Cannabidiol exerts species selective cardioprotective effects in anthracycline-induced cardiotoxicity.

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2019

Cannabidiol exerts species selective cardioprotective effects in anthracycline induced cardiotoxicity

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Background: Anthracyclines such as doxorubicin are effective chemotherapy agents, however a severe and common side effect of this treatment is anthracycline induced cardiotoxicity (AIC), which damages the heart muscle severely impacting its ability to pump and can lead to heart failure [1-3]. Although the exact pathophysiological mechanisms of AIC have yet to be fully elucidated experimental studies have suggested that they involve increased oxidative stress, intracellular calcium ($[Ca^{2+}]_i$) dysregulation, and cardiomyocyte apoptosis [4-7]. At present limiting anthracycline exposure is the only proposed strategy for reducing the risk of developing AIC, however it is not entirely effective and other means of prevention require investigation. Cannabidiol (CBD), a constituent of the *Cannabis sativa* plant, has recently been licensed for the treatment of spasticity in patients with Multiple Sclerosis and is currently being trialled as an anti-epileptic agent. Previous work has demonstrated that CBD is cardioprotective in rodent models of AIC by virtue of its antioxidant and anti-inflammatory effects and its ability to restore intracellular Ca^{2+} homeostasis [8-10], however, it is not known whether these findings extrapolate to humans. The aim of the present study was to investigate the effects of CBD in both rat and human *in vitro* models of AIC.

Methods:

Cell viability experiments: Rat H9c2 myoblast cells, human induced pluripotent stem cell cardiomyocytes (HiPSC-CM), and MDA-MB-231 cells (triple negative breast cancer cell line) were plated for the assessment of cell viability using the MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) assay. Concentration response curves were carried out to calculate the IC_{50} for doxorubicin (10^{-9} – 10^{-4}M) for each cell type and this concentration was subsequently used to investigate the effects of CBD (0.1-10μM) on AIC. Following treatment with pharmacological agents, media from HiPSC-CMs was stored for the measurement of cardiac troponin I (cTnI) release via ELISA.

Superoxide production: All cell types were plated and treated with doxorubicin +/- CBD as per the cell viability experiments and superoxide production detected using the ROS-ID® Total ROS/Superoxide Detection Kit (Enzo Life Sciences). In brief, the superoxide detection solution (1:2,500) was added to all wells (for 1hr at 37°C) following 48hr treatment with pharmacological agents. Plates were then read using a fluorescence microplate reader with a standard rhodamine (Ex=550nm/Em=610nm) filter set.

Detection of intracellular calcium: All cell types were plated and treated with doxorubicin +/- CBD as per the cell viability experiments and intracellular calcium detected using the calcium-sensitive fluorogenic dye, Cal-520 AM. Following treatment with pharmacological agents, media was removed from all wells and 10μM Cal-520 AM added for 2hr at 37°C. Cells were then washed with DPBS and fluorescence measured at Ex=492nm/Em=514nm.

Statistical analysis: Concentration response curves were fitted using a three-parameter Hill equation and log IC_{50} values compared using global comparison of fits within GraphPad Prism. The effects of pharmacological agents on cell viability were compared using a repeated measures ANOVA with Dunnett's post hoc test and cTnI levels using a One-way ANOVA and Dunnett's post hoc test. The effects of pharmacological agents on both superoxide production and intracellular calcium were compared using a Two-way ANOVA and Bonferroni post hoc test.

Results: Cell viability

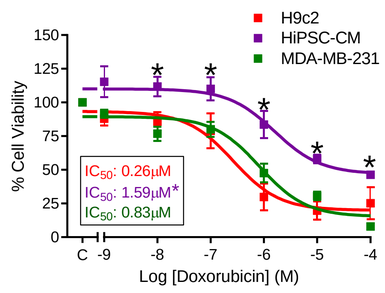

Figure 1. Concentration response curves to doxorubicin. Cell viability was assessed via MTT assay and calculated as a percentage of control cells (100%). Data is expressed as mean±SEM from 3-4 independent experiments. *P<0.01 vs. H9c2 cells.

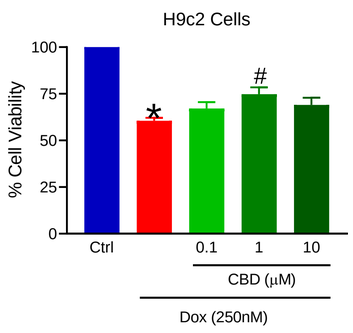

Figure 2. Effect of CBD on doxorubicin induced toxicity in H9c2 cells (rat cardiomyocytes). Cell viability was assessed via MTT assay and calculated as a percentage of control cells (100%). Data is expressed as mean±SEM from 4 independent experiments. *P<0.001 vs. Ctrl; #P<0.05 vs. Dox alone.

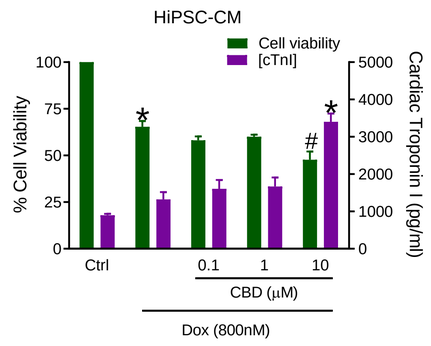

Figure 3. Effect of CBD on doxorubicin induced toxicity in HiPSC-CM (human cardiomyocytes). Cell viability was assessed via MTT assay and calculated as a percentage of control cells (100%). Cardiac troponin I (cTnI) release from cells was measured via ELISA. Data is expressed as mean±SEM from 3 independent experiments. *P<0.001 vs. Ctrl; #P<0.01 vs. Dox alone.

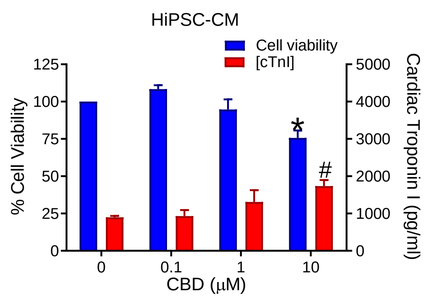

Figure 4. Effect of CBD on HiPSC-CM viability and cardiac troponin I release. Cell viability was assessed via MTT assay and calculated as a percentage of control cells (100%). Cardiac troponin I (cTnI) release from cells was measured via ELISA. Data is expressed as mean±SEM from 3 independent experiments. *P<0.001 & #P<0.05 vs. 0μM CBD.

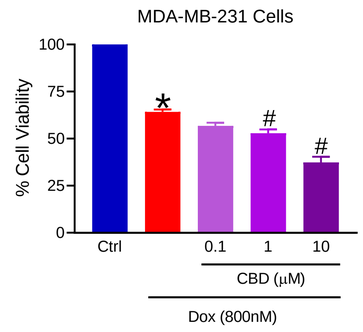

Figure 5. Effect of CBD on doxorubicin induced toxicity in MDA-MB-231 cells (human breast cancer cell line). Cell viability was assessed via MTT assay and calculated as a percentage of control cells (100%). Data is expressed as mean±SEM from 3 independent experiments. *P<0.001 vs. Ctrl; #P<0.01 vs. Dox alone.

Results: Superoxide production

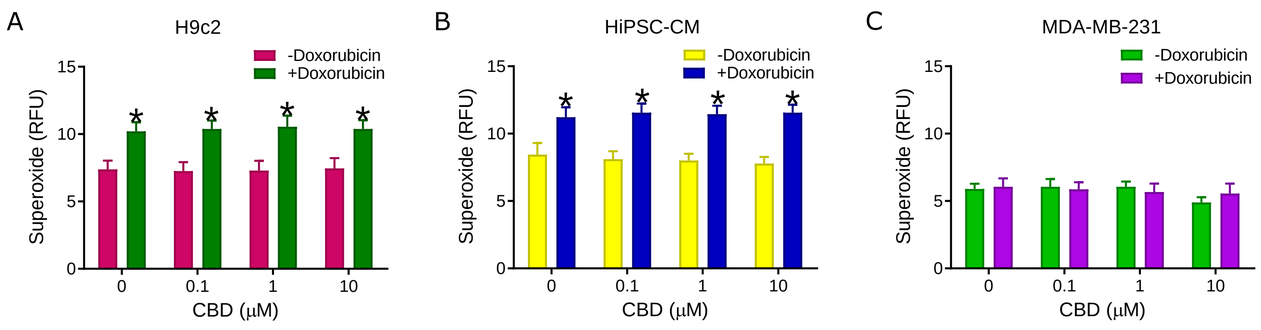

Figure 6. Effect of CBD on doxorubicin induced superoxide production in both cardiomyocytes and breast cancer cells. Following treatment with doxorubicin (250nM for H9c2 cells and 800nM for both HiPSC-CM and MDA-MB-231 cells) in the absence and presence of increasing concentrations of CBD, superoxide production was detected in H9c2 (A), HiPSC-CM (B), and MDA-MB-231 (C) cells using the ROS-ID® Total ROS/Superoxide Detection Kit. Superoxide production was quantified in terms of fluorescence signal above background (cell free wells with superoxide detection solution). Data is expressed as mean±SEM from 3-4 independent experiments. *P<0.05 vs. –Doxorubicin (equivalent CBD concentration).

Results: Intracellular calcium

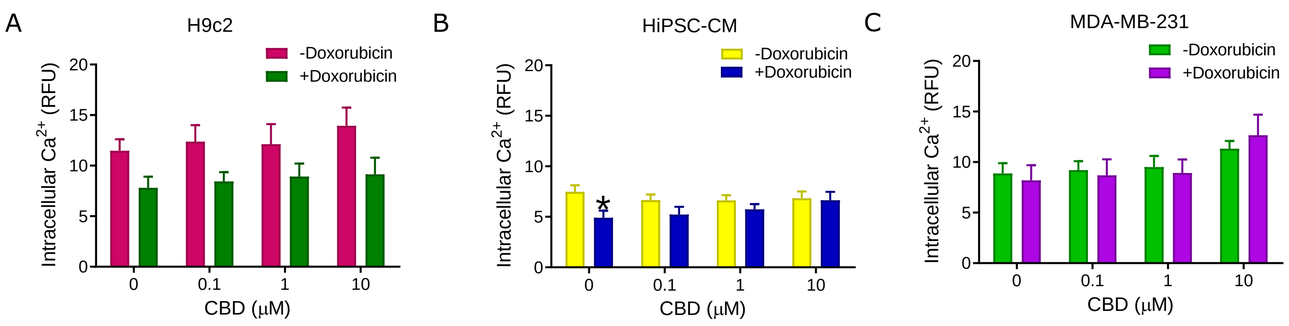

Figure 7. Effect of CBD on doxorubicin induced changes in intracellular calcium in both cardiomyocytes and breast cancer cells. Following treatment with doxorubicin (250nM for H9c2 cells and 800nM for both HiPSC-CM and MDA-MB-231 cells) in the absence and presence of increasing concentrations of CBD, H9c2 (A), HiPSC-CM (B), and MDA-MB-231 (C) cells were stained with the fluorogenic dye Cal-520 AM to detect calcium. Intracellular calcium was quantified in terms of fluorescence signal above background (cell free wells with Cal-520 AM dye). Data is expressed as mean±SEM from 3-4 independent experiments. *P<0.05 vs. –Doxorubicin (equivalent CBD concentration).

Summary of results:

- CBD protects rat cardiomyocytes from the toxic effects of doxorubicin
- In contrast, CBD failed to protect human cardiomyocytes and was toxic at high concentrations i.e. 10μM
- CBD enhanced the cytotoxic effect of doxorubicin in breast cancer cells at a concentration that was not toxic to human cardiomyocytes i.e. 1μM
- CBD does not appear to protect rat cardiomyocytes via an antioxidant effect
- Finally, CBD does not appear to protect rat cardiomyocytes via modulation of intracellular calcium concentration

Conclusion:

Cardioprotective effects of CBD previously observed in rodent models of AIC do not appear to extrapolate to human tissues

This work was supported by Tenovus Scotland.

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