Novel mitochondrial targeted antioxidant delivery systems for neuroprotective and nootropic applications

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Background

Oxidative stress in the brain as a result of cellular reactive oxygen species (ROS) is known to cause morphological abnormalities, membrane damage, and altered neurotransmitter function resulting in neuron degeneration and consequent cognitive decline. Most mammalian cells possess endogenous antioxidant defensive enzymes such as glutathione, superoxide dismutase and catalase. However, with increasing age or under persistent pro-oxidant attack these defence systems become overwhelmed and diminish. Such sustained oxidative stress has been associated with the initiation of dementia and the development of neurodegenerative diseases such as Alzheimer's and Parkinson's disease. Although there are both natural polyphenol antioxidants (e.g. curcumin) and synthetic antioxidant based supplements (e.g. N-acetylcysteine) currently marketed to counter oxidative stress, they are limited by low activity, low stability, poor absorption and limited ability to access the brain. Formulation science strategies such as polymer or liposomal based delivery systems have been utilised to overcome these issues. However, many of these cannot pass the blood brain barrier (BBB) and target the mitochondria where the majority of neuronal ROS formation occurs.

Objectives

This study therefore aimed to develop novel mitochondria targeted micellar nanocarrier delivery systems for the antioxidants curcumin (CU), hydroxytyrosol (HT) and N-acetylcysteine (NAC) and examine their ability to protect against oxidative stress and neuronal degeneration in an in vitro neuronal cell line model.

Methods

Nanocarriers entrapping the antioxidants CU, HT or NAC, alongside corresponding blank formulations were prepared using the block co-polymer Pluronic® ® (P68) and the amphiphilic cation dequalinium (DQA) by modified thin-film hydration. Dynamic light scattering and transmission electron microscopy were used to measure particle size and assess morphology (respectively). A UV spectrophotometry method was used to assess loading efficiency. In vitro assessments were conducted using the human neuroblastoma cell line SH-SY5Y. The potential toxic effects of the formulations and their ability to protect against neurodegeneration was evaluated by a tetrazolium dye-based cytotoxicity assay. A cellular antioxidant activity (CAA) assay employing a potent pro-oxidant (ABAP) was used to assess nanocarrier antioxidant activity and ability to counteract neuronal oxidative stress.

Results

All formulations demonstrated high encapsulation efficiency (85-98%) and retained spherical morphology. Nanocarrier size was measured to be < 200nm. No cytotoxicity was observed for any antioxidant nanoformulation. All antioxidant formulations were able protect against neurodegeneration, in most cases maintaining cell viability at ≥80% compared to the 50% reduction induced by rotenone ($p \leq 0.005$). Each concentration of all formulations (CU, HT and NAC) exhibited higher CAA under conditions of cellular oxidative stress compared to the free CU (13% & 17%), HT (64% & 10%) and NAC (67% & 76%) alone. In all assessments the NAC formulation was superior to free NAC as well as the CU and HT formulations.

Key Point Summary

This study demonstrates for the first time the development of novel mitochondria targeted micellar antioxidant nanocarriers.

The study highlights the ability of these carrier systems to stabilise and deliver natural antioxidants to neurons to counter cellular oxidative stress.

These mitochondria targeted nanocarriers could provide an attractive approach for enhancing the protective anti-ageing effects of antioxidants on neuronal and cognitive health.

1. Our nanoformulation approach for neuronal delivery

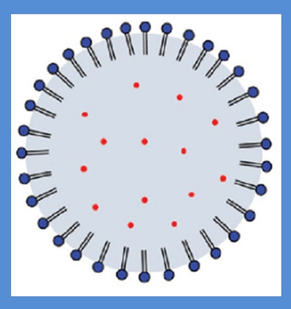
Natural polyphenol antioxidants (e.g. CU and HT) and synthetic antioxidant based supplements (e.g. NAC) are limited by low activity, low stability, poor absorption and limited ability to access the brain. Nanotechnology based micellar formulations (nanoformulations) can help preserve beneficial characteristics of a substance while enhancing absorption, potency and access into the brain.

We use the approved block co-polymer Pluronic® ® F68 (P68) and the amphiphilic cation dequalinium (DQA) as encapsulating materials for CU, HT and NAC antioxidants for the following reasons:

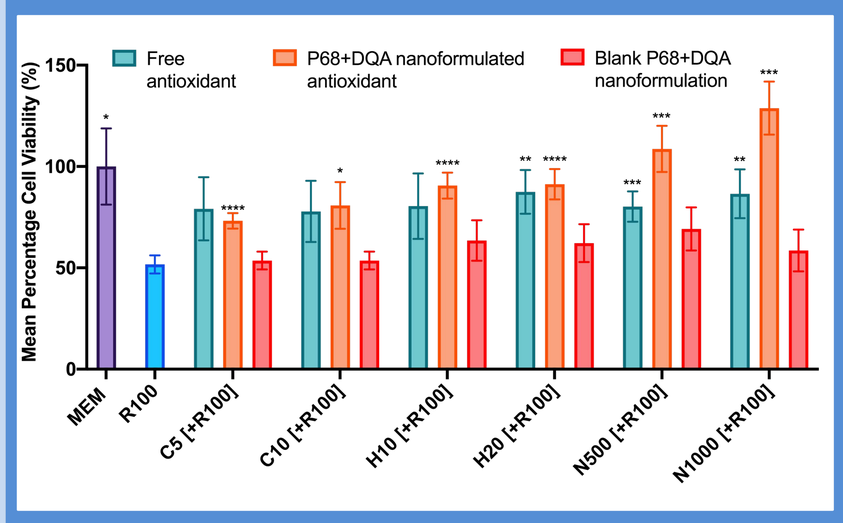
✓ Protection of 'entrapped' cargo from reacting with external environment (e.g. humidity and light) and internal conditions (e.g. pH and enzymatic damage).

✓ High cellular absorption and targeting to mitochondria

✓Small size and neutral charge to support access into the brain



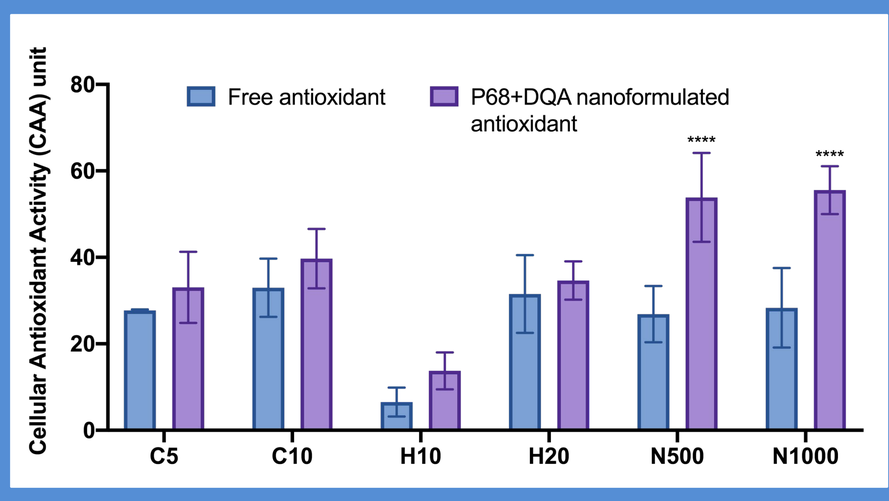
2. P68+DQA nanoformulations are protective against neuronal toxicity



The ability of CU, HT and NAC loaded P68+DQA nano-formulations and the unloaded blank formulations to protect against neurodegeneration was evaluated using the MTT cytotoxicity assay after 24h 100μM rotenone treatment.

All antioxidant loaded nano-formulations were able to protect against the neurotoxicity induced by 100μM rotenone.

3. P68+DQA nanoformulations facilitate enhanced cellular antioxidant activity



The ability to counteract cellular oxidative stress and the antioxidant potential of the nano-formulations in the SH-SY5Y neuronal cell line was compared by carrying out a cellular antioxidant assay (CAA) using ABAP as a pro-oxidant (****P<0.0001).

P68+DQA nanoformulations of each antioxidant (CU, HT and NAC) exhibited higher CAA under conditions of cellular oxidative stress compared to the corresponding unencapsulated 'free' antioxidants. Only NAC formulations exhibited significantly higher CAA at each concentration (67% & 76%).

4. P68 DQA nanoformulations have nanometre dimensions

Nanocarriers entrapping NAC, HT and CU and corresponding blank formulations were prepared using the block co-polymer P68 and the amphiphile DQA by an in-house thin-film hydration method.

Electron microscopy images illustrate that the antioxidant loaded P68+DQA nanoformulations are of nanometre size and have smooth, spherical morphology.

The authors have no conflicts of interest to declare

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