Targeted Delivery of Antioxidant-loaded Biodegradable Nanoparticles for Neuroprotection

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Statement of Purpose:

In many neurodegenerative diseases such as chronic traumatic encephalopathy (CTE), Parkinson's (PD), Huntington's and Alzheimer's disease, there is a progressive loss of structure or function of neurons, including death of neurons. Mitochondrial dysfunction and oxidative and nitrative stress have been implicated in a number of neurodegenerative diseases. Current treatment approaches for these diseases are symptomatic and fail to prevent the progression of the neurodegenerative process. Mitochondrial-targeted antioxidants have been recently shown to protect against the Parkinsonian toxicant, 1-methyl-4-phenylpyridinium (MPP^+)-induced production of reactive oxygen/nitrogen species (ROS/RNS) production (1).

Polyanhydride-based delivery systems can elicit unique cellular responses, such as particle internalization and directed intracellular trafficking (2). Through encapsulation of the payload within the particles, sustained release of these compounds can be achieved. Altering the polymer chemistry, functionalizing the particle surface, and/or controlling particle size enable the programmed release of payload to targeted intracellular compartments.

In this work we investigated the ability of biodegradable polyanhydride nanoparticles to enhance the delivery of mitochondrial-targeted antioxidants to protect against oxidative stress induced by hydrogen peroxide (H_2O_2) and MPP^+.

Methods:

A 20:80 copolymer of 1,6-bis-(p-carboxyphenoxy) hexane and sebacic anhydride (CPH:SA) was synthesized and characterized by ^{1}H-NMR. The antioxidant utilized in these studies was mitoapocyanin, which was based on apocyanin functionalized with a mitochondrial targeting moiety (mAPO) (3). Nanoparticles containing mAPO or quantum dots (QDs) were synthesized using an anti-solvent nanoencapsulation method (4). Surface modification of the nanoparticles with folic acid was performed by carbodiimide coupling. The particles were characterized by SEM, DLS, and XPS. The release of mAPO from the nanoparticles was quantified using HPLC.

Mitoapocyanin-loaded nanoparticles were evaluated for toxicity and protection using both a neuronal cell line (N27) and primary mouse cortical neurons. Cell death was determined by the MTS assay. Cellular uptake of QD-loaded nanoparticles was evaluated by confocal microscopy and flow cytometry. The intracellular fate of the QD-loaded nanoparticles was investigated with TEM.

Results:

Particles were synthesized with sizes ranging from 250-320 nm. Folic acid modification of the nanoparticles resulted in increased particle size. The nanoparticles provided controlled release of mAPO.

All nanoparticle formulations were non-toxic to N27 and primary cortical cells at concentrations up to 100 μg/mL. The 5% mAPO loaded CPH:SA nanoparticles with folate modification were protective against H_2O_2 insult as was soluble mAPO (Fig. 1). We observed internalization of all the nanoparticles, with the folic acid modified nanoparticles showing the highest level of internalization via flow cytometry.

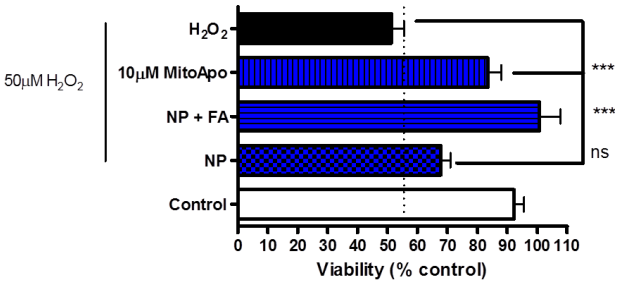

Fig. 1. Protection of primary cortical cells by nanoparticles containing mAPO against assault with H_2O_2. Cells pretreated with soluble mAPO or nanoparticle formulations containing 5% mAPO. Viability determined after H_2O_2 insult by MTS assay.

Conclusions:

Controlled release of mAPO was obtained by encapsulation into polyanhydride nanoparticles. Surface modification of the nanoparticles with folic acid resulted in protection of neuronal cells against insult with H_2O_2. This work suggests that these nanoparticles have the potential to protect neuronal cells against mitochondrial dysfunction stemming from ROS/RNS and lays the foundation for future in vivo studies.

References:

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