EFFECT OF CANNABIGEROL, A NON-PSYCHOTROPIC PHYTOCANNABINOID, ON AKT AND MAPK ACTIVATION IN CANCER CELLS.

1)D'aloia A. 2)Ceriani M. 3)Stucchi S. 4)Costa B.

Dept. of Biotechnology and Bioscience

The plant Cannabis sativa produces over 421 chemical compounds, including about 80 terpeno-phenol compounds named phytocannabinoidis that have not been detected in other plant. Phytocannabinoids include psychotropic compounds such as Δ9-tetrahydrocannabinol and many other non-psychotropic compounds of therapeutic interest, such as cannabigerol (CBG).The exact mode of action of major phytocannabinoids has to be elucidated, but both, receptor and non-receptor mediated effects are to be clarified. CBG appears as a relatively low concentration intermediate in the plant, although recent breeding works have yielded Cannabis chemotypes expressing 100% of their phytocannabinoid content as CBG (de Meijer et al., 2003). According to recent literature, the main cannabinoid receptors, CB1 and CB2, contain seven transmembrane spanning domains; they are both coupled by Gi/o proteins to adenylyl cyclase in a negative way, and also to the mitogen-activating protein (MAP) kinase in an activating manner (Howlett et al., 2002). However, CB1 can also act through GS proteins to activate adenylate cyclase (Pertwee, 2006). When receptors are activated by phytocannabinoids, the cyclic adenosine monophosphate (cAMP) level is decreased by inhibiting adenylate cyclase and stimulating MAP kinase. Older and recent studies support analgesic, antierythemic, antibacterial, antidepressant and antihypertensive actions for CBG. Recent development suggest that non-psychotropic phytocannabinoids exert a wide range of pharmachological effects; in particular, CBG has shown interesting anti-proliferative and pro-apoptotic properties in a panel of tumor cell lines (Izzo et al., 2009). The high frequency of RAS mutations in human cancers (33%) has stimulated intense interest in the development of anti-Ras inhibitors for cancer therapy. Currently, the major focus of these efforts is centered on inhibitors of components involved in Ras downstream effector signaling: phosphatidylinositol 3-kinase (PI3K) which activate protein kinase B/Akt involved in, cell growth, proliferation, differentiation, motility, survival and intracellular trafficking; Mitogen-activated protein kinase (MAPK), which regulate cell functions including proliferation, gene expression, differentiation, mitosis, cell survival, and apoptosis and the guanine nucleotide exchange factors of the Ras-like (Ral) small GTPases (RalGEFs) that activate Ral GTPase involved in including proliferation, vesicular transport, cytoskeletal organization, tumorigenesis invasion and metastasis in in vitro and animal model.

Akt, a serine/threonine-specific protein kinase plays a key role in multiple cellular processes such as glucose metabolism, apoptosis, cell proliferation and cell migration. Due to these roles we have has been demonstrated that AKT is involved in tumor development and progression. The main aim of this project is to clarify the mechanisms of action of CBG in NIH and NIH kRas cells to determine the signal transduction pathway that are regulated by this phytocannabinoid.

Initially it has been performed an in vitro Akt/PKB Kinase assay (Cyclex) to evaluate the inhibitory activity of CBG on purified Akt; through this assay it has been demonstrated that Akt activity is inhibited by 75% and that the EC50 of CBG is 20,98 µM.

Then CBG activity has been tested on cell lines using different concentration of this cannabinoid (1-5-10 µM); in particular we use NIH 3T3 and NIH 3T3-KRas cells to evaluate the action of this cannabinoid on normal and activated c-K-ras cells to test its activity on tumor cells. Akt activation in vivo is inhibited by CBG at 10µM in NIH wt cells while in NIH 3T3-KRas cells CBG inhibits Akt activation at 1 µM. MAPK are not inhibited by CBG both in wt and in Kras cells. Kinetics assays has demonstrated that the treatment of cells with CBG negatively regulate NIH3T3 k-ras growth. CBG activity on Ras and Ral GTPase activation and on morphological changes in wt and K-Ras cells are in progress; furthermore we will determine which cannabinoid receptors is responsible for CBG effect. All these data will give new insight in CGB action and will permits to identify the pathways in which it is involved.

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