Cannabinoids modulate cytotoxicity and neuritogenesis in Amyloid-β treated neuronal cells.

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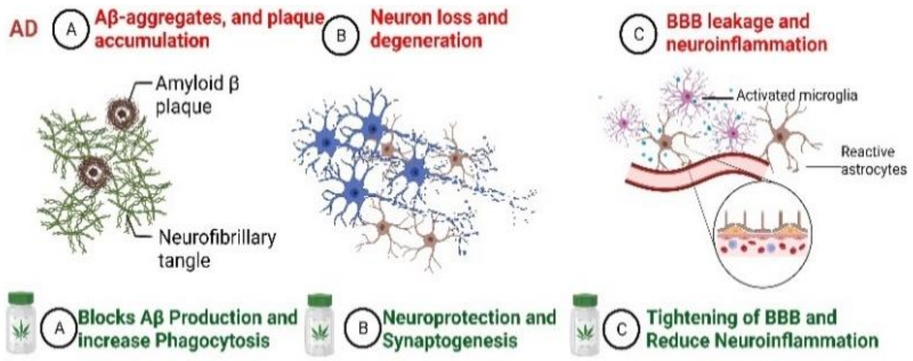
Introduction

- The impact of neurodegenerative disorders, including Alzheimer's Disease (AD), on the Canadian economy is around $28 billion and is estimated to increase by ten folds in the next few years.
- AD is caused by toxicity and proteostatic collapse due to misfolded Amyloid beta (Aβ) protein.
- Studies have shown that Cannabinoids, via their cognate receptors (CB1R and CB2R), reduce Aβ toxicity, decrease p-*tau*, and inflammatory response, thus improving neuronal viability.



- Therefore, in the present study, we examined the role of cannabinoids on Aβ-induced toxicity using *in vitro* models.

Methods

- We used human SH-SY5Y neuroblastoma cells (Sigma-Aldrich) differentiated with Retinoic acid (RA, 10μM) for 5 days. Post-differentiation, cells were treated with pCBx for 24 hrs in a dose-dependent manner alone or under Aβ -mediated cytotoxic insult and processed accordingly.
- **MTT Assay:** The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay was performed to determine cell viability.
- **Neurite Measurements:** Phase Contrast Images were analyzed for Neurite Tracing using ImageJ/Fiji software (NeuronJ)
- **Immunocytochemistry:** Cells were grown to confluence on a glass coverslip pre-coated with matrigel. Cells were treated and processed for immunostaining.
- **Western Blot Analysis:** Cells were treated, and the lysate was quantified. 15μg of total protein was fractionated and transferred onto a nitrocellulose membrane and probed with target-specific antibodies.

Results

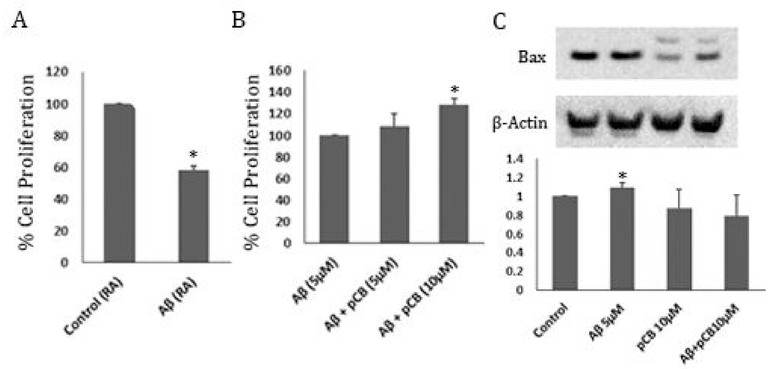


Fig 1: Phyto-cannabinoids (pCBx) promote neuroprotection. (A & B) *Aβ (5μM) induces cytotoxicity in SHSY-5Y cells, whereas pCBx attenuated Aβ mediated cell death in a dose-dependent manner. Cell viability was determined by MTT assay.* ***(C)*** *Representative immunoblot analysis displaying inhibition of Aβ induced Bax protein in pCBx (10μM) presence. Statistical analysis was performed using one-way ANOVA and a post hoc Dunnett's test. *p < 0.05 (compared with control; n=3).*

Results

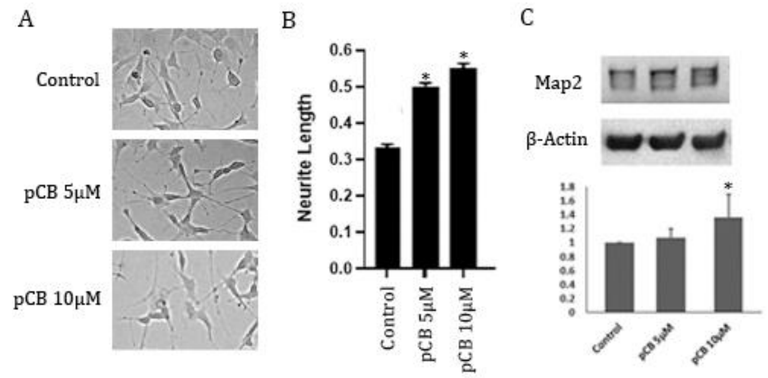


Fig 2: Phyto-cannabinoid (pCBx) promote neuritogenesis. (A & B) *Representative bright field photographs of SH-SY5Y cells post-differentiation and followed by treatment with pCBx displayed morphological changes in the shape and size of cells along with neurite out-growth. Note that pCBx, in a dose-dependent manner, significantly increased the length of the neurites in SHSY5Y cells.* ***(C)*** *Changes in the prominent intracellular marker of neurite growth – MAP2. Statistical analysis was performed using one-way ANOVA and a post hoc Dunnett test. *p < 0.05 (compared with control).*

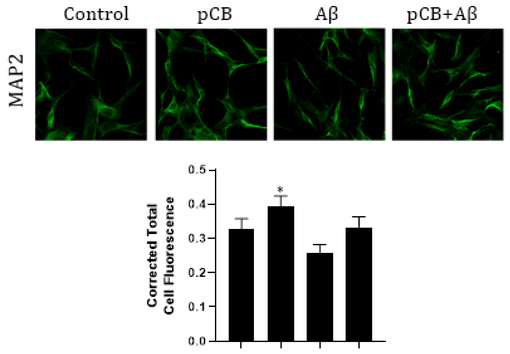


Fig 3: Morphological characterization of pCBx-mediated changes in MAP2 expression. *Representative confocal photomicrographs showing changes in MAP2 expression following treatment with pCBx in the presence or absence of Aβ (5μM). Note that Aβ (5μM) decreased and pCBx up-regulated the expression of MAP2, whereas pCBx abrogates the down-regulation MAP2 expression as induced by Aβ (5μM) treatment. Statistical analysis was performed using one-way ANOVA and a post hoc Dunnett test. *p < 0.05 (compared with control).*

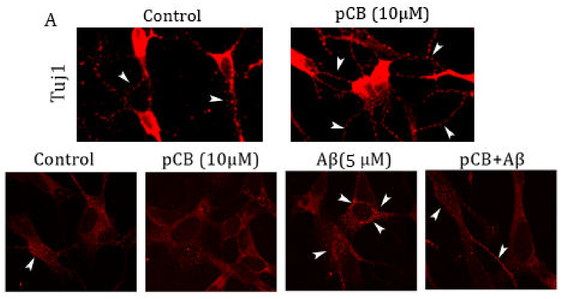


Fig 4: Phyto-cannabinoid (pCBx) promotes neuritogenesis and modulates tau expression. (A) *Photomicrographs illustrating Tuj1 immuno-reactivity in control and pCBx (10μM) treated cells. The formation of extended neurites and arborization is evident upon pCBx treatment.* ***(B)*** *Photo-micrographs illustrating tau immunoreactivity in control or cells treated with Aβ (5μM) and pCBx (10μM) alone or in combination. Note the decreased tau expression in the presence of pCBx with or without Aβ (5μM).*

Summary

- In the present study, we observed that pCBx mediated a significant increase in cell survival under Aβ induced cytotoxic insults.
- Moreover, pCBx treatment attenuates increased BAX and *tau* expression in the presence of Aβ (5 μM).
- pCBx treatment improved neuritogenesis, as evidenced by increased neurite length or enhanced expression of neurite markers, Tuj1 and MAP2 in control or Aβ treated cells.

Conclusions

The results presented here demonstrate the anti-apoptotic effects of pCBx and its role in neuritogenesis in the cells of neuronal origin and support the role of pCBx as a potential therapeutic intervention in neurodegenerative diseases.

COI: InMed Pharmaceuticals is commercializing cannabinoid-based therapies. RKS is a Research Consultant.

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