CANNABINOIDS INHIBIT HUMAN COLON CANCER CELL PROLIFERATION THROUGH CB_2-RECEPTOR AND CERAMIDE PATHWAY ACTIVATION

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Numerous recent studies have pointed to cannabinoids as potential anticancer agents. One of the possible molecular mechanisms underlying the anti-tumour effect of these compounds is the ability to induce tumour cell apoptosis through the activation of their receptors, namely CB_1 and CB_2. Moreover, the endocannabinoid system has been shown to modulate cell-signalling pathways involved in cancer cell growth.
The aim of this study was to investigate the presence of cannabinoid receptors in human colorectal cancer specimens and in human HT29 and DLD1 colon cancer cell lines, and to evaluate the effects of anandamide (AEA), 2-arachidonylglycerol (2AG), two endogenous CB_1/CB_2 agonists, and of ACEA and 3g, a CB_1 and a newly synthesized CB_2 agonists, on apoptosis and cell proliferation in colon cancer HT29 and DLD1 cell lines, *in vitro* and *in vivo*, in Balb/c nude mice.
Expression of CB_1 and CB_2 receptors was evaluated by Western blot analysis and RT-PCR. Cell proliferation was investigated using ^{3}[H]-thymidine incorporation; cell apoptosis was evaluated by a flow cytometric method, using Annexin V detection kit and by a flourometric method for caspase-3 determination. We also investigated the effect of the enzymatic inhibition of ceramide formation, using fumonisin B1 (FB1) on cell proliferation and apoptosis.
$CB_1$and CB_2 receptor genes and proteins were found both in tumour tissue and in normal colon mucosa. CB_1 and CB_2 receptor expression was related with tumour stage: they were higher in tumours with lymph node metastases (stage III) than in those tumours without metastases (stage I-II). Both the HT29 and the DLD1 cell lines expressed constitutively CB_1 and CB_2 receptors. Tumour cell proliferation was slight inhibited by ACEA (100nM) but mostly by 3g (100 nM); these effects were antagonized by AM 251 (100 nM) and by AM 630 (100 nM) a CB_1- and a CB_2- antagonist, respectively. The administration of FB1 (10 μM) prevented cell growth inhibition and apoptosis induced by 3g alone or in combination with ACEA. The pretreatment of HT29 cells, which constitutively express COX-2, with celecoxib (10 μM), a selective COX-2 inhibitor, potentiates the pro-apoptotic effect of 3g.
In conclusion, our data indicate that cannabinoids induce apoptosis and inhibit cell proliferation in human colon cancer cells through the activation of CB_2 receptors: These effects seem to depend on COX-2 enzyme activity and on ceramide pathway involvement.