ENDOCANNABINOID SYSTEM MODULATION: SCREENING OF THE EFFECT OF PHYTOCANNABINOIDS IN NEURONAL MODELS

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INTRODUCTION

Neurodegenerative disorders (ndds) are characterised by progressive atrophy of nerve cells in both central nervous system as well as peripheral nervous system. Few among the many causative factors of neuronal degeneration include neuroinflammatory response due to microglial imbalance (salter & stevens, 2017), mitochondrial dysfunction and related disruption to molecular mechanisms (kay et al., 2018), neurotoxic metals or ionic imbalance, and genetic mutations. As per the statistics of the world health organisation (who, 2018) of all reported ndd incidences, approximately 1.8% population accounts for parkinson's disease (pd) and 12% accounts for alzheimer's disease (ad), making the two conditions major socioeconomic burdens in both the developing as well as in developed world. Parkinson's disease (pd) is biochemically characterised by the progressive degeneration of dopaminergic neurons in substantia nigra pars compacta, and intracellular accumulation of lewy bodies consisting mutated alpha-synuclein proteins (jankovic, 2008). Alzheimer's disease (ad) is characterised by severe degeneration of cortical neurons anatomically, and amyloid-beta plaques and intracellular accumulation of tau neurofibrillary tangles (nussbaum & ellis, 2003).

For the development of therapeutic strategies for neurodegenerative disorders, the endocannabinoid system (ecs) has played a vital role in the past several decades. The brain's ecs consists of two primary endocannabinoid neurotransmitters, anandamide and 2-arachidonylglycerol, acting as ligands to the two primary endocannabinoid receptors type-1 and -2 (cb1r, cb2r) (di marzo et al., 2004). The cb1r and cb2r also act as receptors to the external cannabinoids (phytocannabinoids), delta-9-tetrahydrocannabinol (thc) and cannabidiol (cbd), the two primary phytocannabinoids of the plant *cannabis sativa*.

Research problem and objectives

The effects of the phytocannabinoids thc and cbd are currently being studied at local and international levels, however, the research are primarily focused at clinical setting thus creating a significant research gap in the molecular mechanism of thc and cbd at cellular level (cooray et al., 2020). Therefore, our current study aims to determine the phytocannabinoid effect in two primary disease models: ad and pd, in comparison to healthy models, over 4 weeks period, providing a fundamental initial step in understanding the long-term cannabinoid response thus the genetic and molecular mechanism to phytocannabinoid dynamics, in neurons.

METHODOLOGY

Ethics approval

The ethical approval for the use of human neuronal cell lines was obtained from Deakin University Human Research Ethics Committee, Australia.

Neuronal cell culture

Two neuroblastoma cell lines: SH-SY5Y (ATCC CRL2266) and BE-(2)-M17 (ATCC CRL267), were cultured in Dulbecco's Modified Eagle Medium (DMEM) + Ham's F12 (Sigma) at 1:1 ratio with 100U/mL Pen-Strep, 10% foetal bovine serum (FBS), and were incubated at 37 ^{0}C with 5% CO_2. Culture media were changed in every 48 hours or as necessary.

Neuronal differentiation to AD, PD and H models

At 60% confluency, the cells were differentiated to AD model with retinoic acid (RA) according to the adapted method by (König et al., 1990). In brief, fresh complete media with 10 mM RA (Sigma) were added to the flasks before placing in the incubator. Five total RA treatments were followed under careful microscopic observation for neuronal morphology changes. Following differentiation of AD model, the cells were treated with 10 uM MPP+ (1-methyl-4-phenylpyridinium) for PD model (Carroll et al., 2012).

Cannabinoids treatment

At 60% following differentiation, the cells were plated into 96-well plate for cannabinoid treatment for each SH-SY5Y and M17 cell line. Four different treatment groups (T1-T4) and one control group (C) were set up with different THC: CBD ratios as: T1 - 1:0 (10 uM : 0), T2 - 0:1 (0 : 5 uM), T3 - 1:1 (10 uM : 5 uM), T4 - 1:2 (10 uM : 10 uM) and C - 0:0 (0: 0), (equal volumes of DMSO was added to each well accordingly).

MTT cell viability assay

Cell viability was measured with the MTT assay (ThermoFisher) according to the manufacturer's protocol. In brief, cells were cultured and differentiated into each model in 96 well-plates and treated with cannabinoids as mentioned previously. At the end of each week of observation cells were subjected to MTT assay. The used media was replaced with fresh 100 uL serum-free media with 10 uL 12 mM MTT solution (1 mL of 1X sterile PBS + 5 mg MTT). The plates were incubated at 37 ^{0}C on 60 rpm shaking incubator for 4 hours. Following incubation 85 uL media was removed and 50 uL for DMSO was added to each well and plates were incubated at 37 ^{0}C on 60 rpm for 10 min before proceeding to colourimetry. The absorbance at 540 nm was measured with microplate reader (Verioskan LUX microplate reader, ThermoFisher Scientific).

Data analysis

The viability percentage was calculated as below.
Viability % = $(Ab_{test} - Ab_{blank}) / (Ab_{control} - Ab_{blank}) \times 100$ (Ab: Absorbance)

The statistical significance of data was analysed with one-way ANOVA (Version 27) and post-hoc Tukey's test.

RESULTS AND DISCUSSION

Cellular differentiation

Following RA differentiation, both M17 and SH-SY5Y cells showed expected morphological characteristics of neurite outgrowth and neuronal networking. The cellular proliferation was minimal.

Model AD and Model PD showed neuroprotection in response to THC and CBD

Compared to the untreated (C) groups, treated groups (T1-T4) showed increasing neuroprotection until 3 weeks (3W) with viability diminishing towards week 4 (4W). This pattern was consistent in model AD (Figure 1) and Model PD in both SH-SY5Y and M17 cells (data not shown). These results align with the previous findings of cannabinoid mediated neuroprotection (Carroll et al., 2012).

Interestingly, the T1 and T2 groups, which were the THC or CBD independently, showed overall better significant increase in viability, *i.e.*, the neuroprotective effect than either of the cannabinoid combinations of T3 and T4.

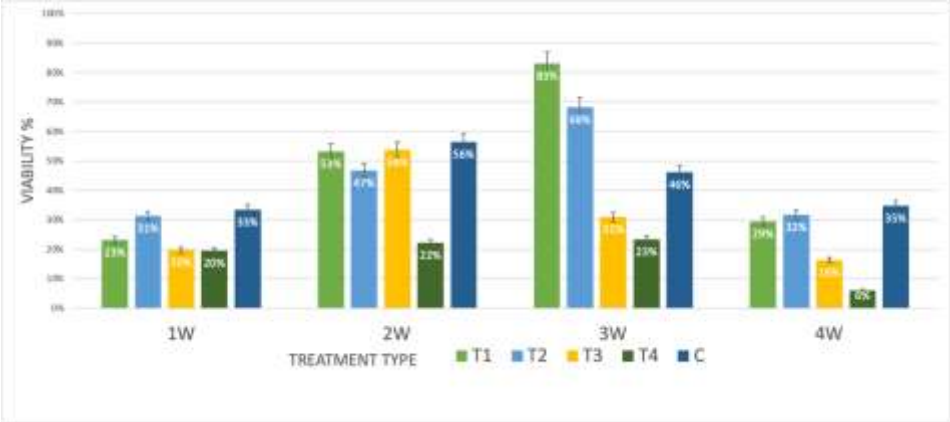

Figure 1: Viability % of cannabinoids treated Model AD (SH-SY5Y), over 4 weeks.

Model H (M17) caused severe neuronal death in response to THC and CBD combinations.

In the Model H of SH-SY5Y overall good neuroprotection was observed with THC and CBD groups (Figure 2). For combined treatments, 1:1 combination showed good neuroprotection.

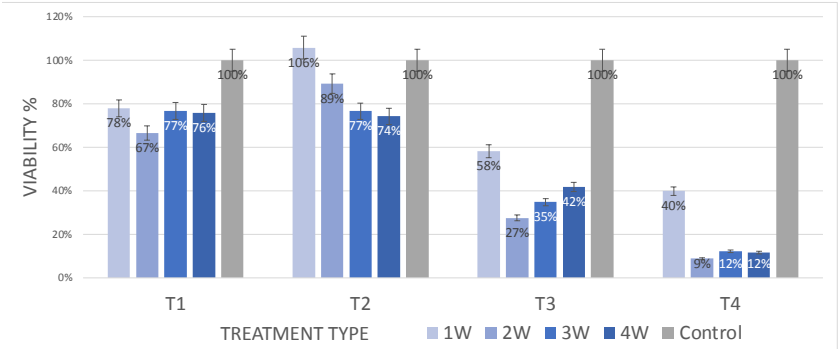

Figure 2: Viability % of cannabinoids treated Model H (SH-SY5Y), over 4 weeks. Blue bars: Model H, Gray bars: Healthy control.

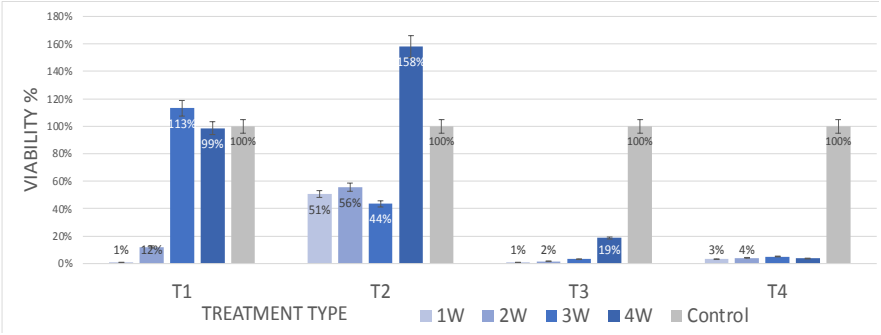

Figure 3: Viability % of cannabinoids treated Model H (M17), over 4 weeks. Blue bars: Model H, Gray bars: Healthy control.

Interestingly, in Model H of M17, with only THC and CBD, some level of significant neuroprotection was observed (Figure 3). However, both the combinatory treatments, caused severe neuronal death.

Even though the precise mechanism is not known, previous studies evidence the altered behaviour of the ECS in old vs young rat models (Marchalant et al., 2008), indicating the age-dependency of the cannabinoid treatment.

CONCLUSIONS/ RECOMMENDATIONS

The cannabinoids THC and CBD may provide significant neuroprotection in their purified form in AD and PD scenarios, however, the effectiveness of combinatory treatments, as well as their effect on healthy brain cells need further investigation prior to prescribing for medicinal purpose. Furthermore, molecular and genetics studies are suggested to demystify the alternative behaviour of the phytocannabinoids in healthy vs disease cell models.

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