Effects of CB83 a new resorcinol-anandamide 'hybrid' derivative on HT29 colon cancer cells

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The two most abundant cannabinoids in *Cannabis sativa* are tetrahydrocannabinol (THC) and cannabidiol (CBD). THC activates the CB1 and CB2 receptors and for the binding with the CB1 in CNS it is the psychoactive component of *Cannabis Sativa*. CBD does not interact efficiently with CB1 and CB2 receptors and it is not psychoactive. A recent area of investigation for the therapeutic applications of THC and CBD has been their anti-tumour activity against a variety of aggressive cancers. Each compound, however, has its own unique mechanism of action. In culture and *in vivo*, the primary mechanisms leading to the inhibition of tumour progression by THC include *de novo* synthesis of ceramide, this leads to stress endoplasmic reticulum and autophagy-mediated cell death. The pathways responsible for anti-tumour activity of CBD have not been well defined; *in vitro* the most unifying theme is the production of reactive oxygen species (ROS). On this basis, we have investigated the anti-tumour activity of a new resorcinol-anandamide 'hybrid' derivative named CB83 that can be considered a new class of stable cannabinoid receptor ligands on HT29 colon cancer cell line. In this study we compared CB83 anticancer activity *vs* 5-fluorouracil (FU), the standard antitumor drug used in colorectal cancer therapy. HT29 cells were cultured in RPMI 1640 medium supplemented with 10% FBS, 1% penicillin/streptomycin and maintained in a humidified atmosphere at 37°C and 5% CO_2. MTT-assay was performed to establish IC_{50} doses. Later we exposed the HT29 cells at obtained dose (CB83 IC_{50}= $1.5x10^{-6}$M, FU IC_{50}= $3.5x10^{-5}$M) for 24 hours to evaluate their potential antiproliferative effect using CyQuant cell proliferation assay and LDH cytotoxicity test. Finally, after 24 hours of exposition, to establish if CB83 can modulate cell survival/death through induction of oxidative stress in HT29 cells, we have measured the enzymatic and non enzymatic parameters of oxidative stress: catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and the levels of reduced (GSH) and oxidized glutathione (GSSG), the content of ascorbic acid (AA) and malondialdehyde (MDA).
The concentration of lactate dehydrogenase was significantly increased in cell supernatants after CB83 and FU treatment, suggesting cell death ($P<0.05$ and $P<0.01$ respectively). Furthermore, CyQuant assay show a significant decrease of cell proliferation at 24 hours after incubation with CB83 (59%; $P<0.01$) and FU (49%; $P<0.05$). The enzymatic antioxidant cellular system shows a decrease of CAT activity in CB83 and FU treated cells of 59% ($P< 0.05$) and 82.2% ($P<0.05$) respectively while GPX and GR activities were reduced only in FU treated cells (-53%; $p<0.02$ and -50% $P<0.05$ respectively). GPx activity was increased (+57%, $P<0.01$) while AA level decreased (-24.68%, $P<0.01$) in CB83 treated cells. Not different in GSH, GSSG and MDA levels were observed in CB83 and FU treated *vs* HT29 control cells. The results obtained showed no evidence of oxidative damage in HT29 treated cells and this let suppose that oxidative stress is not the mechanism involved in cellular damage induced from CB83 and FU in this *in vitro* study. The HT29 cellular antioxidant system is able to counteract the ROS formation in treated cells thus we have to considered other pathways involved in HT29 cellular death mediated by a ROS-indipendent mechanism. In conclusion, CB83 is a new potent cannabinoid analogue compound with a promising anti-tumour activity in colon cancer but the molecular mechanism of its effect is still unknown and its clarification needs additional investigations.