (RANKL) signaling contributes to the progression of this disease. Furthermore, denosumab, an antibody against RANKL already used in patients with other bone diseases, inhibited osteosarcoma in mouse models and so is a viable candidate for future testing in human patients.

Capsule

Complementarity and redundancy of IL-22-producing innate lymphoid cells

Intestinal T cells and group 3 innate lymphoid cells (ILC3 cells) control the composition of the microbiota and gut immune responses. Within the gut, ILC3 subsets coexist that either express or lack the natural cytotoxicity receptor (NCR) NKP46. Rankin et al. identified the transcriptional signature associated with the transcription factor T-bet-dependent differentiation of NCR+ ILC3 cells into NCR− ILC3 cells. Contrary to the prevailing view, the authors found by conditional deletion of the key ILC3 genes Stat3, Il22, Tbx21 and Mc11 that NCR+ ILC3 cells were redundant for the control of mouse colonic infection with Citrobacter rodentium in the presence of T cells. However, NCR+ ILC3 cells were essential for cecal homeostasis. These data show that interplay between intestinal ILC3 cells and adaptive lymphocytes results in robust complementary failsafe mechanisms that ensure gut homeostasis.

Capsule