DEVELOPMENT, CHARACTERISATION AND NEUROPROTECTIVE EFFECTS OF NOVEL POLYMER-DRUG CONJUGATE NANO-POLYPLEX: WORKING TOWARDS A MULTI-TARGET TREATMENT FOR NEURODEGENERATIVE DISEASES

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Background: The multifactorial aetiology of neurodegenerative diseases like Alzheimer's disease (AD) requires a multitargeted treatment strategy. Polymer-drug conjugates (PDCs) offer a promising approach by combining multiple therapeutic compounds with improved drug delivery. This study aimed to develop a PDC nano-polyplex by combining antioxidant and anticholinesterase polymers, to explore its potential for enhancing multi-target therapeutic efficacy and improving drug delivery in the treatment of NDs.

Methods: A nano-polyplex was formulated by mixing cationic antioxidant-polymer and anionic anticholinesterase-polymer conjugates. Particle size was measured using a Zetasizer and confirmed by cryogenic transmission electron microscopy (Cryo-TEM). The antioxidant activity was evaluated using the oxygen radical absorbance capacity (ORAC) assay, while cholinesterase inhibition was assessed *via* Ellman's assay. Cytotoxicity, neuroprotective, and anti-inflammatory effects were studied in undifferentiated SH-SY5Y human neuroblastoma and BV-2 murine microglial cell lines using the MTT assay. The Thioflavin T (ThT) assay and TEM were used to analyze amyloid beta (Aβ) aggregation in a cell-free assay, and western blotting was conducted to evaluate tau protein expression in undifferentiated SH-SY5Y cells induced with okadaic acid. *In vivo,* toxicity and efficiency studies were performed using *Drosophila melanogaster* tauopathy and wild-type models.

Results: The nano-polyplex formed uniform nanoparticles of 30.5±7.9 nm. Significantly enhanced antioxidant activity ($p \leq 0.01$) and cholinesterase inhibition ($p \leq 0.01$) were exhibited with the nano-polyplex compared to parent drugs alone. The nano-polyplex was non-toxic to undifferentiated SH-SY5Y cells (cell viability >90%) but showed significant toxicity in BV-2 cells (cell viability 75%, ***$p \leq 0.001$). The nano-polyplex significantly protected undifferentiated SH-SY5Y cells against hydrogen peroxide-induced oxidative stress (45% protection, $p \leq 0.0001$), reduced lipopolysaccharide-induced inflammation in BV-2 cells by more than 20% ($p \leq 0.01$), and reduced Aβ aggregation by over 10% ($p \leq 0.01$) in a cell-free assay. Additionally, the nano-polyplex decreased okadaic acid-induced neurotoxicity by 50% ($p \leq 0.0001$) and reduced total tau protein expression ($p \leq 0.01$) in undifferentiated SH-SY5Y cells stressed with okadaic acid. Additionally, PAA modestly reduced tau phosphorylation at the pSer202/pThr205 site. In *Drosophila* studies, N5NM15 and PAA were toxic to *GMR*-GAL4 adult flies but well-tolerated in *ELAV*-GAL4 flies. Furthermore, no significant improvement was observed in ommatidial patterning, eye bristle number, or eye length in *Drosophila* tauopathy models when treated with N5NM15. Climbing and survival assays demonstrated mild neuroprotective effects at lower doses in early disease stages, whereas higher concentrations were significantly toxic in both wild-type ($p \leq 0.0001$) and tauopathy flies ($p < 0.05$).

Conclusions: This study highlights the potential of multifunctional nano-polyplexes as multi-target therapeutic agents for NDs. However, findings also emphasise the need for dose optimisation and reformulation using non-toxic buffers to enhance therapeutic efficacy while reducing toxicity in both normal and disease *Drosophila* models of tauopathy.