ORAL PRESENTATION • DRUG DISCOVERY AND DEVELOPMENT

[OPD1]

NEUROPROTECTIVE EFFECTS OF BETA-SITOSTEROL AGAINST BETA-AMYLOID-INDUCED MICROGLIA-MEDIATED NEUROINFLAMMATION AND NEUROTOXICITY

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Recent studies have shown that neuroinflammation plays a bigger role in the pathogenesis of Alzheimer's Disease (AD). β-sitosterol, a naturally occurring phytosterol has been shown to exhibit anti-inflammatory properties. However, the anti-neuroinflammatory effects of β-sitosterol have yet to be investigated. The research question for this study is "does β-sitosterol exhibit neuroprotective effects against Aβ-induced microglia-mediated neuroinflammation and neurotoxicity?". Hence in this study, the effects of β-sitosterol on Aβ-induced microglia-mediated neuroinflammation and neurotoxicity were investigated.

The β-sitosterol cytotoxicity on microglia and neuronal cells was determined using diphenyl-2H-tetrazolium bromide (MTT) cell viability assay. Effects of β-sitosterol on the production of pro-inflammatory cytokines (IL-1β and TNF-α) were assessed using enzyme-linked immunosorbent assay (ELISA) kits. Subsequently, Promega Cell Viability Assay kit was used to evaluate the neuroprotective effects of β-sitosterol on neuronal cells against microglia-mediated neurotoxicity. Western blot analysis was conducted to investigate the regulation of inflammasome expression, tau production, and mitogen-activated protein kinase (MAPK) signalling pathway. Data were analysed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test.

Our data showed that β-sitosterol down-regulated production of pro-inflammatory cytokines (IL-1β and TNF-α). Furthermore, β-sitosterol increased cell viability of neuronal cells in comparison to conditioned media containing Aβ alone, thus conferring a neuroprotective effect on neuronal cells. These findings corresponded to the inhibitory effects of β-sitosterol on production of pro-inflammatory cytokines. Besides that, it downregulated the p38 MAPK signalling, and NLR family pyrin domain containing 3 (NLRP3) expression induced by Aβ in microglia. In addition, β-sitosterol exerted neuroprotective effects by downregulating the levels of phosphorylated p38 MAPK, phosphorylated Tau and NLRP3 in Aβ-induced neuronal cells.

In conclusion, the present study suggests that β-sitosterol could be a potential candidate for AD drug development due to its ability to reduce neuroinflammation via reducing production of pro-inflammatory cytokines and confer neuroprotective effects on neuronal cells.

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[OPD2]

PREDICTING DNA/RNA APTAMER DESIGN FOR TARGETING GRAM-NEGATIVE BACTERIA PROTEINS USING A GENETIC ALGORITHM

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Recent medical research has focused on producing binding agents for small molecules and protein targets, of which antibiotics are a popular method. Antibiotic-resistant bacteria continuously pose a global threat, meanwhile the antibiotic industry is costly and hosts a challenging manufacturing process. Hence, there is an urgent need for alternative, sensitive, and rapid-developing diagnostic and therapeutic agents. Lately, studies have touted aptamers as an efficient and promising alternative. Aptamers are short single-stranded DNA, RNA molecules which bind, with high affinity and selectivity, to several biological targets such as cells, viruses, and proteins. Coupled with Systematic Evolution of Ligands by EXponential enrichment (SELEX) technology, generating a multitude of unique aptamers is possible. But as SELEX is restrictive and labour-intensive, in silico methods have attempted to improve the enrichment rate. Fortunately, advanced machine-learning models have shown promise in predicting the binding abilities within target-ligand complexes, though research is limited.

Thus, this project expands upon this endeavour by utilising a genetic algorithm (GA) with an affinity predictor fitness function to predict in silico design of DNA aptamers. As a case study, aptamer datasets were used to train the GA to elucidate rules for the antibacterial design of the b-barrel assembly machine complex protein. Firstly, Discovery Studio and CASTp 3.0 were employed to identify active sites and differentiate high- and low-affinity features toward the target protein. Then, 103 aptamers were used to conduct molecular docking via Haddock. Eight aptamers were identified with the highest binding scores; thus, six attributes were chosen (e.g., Haddock score, electrostatic energy) as vectors in building the GA's fitness function. Thus, it is predicted that the best-performing aptamer would mirror its in silico results through in vitro validation. This proposed method could provide a relevant, simplified contribution to aptamer-protein interaction design, using machine-learning techniques to potentially accelerate aptamer screening.

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[OPD3]

AN *IN VITRO* STUDY ON THE NEUROPROTECTIVE EFFECT OF TGF-βR1 INHIBITOR (REPSOX) AGAINST BETA-AMYLOID-INDUCED NEUROTOXICITY

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Alzheimer's disease (AD) is characterised by the manifestation of extracellular beta-amyloid (Aβ) plaque accumulation and intracellular neurofibrillary tangles (NFTs) consisted of hyperphosphorylated tau. Notably, hyperphosphorylation of tau is increasingly evident to contribute to Aβ-induced neurotoxicity and phosphorylated tau (p-Tau) is highly associated with cognitive decline. Recently, dysregulation of transforming growth factor-β (TGF-β) signalling is shown to play a significant role in AD pathogenesis while increased TGF-β1 level was observed in AD patients. The silencing of the *TGFBR1* gene resulted in neuronal survival from Aβ-induced neurotoxicity. Therefore, in this study, the neuroprotective effect of RepSox (selective TGF-β receptor type 1 (TGF-βR1) inhibitor) against Aβ-induced neurotoxicity and its modulation on the expression of Aβ-induced tau hyperphosphorylation were investigated on human SH-SY5Y neuroblastoma cells.

The cytotoxicity effect of RepSox on SH-SY5Y cells was determined using methyl thiazolyl tetrazolium (MTT) assay. Next, the neuroprotective effect of RepSox against Aβ-induced neurotoxicity was investigated at three different concentrations (50 nM, 100 nM and 150 nM) over 72 h using RealTime-Glo™ MT Cell Viability Assay. Western blotting was used to examine the expression of phosphorylated tau (pT231) and total tau proteins (Tau-5) in treated SH-SY5Y cells.

RepSox at the tested concentration range of 1.5625 ng/mL to 200 ng/mL showed no cytotoxicity effect on SH-SY5Y cells. Furthermore, RepSox exhibited neuroprotection on SH-SY5Y cells against Aβ-induced neurotoxicity by significantly increased the cell viability in comparison to SH-SY5Y cells treated with Aβ only. Importantly, the pT231 expression was significantly attenuated by RepSox in Aβ-induced SH-SY5Y cells while the Tau-5 expression remained unchanged. In conclusion, this study demonstrates that treatment with RepSox, a selective TGF-βR1 inhibitor, exhibits the neuroprotective effect on SH-SY5Y cells against Aβ-induced neurotoxicity by attenuating Aβ-induced tau hyperphosphorylation. Hence, RepSox could be a potential therapeutic agent for AD.