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AM630, a CB2 cannabinoid receptor antagonist, inhibits proliferation of human U87 glioblastoma cells by targeting the mitochondrial unfolded protein response

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INTRODUCTION The brain endocannabinoid system is a potent modulator of brain cancer cells fate, thus provides a novel therapeutic target for brain cancer. The gene encoding CB2 cannabinoid receptor has been identified and characterised as a novel oncogene (1). This study aims to investigate a new therapeutic strategy targeting CB2 cannabinoid receptor signalling pathways in human U87 glioblastoma cells.

METHODS We investigated the anticancer activity of AM630 (iodopravadoline), a highly potent competitive CB2 cannabinoid receptor antagonist in human U87 glioblastoma cells. The anti-cancer effects of AM630 were measured using an MTT cell viability assay (2). Cytotoxicity was evaluated by release of the cytosolic enzyme LDH by dead and dying cells (CytoTox-96 LDH assay; Promega) *in vitro*. The potential underlying molecular mechanisms were investigated by measuring the mitochondrial Unfolded Protein Response (UPRmt) transcription factor (TF) regulation (Mitochondrial UPR TF Activation Profiling plate array, Signosis Inc). All values obtained were transformed in percentages and compared to the control group represented by100%. The statistical differences were measured with the One-way ANOVA and the Independent Samples T-Test with a level of significance designed to include any value bellow P=0.05.

RESULTS AM630 (2-10μM, t=24-48h, n=6-8) inhibits U87 cells proliferation in a dose dependent manner. Maximum cell loss was observed at concentrations >6μM AM630 and markers of cell damage began to appear after 24h exposure. MTT production was inhibited in the presence of AM630 ($P<0.05$). Similarly, AM630 (10μM, t=24-48h, n=4-6) caused a substantial increase in LDH release above control levels ($P<0.05$). Other cannabinoid inhibitors (RHC80267, JZL184 or URB597) were unable to modify U87 cells proliferation rate. We found that AM630 (10μM, t=24h, n=4) inhibits growth and survival of U87 cells through inducing mitochondrial dysfunction in the UPRmt. In response to AM630 treatment, we observed very significant decreases in the activation of the transcription factors XBP (approx. 69%, $P<0.0003$ vs. control ($P>0.05$)), HSF (approx. 64%, $P<0.0019$ vs. control), E2F1 (approx. 47%, $P< 0.0078$ vs. control) and to a lesser extent in AP-1 ($P<0.0236$ vs. control), ATF4 ($P<0.0292$ vs. control), C/EBP ($P<0.0139$ vs. control), NRF1 ($P<0.0365$ vs. control) and NRF2/ARE ($P<0.0171$ vs. control).

CONCLUSION These data indicate that modulation of the signalling pathways associated to the CB2 cannabinoid receptor directly target the activation of the UPRmt and represent a potential novel approach for glioma therapy.

REFERENCES

(1) Alberich-Jorda M et al. (2003) Annals of New York Acad Sci. 996-1.

(2) Carmichael J et al. (1987) CancerRes. 47, 936-942.