Investigating Causative Factors of Neurodegenerative Disease

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Introduction

Neurodegenerative disease affects millions worldwide and includes conditions such as Alzheimer's, Parkinson's and Huntington's which are progressive and as yet incurable. Current therapeutics treat only the symptoms of the disorder but cannot stop the progression of disease.

TRPM2 is a channel protein which is highly expressed in the membrane of microglial cells (the immune cells of the brain) and allows ions (e.g. calcium) to move into/out of the cell.

When dysregulated these ion currents can cause pathological microglial activation, as can occur when harmful signals in the fluid bathing the neurones (e.g. hydrogen peroxide) are detected. Microglia release their own signals in an inflammatory response to elicit local neuronal death and attract more microglia. With improper regulation this propagates a chronic state of neuroinflammation, and subsequent neurodegeneration.

We do not know the exact mechanisms of signalling downstream of TRPM2 so the development of treatments to utilise them has not yet been possible.

The understanding of how TRPM2 fits into this puzzle could be key to developing novel therapeutics.

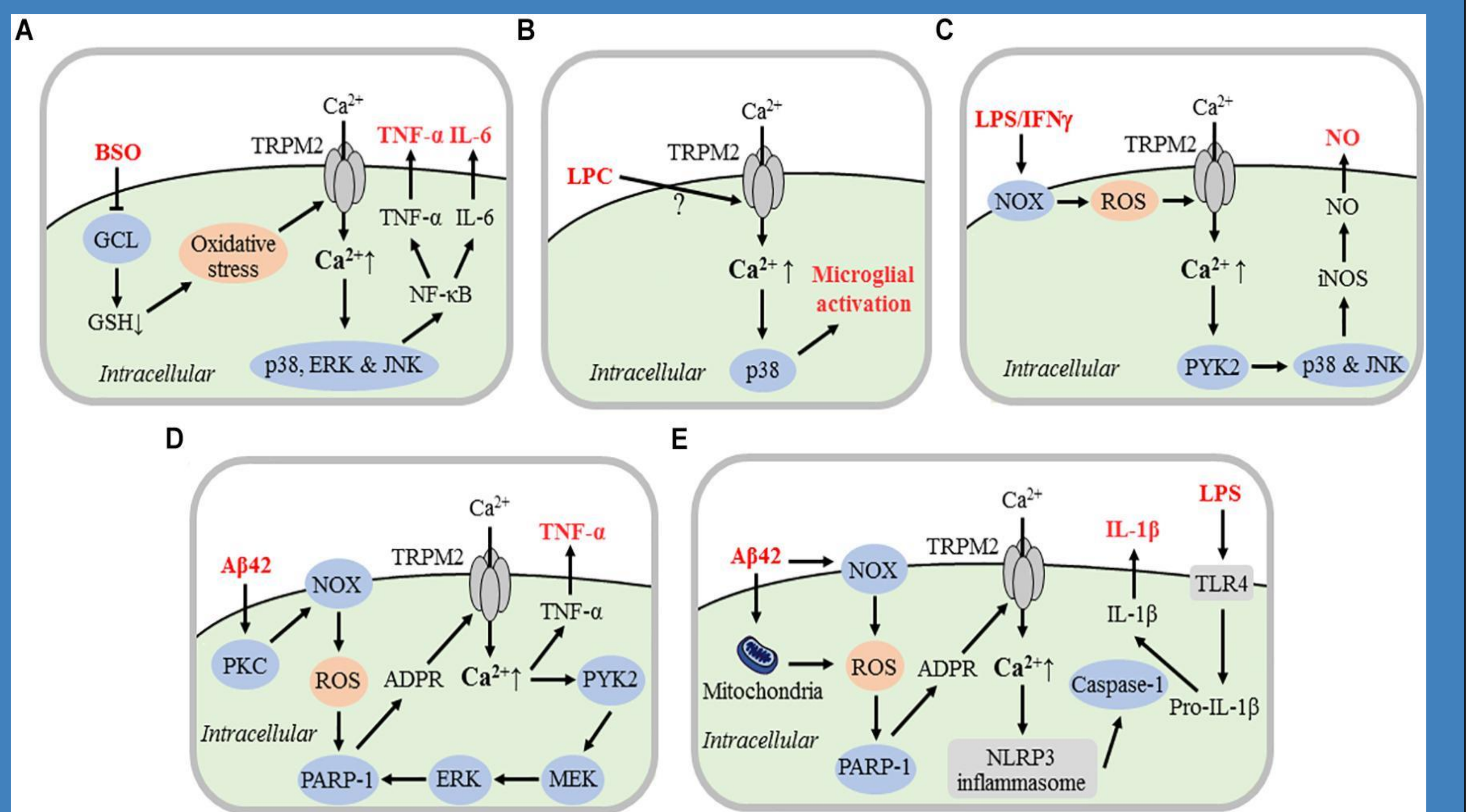


Figure 1: A diagram showing the numerous mechanisms of neuroinflammatory pathogenesis where TRPM2 has been confirmed to play a role, thus demonstrating it's integral function in these disease processes.

Aim

To evaluate the inhibitory potential of tetracyclines as proposed TRPM2 blockers and explore their utility as novel anti-neuroinflammatories.

Methods

To measure the concentration of calcium within our cells we used a Flexstation and a dye which fluoresces brighter in a calcium-rich environment.

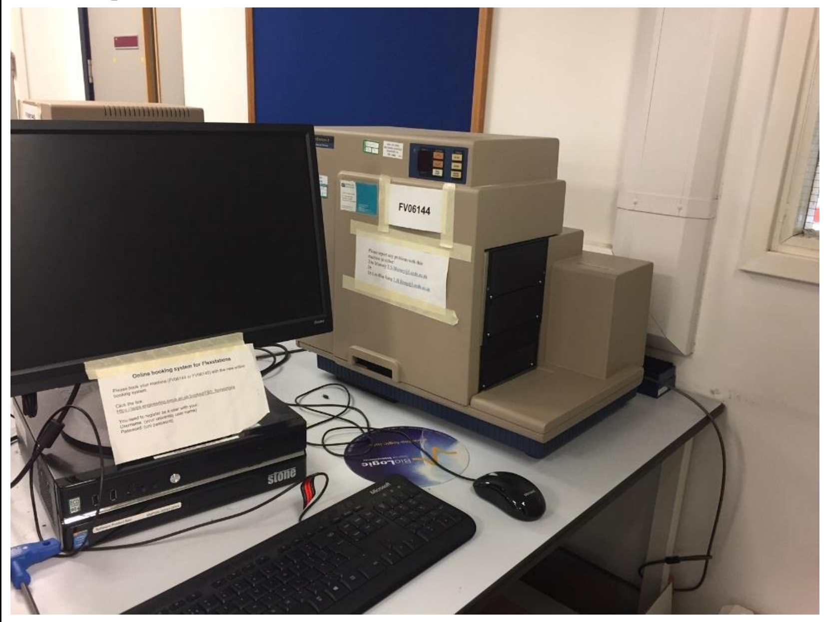


Figure 2: A Flexstation emits a laser at 340nm and 380nm to excite the Fura-2 stain, which when bound to calcium will absorb more light energy at 340nm compared to 380nm and hence fluoresce more strongly. This creates an excitation ratio (F340/F380) which can be used to quantify the intracellular calcium load.

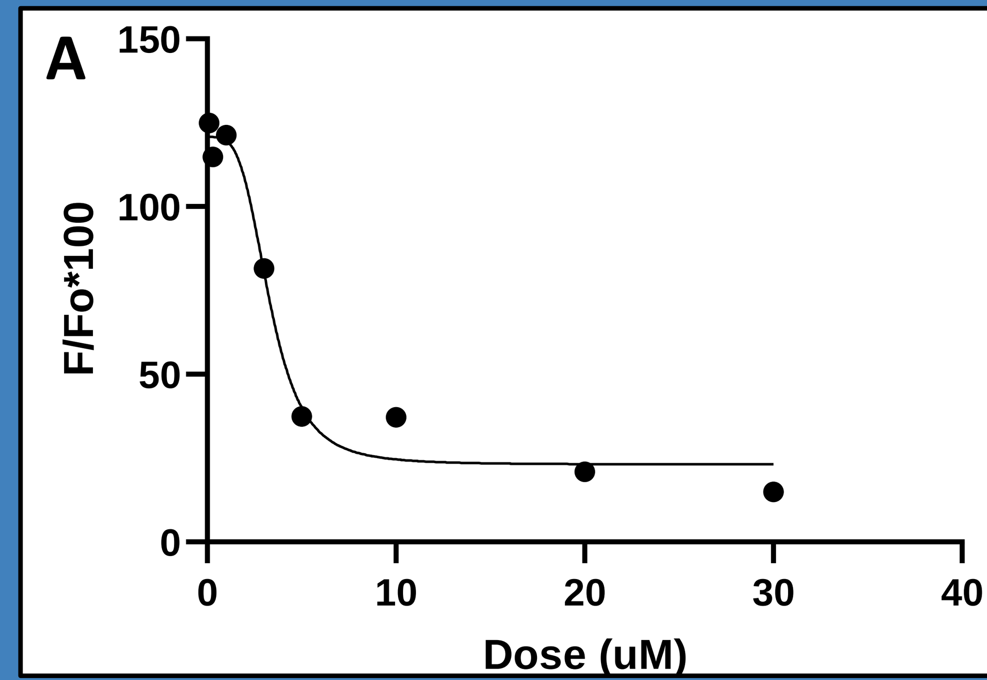
To model the microglial-injury response we stimulated the cells with hydrogen peroxide before adding the tetracyclines and observing their effects.

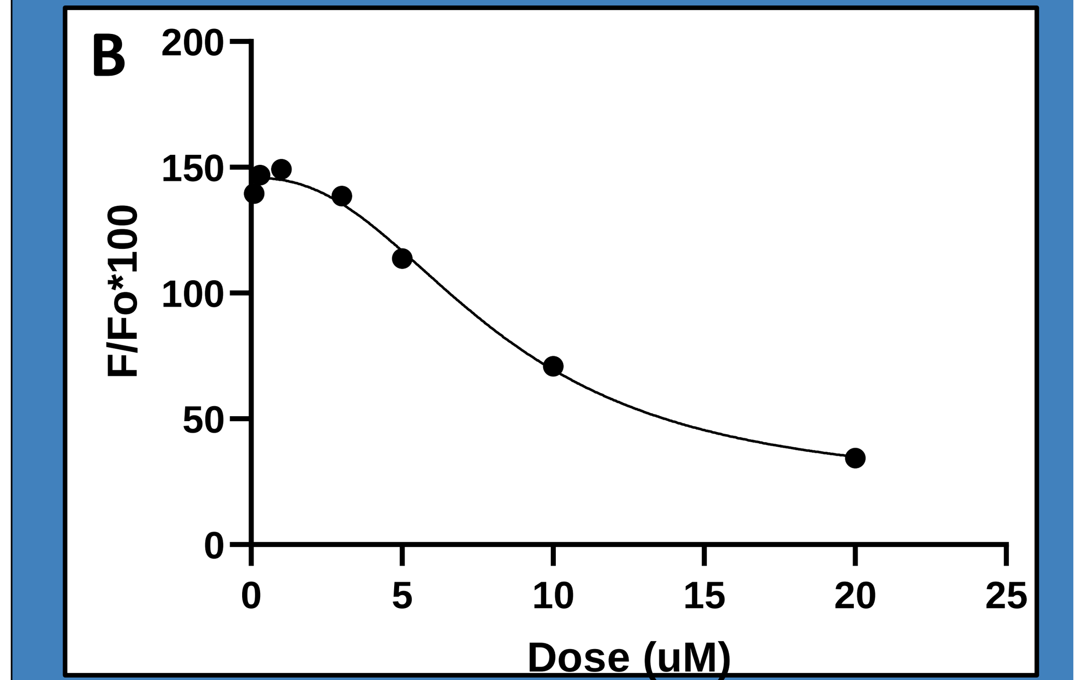
Impedance of calcium accumulation within the cell was evaluated for each tetracycline to assess their potential therapeutic efficacy.

Results

At low concentrations (0.1-3μM) both chlortetracycline and tigecycline produced a novel and as yet unexplained increase in the intracellular calcium concentration above the control levels.

At sub-micromolar concentrations (50% inhibition occurred at 0.32μM) doxycycline treatment yielded a strong inhibitory effect. Furthermore, chlortetracycline brought about a similar level of inhibition with a 10x greater dose. While inhibition was present with tigecycline it was significantly weaker, requiring 8.23μM to achieve comparable inhibition.





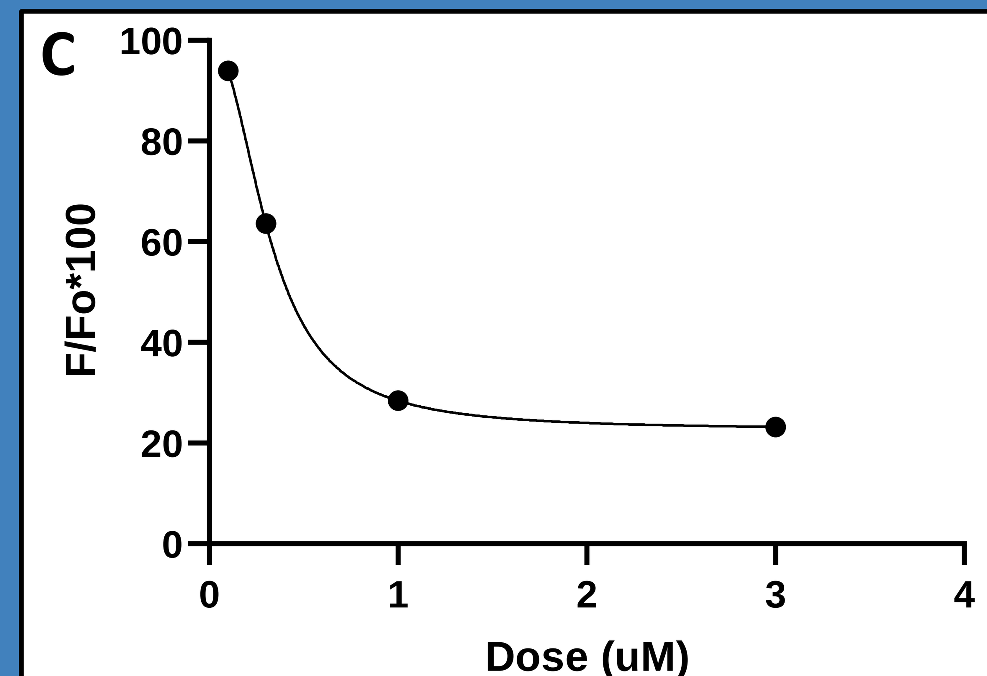


Figure 3: A series of IC50 plots demonstrating the dose-response curves of the 3 trialled tetracycline compounds; (A) chlortetracycline (IC_{50}=3.31μM, 95% CI= 2.228-5.484); (B) tigecycline (IC_{50}=8.23μM, 95% CI=5.888-30.33); (C) doxycycline (IC_{50}=0.3183, insufficient data to define a 95% CI value). All data are normalised to control (fixed at 100%).

Conclusions

This data suggests that tetracyclines act as in vitro inhibitors of microglial activation, with doxycycline acting at a concentration favourable for clinical translation. However, further research involving electrophysiological techniques, cell death assays and genetically engineered mouse models will be required to elucidate the specific role of TRPM2 in this action.

References: Malko et al., 2019. TRPM2 Channel in Microglia as a New Player in Neuroinflammation Associated With a Spectrum of Central Nervous System Pathologies.

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