

Proposal for: Examining the Neuroprotective Ability of N-methyl-4-Isoleucine Cyclosporin in Amyloid Beta (1-42) Neurotoxicity

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Abstract

The amyloid beta ($A\beta$) peptide is the most notorious factor in the aetiology of Alzheimer's disease. The formation of amyloid beta plaques throughout the brain is synonymous with the mass neuronal death that leads to the major cognitive decline attributed to AD. A wealth of literature has attributed amyloid beta's neurotoxic abilities to the formation of mitochondrial permeability transition pores in neurons, which leads to reactive oxygen species generation, a decrease in mitochondrial respiration, release of pro-apoptotic factors, and finally cell death. N-methyl-4-Isoleucine Cyclosporin (NM811), a derivative of the Cyclosporin A, has been shown to inhibit mitochondrial permeability in liver cells and as such is a promising neuroprotective agent. Surprisingly, it has never been tested as a potential treatment for Alzheimer's disease and its molecular basis of action remains uncertain. In this science fair project I plan to perform an *in vitro* analysis of the viability of $A\beta$ 1-42 effected cultured primary hippocampal cells treated with NM811.

1 Introduction

Alzheimer's disease (AD) is a neurodegenerative disease that primarily effects the elderly population. As much as 50% of individuals over 85 years of age suffer from it, with symptomatic manifestations including, but not limited to: increasing permanent memory loss, severe cognitive dysfunction, social impairment, and death [1, 2]. Currently, there is no known method of cure or (significant) prevention for AD, only treatments that delay the inevitable. These facts, combined with the fact that the complex nature of AD's pathology, cause it to be a highly researched topic in the field of psychopharmacology and biomedicine.

The two "hallmark" pathologies for AD are extracellular deposits of amyloid-beta ($A\beta$) peptides and neurofibrillary tangles [2]. In recent years, research has shown that $A\beta$ peptides exert neurotoxic effects by causing mitochondrial dysfunction and oxidative stress within neurons [3, 4]. This research has led to the creation of the mitochondrial cascade hypothesis which, simply put, states that mitochondrial dysfunction mediates the progression of AD [5, 6]. Although this hypothesis has been critiqued for "missing the bigger picture" (as mitochondrial dysfunction is likely not the sole driver of AD progression), it would be naive to discredit the role of mitochondrial dysfunction in such a complex aetiology. Indeed, a large amount of experimental evidence exists to verify this, as it has been shown that the aforementioned amyloid beta oligomers cause neuronal death through formation of mitochondrial permeability transition pores (mPTPs), pathological openings in the mitochondrial membrane that can ultimately lead to apoptotic and necrotic cell death via a variety of methods [7, 5, 8, 9, 10].

Consistent with this hypothesis, multiple known neuroprotective agents have been shown to reduce mitochondrial permeability, and multiple mitochondrial permeability mediators have been shown to have neuroprotective effects [11, 12]. One such mediator with possible neuroprotective effects is Cyclosporin A (CsA), an immunosuppressive drug that happens to bind to Cyclophilin-D, a major component in the formation of mPTPs [13]. This ability causes it to be a highly potent mPTP preventative. However, its severe side effects (mostly stemming from its immunosuppressiveness) and carcinogen status have caused it to be used as a last resort treatment in most cases [14, 15, 16].

N-methyl-4-isoleucine cyclosporine (NIM811), a derivative of CsA, has been shown to have the same binding effect on Cyclophilin-D and thus the same mitochondrial protective effect, without any

immunosuppressive effect [17, 18]. Although it has never been studied in the context of AD, NIM811 shows promise as a potential treatment. Thus, the goal of this research is to examine the neuroprotective ability of NIM811 in the context of A β 1-42 induced neurotoxicity, which will give insight into its abilities in preserving neuronal viability and thus cognitive function.

2 Comprehensive Planned Methodology

2.1 ISEF Regulation Accordance

The hippocampal rat neurons used in this procedure will be purchased from an independent supplier instead of extracted for the project in order to avoid ethical and regulation complications with the ISEF. The same shall be done with the NIM811 and amyloid beta peptides.

2.2 Basic Overview

This process has been generally adapted from Sahu et al. (2019) and Sun et al. (2014) [19, 11].

First, a 48-well clear bottom plate of which the neurons are to be placed in will be sterilized with 96% ethanol. Then the ethanol will be wiped off and the wells air dried for 5 minutes, before coating them in 500 μ l of 10 μ g/ml Poly-l-lysine and placing them in a 5% CO₂ incubator for 18 hours at 37° Celsius. After incubation, the wells will be rinsed with 500 μ l of phosphate-buffered saline. After rinsing, neuralbasal medium (including 2% B27, 1% l-glutamine, and 1% penicillin–streptomycin) will be placed in the wells. 100,000 neurons will be placed in two lines of 5 on the well plate. After 24 hours of incubation, the lines will be assigned to one of two groups: control or treated. Either group of cells will be administered 5 μ g of A β 1-42, with treated cells being treated with 5 μ g or 20 μ g of NIM811 two hours beforehand.

Once every two days, half of the serum media in each well plate will be removed and replaced with fresh media of the same origin. After 5-6 days, the cells will be dissociated into a suspension and flow cytometry will be preformed. Cell viability will be measured by flow cytometry via C12-resazurin dye and SYTOX Green dye. Mitochondrial membrane potential will be measured by flow cytometry via JC-1 dye. 3 plates in both groups will be used for viability analysis, and 2 plates in both groups will be used for measuring mitochondrial membrane potential.

3 Research Implications and Possible Findings

Should this research generate a positive outcome, it would underscore the possible therapeutic potential of NIM811 and mitochondrial permeability mediators in the treatment of AD. Moreover, it would prompt greater questioning of if NIM811 is actually a viable treatment for AD, and/or if it has synergistic potential with existing treatments. Beyond AD, mPTP formation prevention has a large number of applications in various diseases, especially other neurodegenerative disorders.

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