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New Drugs

Cannabinoids: A new hope for breast cancer therapy?

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ABSTRACT

Breast cancer is a very common disease that affects approximately 1 in 10 women at some point in their lives. Importantly, breast cancer cannot be considered a single disease as it is characterized by distinct pathological and molecular subtypes that are treated with different therapies and have diverse clinical outcomes. Although some highly successful treatments have been developed, certain breast tumors are resistant to conventional therapies and a considerable number of them relapse. Therefore, new strategies are urgently needed, and the challenge for the future will most likely be the development of individualized therapies that specifically target each patient's tumor. Experimental evidence accumulated during the last decade supports that cannabinoids, the active components of *Cannabis sativa* and their derivatives, possess anticancer activity. Thus, these compounds exert anti-proliferative, pro-apoptotic, anti-migratory and anti-invasive actions in a wide spectrum of cancer cells in culture. Moreover, tumor growth, angiogenesis and metastasis are hampered by cannabinoids in xenograft-based and genetically-engineered mouse models of cancer. This review summarizes our current knowledge on the antitumor potential of cannabinoids in breast cancer, which suggests that cannabinoid-based medicines may be useful for the treatment of most breast tumor subtypes.

Introduction

Cannabinoids are a family of unique compounds synthesized by *Cannabis sativa* (marijuana) that have not been found as yet in any other plant. The main representative cannabinoid, owing to its high abundance in the plant and its strong biological activity, is Δ^9-tetrahydrocannabinol (THC).[1] For decades, and mainly due to their lipophilic nature, it was assumed that cannabinoids exerted their effects by perturbing the biophysical properties of biological membranes.[2] This scenario changed dramatically in 1990, when the first cannabinoid-specific membrane receptor (CB_1) was characterized and cloned.[3] This discovery boosted the scientific research on cannabinoids, which currently constitutes a very active field in biomedicine. The term cannabinoid includes now not only the marijuana-derived compounds that are structurally related to THC (phytocannabinoids), but also the synthetic molecules that activate the same primary targets as THC (synthetic cannabinoids) and a family of endogenously produced compounds that engage cannabinoid receptors (endocannabinoids), of which anandamide (arachidonoylethanolamide, AEA)[4] and 2-arachidonoylglicerol (2-AG)[5,6] are the main – if not only – representatives. Two G protein-coupled receptors (GPCRs) that are selectively engaged by cannabinoids have been cloned so far (CB_1 and CB_2),[3,7] and some evidence indicates that cannabinoids may act by binding to additional receptors such as the vanilloid receptor 1 (TRPV1) and the orphan GPCR GPR55.[8] The endocannabinoids, together with their receptors and the proteins in charge of their transport, synthesis and degradation, constitute the so called endocannabinoid system, which is involved in the control of a wide variety of biological functions such as motor behavior, memory and learning, pain, the immune response or bone physiology, just to mention a few.[2]

Cannabinoids and cancer

The therapeutic properties of marijuana have been known for millennia, but the use in the clinic of either plant-derived preparations or pure cannabinoids is still very limited. To date, only three cannabinoid-based medicines can be prescribed, and for very specific indications. The orexigenic and anti-cachexic properties of cannabinoids are exploited by Marinol® (oral capsules of dronabinol – synthetically generated THC) to manage weight loss associated to wasting syndrome in patients with AIDS. Sativex® (nabiximols, an oromucosal spray containing plant extracts enriched in THC and cannabidiol at an approximate 1:1 ratio) has been recently approved in several European countries, Canada and New Zealand for the relief of spasticity associated to multiple sclerosis, and in Canada for the treatment of neuropathic pain in the same disease. In the context of cancer, it is well established that cannabinoids have

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antiemetic properties,[9] and, in fact, Marinol® and Cesamet® (oral capsules of nabilone – a synthetic THC analog) can be prescribed to prevent nausea and vomiting elicited by standard chemotherapeutic regimes. In addition, Sativex® is approved in Canada for the treatment of cancer-associated pain.

Aside from these palliative actions, recent preclinical evidence suggests that cancer patients might benefit from cannabinoids in an additional manner: since the late 1990s, an important amount of experimental data has shown that these compounds exert anti-tumor effects in different models of cancer, ranging from cell cultures to xenografted and genetically engineered mice.[10] Cannabinoid anti-tumor action relies on the blockade of several hallmarks of tumor progression. Thus, they inhibit uncontrolled cancer cell growth (by inhibiting cancer cell proliferation and by inducing cancer cell death by apoptosis) and impair tumor angiogenesis and metastasis. Interestingly, some – if not all – of these effects have been observed in tumor cells from very different origin, including gliomas, melanomas, carcinomas of the breast, skin, lungs, liver, pancreas, colon, prostate, and lymphomas amongst others, which indicates that cannabinoid anti-tumor action has a general rather than tumor-type specific nature.[10]

Anti-tumor effects of cannabinoids in breast cancer

Breast cancer is the most common malignant disease among Western women. Although the rates of mortality have declined since the late 1990s, mainly due to adjuvant systemic therapy and earlier detection by palpation and mammograms, certain breast tumors remain resistant to conventional therapies. In addition, current treatments have side effects that substantially affect the patients' quality of life.[11–13] It is therefore obvious that new therapeutic strategies are needed for the management of this condition. As breast cancer is an extremely heterogeneous disease that comprises tumors that are very diverse in terms of molecular portraits, prognosis and treatments,[13] in this review we will distinguish the three main breast cancer subtypes according to classical molecular profiles: hormone receptor-positive, HER2-positive and triple-negative tumors. Although the strength of the experimental data is different in each case, evidence obtained so far suggests that cannabinoid-based medicines may be useful for the treatment of these three breast cancer subtypes.

Cannabinoids and hormone-sensitive breast cancer

The presence of estrogen receptors (ER) and/or progesterone receptors (PR) in breast cancer cells, as determined by immunohistochemistry-based methods, defines a subgroup of breast tumors that may respond to endocrine therapy. Specifically, these patients are treated with surgical and/or pharmacological approaches that block estrogenic signaling, which has pro-proliferative and pro-survival features. The targeted strategies include the removal of the endogenous source of estrogens (the ovaries) and/or the use of selective estrogen receptor modulators, such as the widely used tamoxifen, or inhibitors of aromatase, the main enzyme responsible for estrogen synthesis.[14]

It has been demonstrated that cannabinoids modulate pivotal tumor progression-related aspects of ER^+/PR^+ breast cancer cells. Thus, anandamide inhibits basal[15,16] and prolactin- and nerve growth factor (NGF)-induced proliferation[15,17] of MCF-7 and EFM-19 cells in culture. This effect is mediated by the activation of CB_1 receptors[15–17] and is not accompanied by cancer cell death.[15] Anandamide produces this anti-proliferative action by blocking the progression through the cell cycle, specifically by preventing the transition from the G1 to the S phase,[15] and by inhibiting adenylyl cyclase and thus activating the Raf-1/ERK/MAPK cascade, which, upon sustained activation, ultimately down-regulates prolactin and TrkA NGF receptors[15–17] (Fig. 1).

The proliferation of the ER^-/PR^+ human breast cancer cell line EVSA-T is also decreased in response to THC.[18,19] Once again, the cell cycle is targeted: a blockade in the transition from G2 to mitosis via CB_2 receptors, produced by the inhibition of CDK1, was observed.[19] Cell cycle arrest is ensued by apoptotic cell death,[18,19] and the activation of the transcription factor JunD, owing to upregulation of gene expression and translocation to the nucleus, is essential for these actions[18] (Fig. 1).

Besides cancer cell proliferation, cannabinoids impair ER^+/PR^+ cancer cell migration and invasion in culture. Specifically, selective activation of CB_2 receptors in MCF-7 cells that overexpress the chemokine receptor CXCR4 inhibited chemotaxis and wound healing as induced by the CXCR4 ligand CXCL12[20] (Fig. 1). The CXCL12/CXCR4 signaling axis plays a pivotal role in directing breast cancer cells to distant sites[21] and, therefore, the aforementioned finding suggests that cannabinoids may modulate hormone-sensitive breast cancer metastasis. However, the experimental support for this notion is still weak and further research with more complex models should be performed to validate it.

Cannabinoids and HER2-positive breast cancer

The breast tumors that express the tyrosine kinase receptor HER2, as determined by immunohistochemistry and fluorescence *in situ* hybridization (FISH) approaches, constitute another well defined breast cancer histopathological subtype. HER2 belongs to the epidermal growth factor receptor (EGFR) family, which consists of four members (ErbB1/HER1/EGFR, ErbB2/HER2, ErbB3/HER3 and ErbB4/HER4). They all have intrinsic tyrosine kinase activity and, upon ligand binding and subsequent dimerization, they activate a number of oncogenic processes, including cell proliferation and survival.[22] The outcome of these patients has considerably improved since the incorporation to the clinics of Herceptin® (trastuzumab, a humanized monoclonal antibody against the extracellular domain of HER2). Other compounds are in use or under development to overcome trastuzumab resistance, the most prominent being Tykerb® (lapatinib), a dual EGFR/HER2 tyrosine kinase inhibitor.[13]

Strong preclinical evidence suggests that cannabinoids may be useful for the treatment of this particular subset of patients. Thus, THC produces a significant anti-tumor action in MMTV-neu mice,[23] a well established and clinically relevant model of HER2-positive metastatic breast cancer.[24] THC treatment reduces not only tumor growth but also the number of tumors generated per animal and the percentage of animals with lung metastases.[23] THC action relies on the impairment of cancer cell proliferation, via inhibition of the pro-tumorigenic kinase AKT, and on the induction of cancer cell death by apoptosis.[23] A reduction in the number of tumor blood vessels is also observed, suggesting that tumor angiogenesis is impaired by THC[23] (Fig. 2).

Xenograft-based approaches strengthen the hypothesis that HER2-overexpressing tumors may be sensitive to cannabinoids. Two different cell lines isolated from MMTV-neu-derived tumors were injected either subcutaneously in immune-deficient mice[23] or orthotopically in immune-competent syngenic FVB mice[20] and treated with THC[23] and/or CB_2-selective agonists.[20,23] In both cases, a significant reduction in tumor growth was observed, mediated by the inhibition of AKT in one case[23] and accompanied by a decrease in the levels of activated ERK and CXCR4 in the other[20] (Fig. 2).

Of interest, the anti-tumor effect of cannabinoids in all the HER2-positive breast cancer models used so far is mediated by the activation of CB_2 receptors (Fig. 2). Thus, the anti-tumor action of THC in the MMTV-neu model is mimicked by the CB_2-selective agonist JWH-133.[23] In the same line, the growth-inhibiting effect