The effect of synthetic cannabinoids on human neural stem cell survival

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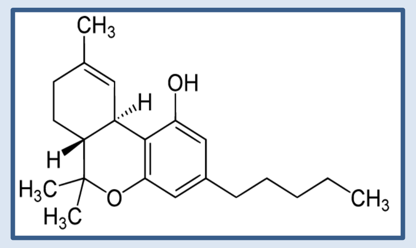
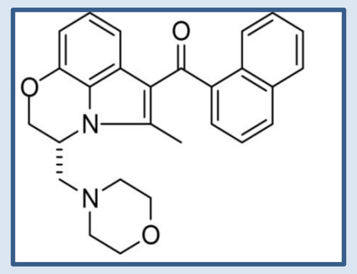
Introduction

Cannabis is the most widely used recreational drug in the world. More recently, synthetic cannabinoids are emerging as a popular alternative. Due to similarity of Δ^9-tetrahydrocannabinol (THC); the main psychoactive component, it is thought that synthetic cannabinoids have a similar psychotropic effect as plant-derived cannabis. Marketed as “spice”, little is known about the toxicity and the harmful effect these compounds have on cognitive function. Relative to THC, synthetic cannabinoids are more potent which can lead to more severe adverse effects. [1]

Pre-natal and adolescent onset of cannabinoids has been linked to significant cognitive decline throughout life, which may be due to cannabinoid exposure having a toxic effect on stem cell reserves located in specific regions of the developing brain. [2,3] WIN-55; a potent agonist of THC will be added to human-neural progenitor cells (hNPCs) in culture, to deduce the potential toxicological effect of synthetic cannabinoids on stem cell viability and differentiation. [4]



THC



WIN-55

Aims and objectives

- To determine the toxicity of WIN-55 on hNPCs and evaluate the mechanism behind WIN-55 mediated cell death
- To see if sub-lethal doses of WIN-55 can affect neural stem cell differentiation by altering gene/protein expression

Methods

- Human-neural progenitor cells were cultured and maintained in cell culture medium
- Cells were exposed to varied concentrations of WIN-55 for up to 14 days and cell viability was measured at day 1, 7 and 14 after exposure
- Primary antibodies of cell death markers were used in Western Blotting to assess the mechanism of possible cell death
- Used florescent microscopy with markers of gene regulation (SATB2) and cell differentiation (Ctip2) to evaluate potential involvement of WIN55 in these processes

References

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2. Suárez I, Bodega G, Fernández-Ruiz J, Ramos J.A, Rubio M, Fernández B.. (2004). Down-regulation of the AMPA glutamate receptor subunits GluR1 and GluR2/3 in the rat cerebellum following pre- and perinatal delta9-tetrahydrocannabinol exposure. *Cerebellum*. 3 (2), 66-74.
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Results

Constant exposure of high concentrations of WIN-55 had a detrimental effect on hNPC survival, illustrated by the toxicity assay which shows significant reduction in cell viability in WIN-55 concentrations above 100nM (see figure 1). However, toxicity assays on matured neurons showed that WIN-55 did not have a significant effect on their survival. (see figure 2)

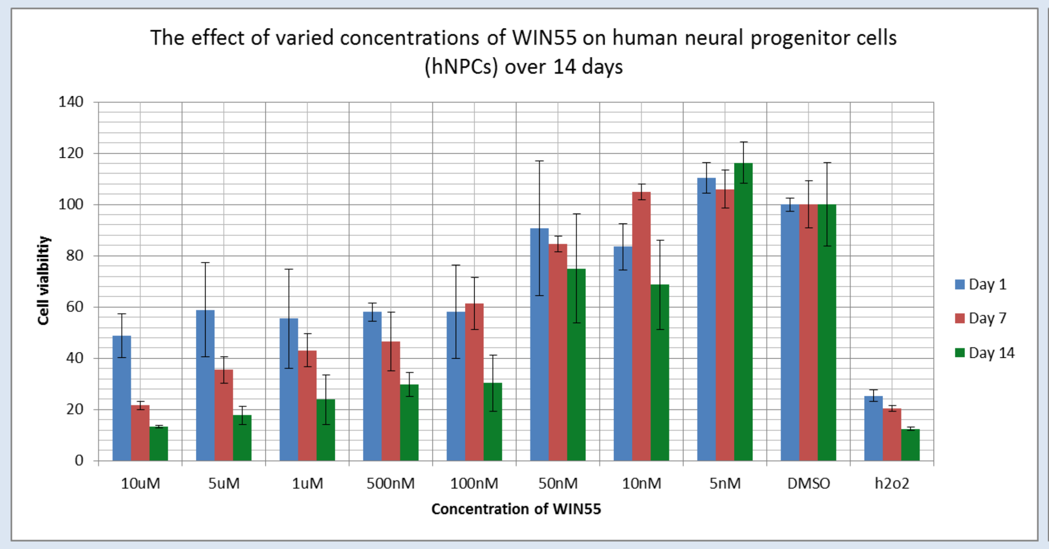

Figure 1. Cell viability of hNPCs after WIN-55 application

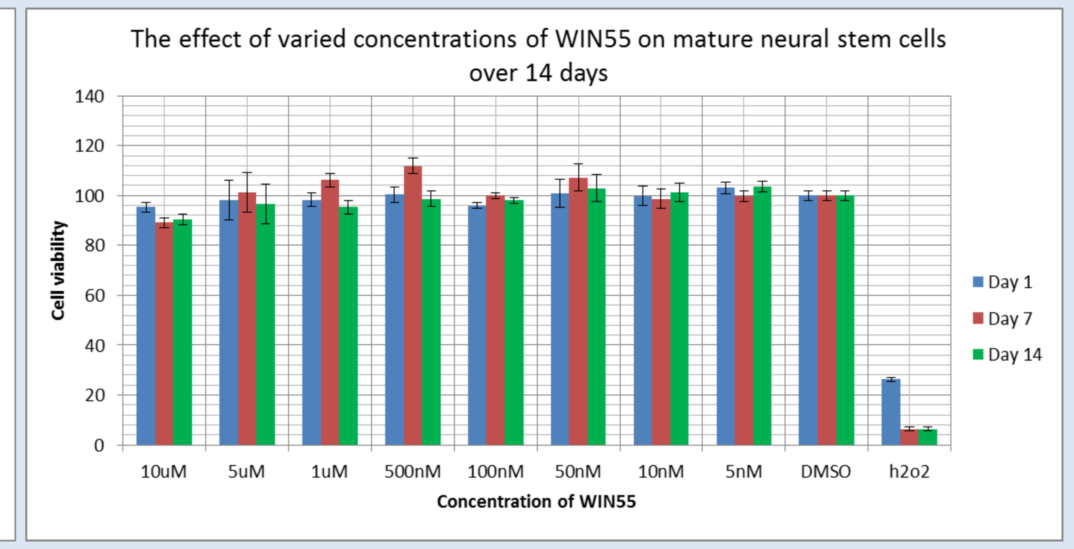

Figure 2. Cell viability of mature neurons after WIN-55 application

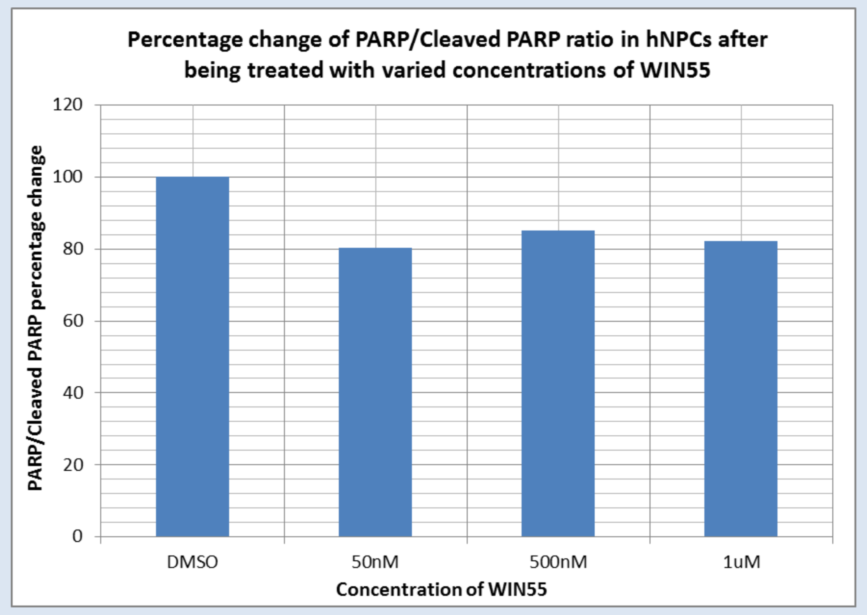

Figure 3. PARP/Cleaved PARP % ratio against WIN-55 concentration

Poly-ADP ribose polymerase (PARP) is a nuclear enzyme that is cleaved by caspase-3 as a result of apoptosis, meaning if a cell dies by apoptosis there should be more cleaved PARP than PARP. Blots of the stem cells show that there is a significant decrease in PARP/Cleaved PARP ratio as WIN-55 concentration increased. (See figure 3 & 4)

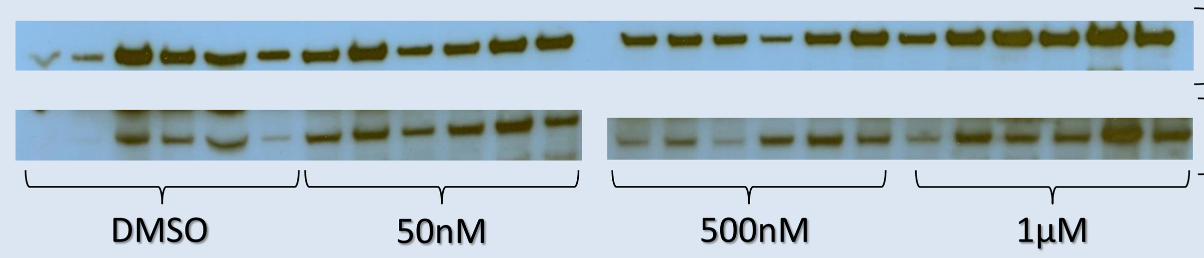

Figure 4. Western blots of PARP and cleaved PARP in hNPCs exposed to WIN-55

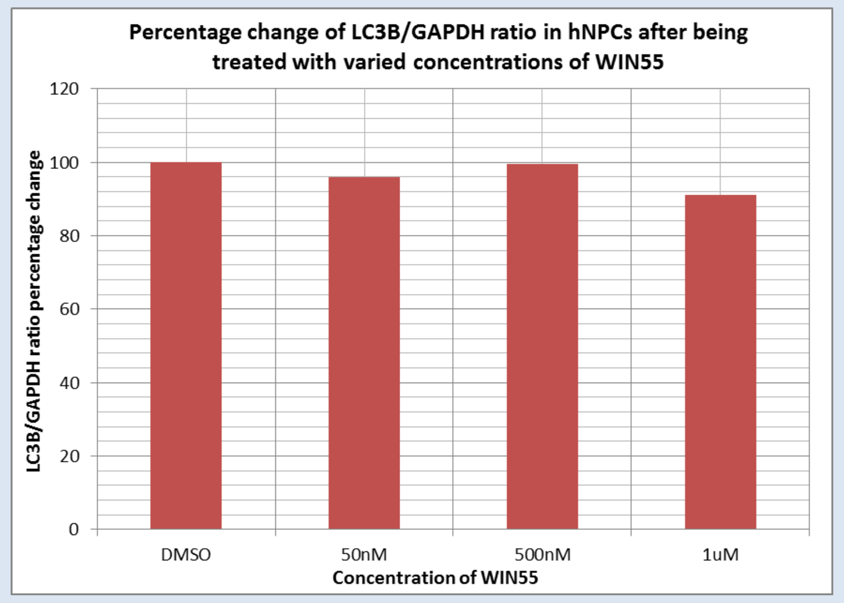

Figure 5. LC3B/GAPDH % ratio against WIN-55 concentration

Microtubule-associated protein 1A/1B-light chain 3B (LC3B) is a soluble protein that is distributed ubiquitously in mammalian cells. LC3B is degraded during cell death by autophagy, therefore it is used as a reliable marker. Stem cell blots reveal a statistically significant reduction of LC3B after WIN-55 application, however this is disputed due to the small percentage decrease (see figure 5), despite the ANOVA test showing the change as significant.

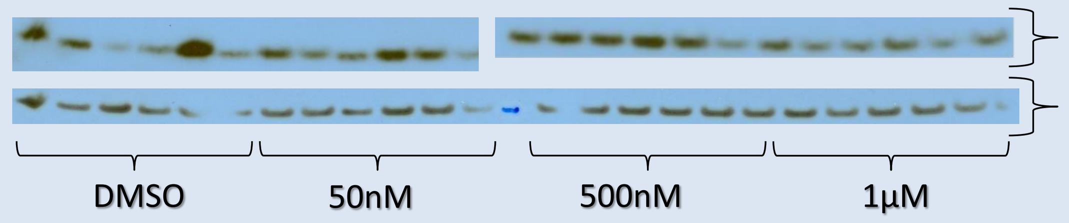

Figure 6. Western blots of LC3B and GAPDH in hNPCs exposed to WIN-55

Fluorescent microscopy shows that SATB2 (stained red/pink) is mostly present in DMSO, 50nM and 1μM of WIN-55 in nuclei stained blue by 4',6-diamidino-2-phenylindole (DAPI). 500nM showed little to no SATB2, which would indicate that WIN-55 represses SATB2 expression/synthesis if it weren't for SATB2 presence in higher concentrations (see figure 7).

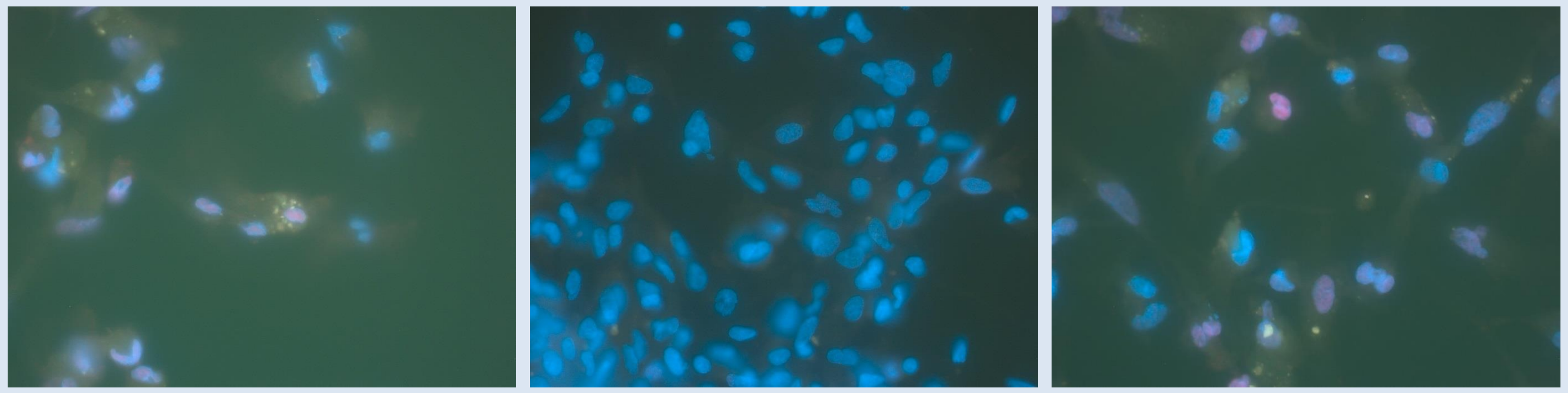

Figure 7. Fluorescent microscopy of WIN-55 treated hPNCs stained with DAPI and SATB2. DMSO (left), 500nM (center), 1μM (right).

Discussion

WIN-55 has a harmful effect on hPNCs when exposed to concentrations above 100nM continuously, causing substantial decrease in cell viability. The mechanism of cell death and how WIN-55 is implicated is unclear. However, due to PARP and LC3B levels decreasing as a result of WIN-55 application, it is suggested that cells die from apoptosis as well as autophagy and that WIN-55 plays a part in their cell death pathways. Whether or not WIN-55 influences hNPC differentiation remains inconclusive. The outcome of fluorescent microscopy is fairly promising but in order to achieve consistent results and to identify a pattern, the experiment would need to be repeated multiple times. It has been shown that WIN-55 and therefore synthetic cannabinoids have a damaging effect on hNPC viability and may alter stem cell differentiation. More research is needed to be done in order to validate the findings in this project and to further explore synthetic cannabinoids and their role in cognitive decline.

Acknowledgements

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