An Assessment of the Efficacious Role of Cannabinoids in an In-vitro Cell-Based Model of Cachexia

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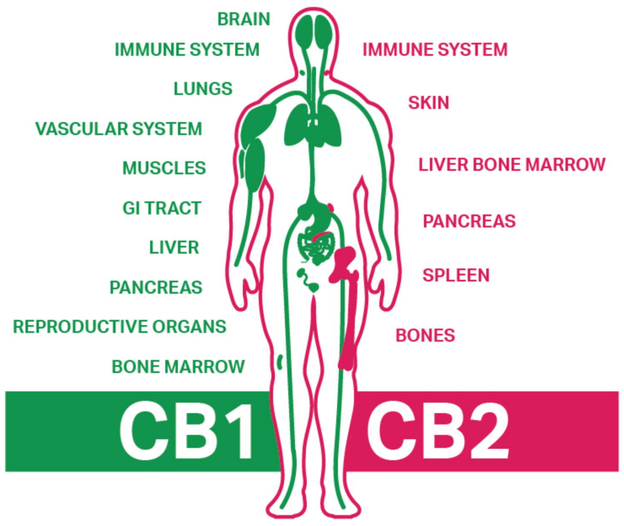
Introduction

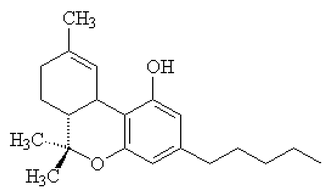
Cannabinoids represent a group of pharmacologically active compounds obtained from hemp plant, *Cannabis sativa*, which have the potential to treat cancer and cachexia associated with various chronic diseases like cancer and AIDS. Cancer and other life-threatening diseases exhibit symptoms which completely compromise both the longevity of life, as well as the quality of life of the patients. For instance, cancer patients are usually predisposed to cachexia associated with substantial muscular pains.



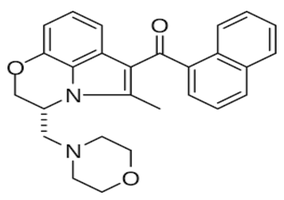
The discovery of the endogenous endocannabinoid system has enabled researchers to utilize cannabinoids for the management of cancer-associated cachexia and other related symptoms. Previous studies have shown that cannabinoids have the potential to improve body weight, appetite, mood, and quality of life in various diseases. The agonists of cannabinoid receptors (CB1 and CB2) play a major role in the treatment of cancer and modulation of associated unwanted symptoms including emesis, anorexia, and cachexia.

Aim

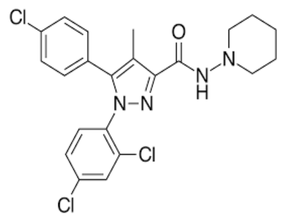
To determine whether cannabinoids such as tetrahydrocannabinoid (THC, CB1/CB2 agonist), Rimonabant SR141716A (RIM) (CB1 antagonist), and WIN55212 (synthetic CB1 agonist) affect metabolism, cell size, mitochondrial abundance and activity, the in-vitro cachexia system.



THC



WIN 55,212-2



Rimonabant

Methods

Cell Culture

C2C12 mouse myoblast cell were cultured at 5-7 x 10^4 cells/cm 2 T-25 flasks (Thermo Fisher Scientific, Dublin, Ireland) in a humidified atmosphere at 37°C, 5% CO 2 using Gibco RPMI 1640 medium (Biosciences, Dun Laoghaire, Ireland) supplemented with 5% foetal bovine serum (FBS) and 1 mM sodium pyruvate (both from Sigma-Aldrich, Dublin, Ireland).

Citrate Synthase Assay

The cells were seeded and allowed to grow until 80-90% confluent. Following this the media was removed and cells were washed with PBS. A solution of Tris (200 mM, pH 8.0) with Triton X-100 (0.2% (v/v)) to the cells (200 µl was prepared and added. The flasks were placed on a rocker for 15 minutes at room temperature. Using a scraper the cells were scraped from flask to obtain lysate and then transfer to a minifuge tube. The cells were centrifuged at 14,000 rpm for 10 minutes at 4°C. The supernatant was kept and used for a bicinchoninic acid assay).
The mitochondrial samples diluted 1:100 with STE so as to have between 0.2 and 1.5 µg per assay. The cells were then homogenates diluted 1:10 in homogenization buffer so as to have ~ 1-6 µg of protein per assay. The whole cell lysates were also kept concentrated. This colorimetric assay works due to a reaction between 5', 5'-Dithiobis 2-nitrobenzoic acid (DTNB) and CoA-SH which then forms TNB, which exhibits its maximum absorbance at 412 nm. The intensity of the absorbance is proportional to the citrate synthase activity. This enzyme is an exclusive marker of the mitochondrial matrix1. Specific activity was calculated by (Rate (abs/min) x 1000 x Total Volume in cuvette (ml))/ Protein Concentration (mg/ml) x ε x Volume of sample (ml) with an extinction coefficient of 13.6.

Statistical analysis

All experiments were carried out in triplicate. Data represent mean ± SEM. The significance of differences between mean values was determined by one-way ANOVA followed by Dunnett's multiple comparisons test. *p ≤ 0.05 was considered significant.

Results and Discussion

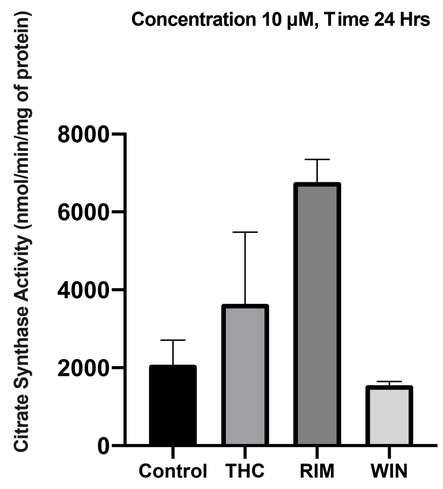


Figure 1a

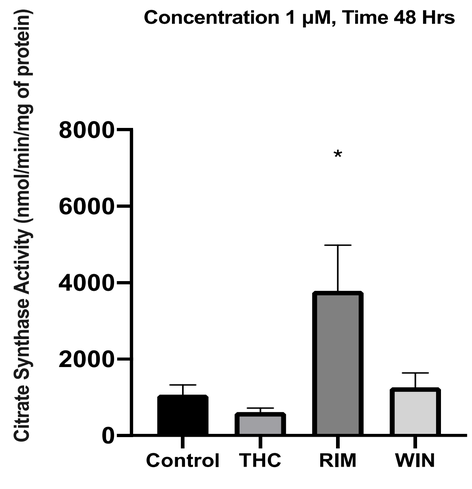


Figure 1b

Figure 1a/b. The effect of THC (tetrahydrocannabinol), RIM (Rimonabant) and WIN (WIN 55,212-2) on citrate synthase activity in C2C12 myotubules at 10 µM following a 24hr incubation period 1 µM at a 48 hour incubation period. N=3 technical replicates . Values of Rimonabant were significantly different (*p ≤ 0.05) in comparison to the control group in Figure 1b. Data represent means ± SEM, n = 3. *p ≤ 0.05.

C2C12 cells incubated with 10 µM of THC (tetrahydrocannabinol), RIM (Rimonabant) showed a non statistically significant increase in citrate synthase activity when compared to the control group. Cells treated with 1 µM concentration of THC and WIN showed similar citrate synthase assay activity when compared to the control group. However RIM showed a significant increase in activity when compared to the control group. In both Figures 1a and 1b RIM shows the highest change in citrate synthase activity when compared to the control group how this change in not statically significant.

Future Projects

- To measure of cannabinoid agonists and antagonists on the bioenergetics of C_2C_{12} myotubes as a model to determine a role for (endo)cannabinoids in modulating skeletal muscle bioenergetics.
- Toxicity assay of (endo)cannabinoids on skeletal muscles

Acknowledgements

Dr Richard Porter Associate Professor (Trinity College Dublin, Biochemistry)
Dr Mary Rooney Research Fellow (Trinity College Dublin, Biochemistry)
Funded by the Laidlaw Programme

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