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Development of biocompatible nanogel for sustained drug release by overcoming the blood brain barrier in zebrafish model

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ABSTRACT

A potential delivery system has to be fabricated for crossing the blood–brain barrier (BBB) to reach the brain fluid for effective delivery of drugs for any neurological disorders. The present study is aimed for the delivery of donepezil through functionalized PNIPAM nanogel by overcoming the BBB using zebrafish model. We had synthesized the poly N-isopropyl acrylamide nanogels with 20 nm size for sustained drug release. The entrapment of donepezil in the nanogel was quantified as 87.5% by HPLC and its sustained drug release pattern was achieved at 37 °C using Janus green dye release assay. Acetylcholineesterase inhibition assay for the donepezil conjugated nanogel (DCN) has confirmed thermoresponsive drug release by obtaining the donepezil peak at 9.3 min retention time in HPLC. Swim behavior and heart beat rates were found to be biocompatible for the functionalized nanogel DCN in zebrafish. Histological analysis revealed increased pial surface in anterior telenchepalon region of zebrafish brain for the DCN administered fishes. DCN treated embryos exhibited minor developmental deformities above 5 μg/ml and thus confirmed its minimal toxicity and its therapeutic efficiency. This study may shed light on the development of neurospecific nanogel for targeted and sustained drug release to brain by crossing the blood–brain barrier.

Introduction

Drug delivery to brain is a major challenging task for any neurodegenerative disease condition (Sharma et al., 2012) of the patients because of the blood brain barrier (BBB). BBB is formed by the brain micro blood vessel endothelial cells and prevents uncontrolled passing of amino acids, ions, peptides and small molecules to brain (Brown et al., 2002). The paracellular transports of molecules are restricted by tight

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junctions of BBB. It plays a key role in Alzheimer's and Parkinson's disease conditions, because the dysfunction of BBB affects the passage of molecules inside brain (Desai et al., 2007). Donepezil is widely used for treating Alzheimer's disease and its related dementia. Though, it is used for treating Alzheimer's disease, the side effects and the relatively decreased efficacies are noted on continuous usage of this drug (Tan et al., 2014). To avoid the over dosage, a controlled drug delivery system is needed for sustained and targeted release (Felice et al., 2014) to the brain.

Nanotechnology based drug delivery vehicles are having the properties of smaller size with effective release of drugs in minimal dosage with target specificity (Ahmad et al., 2014). Poly N-isopropyl acrylamide (PNIPAM) is a thermoresponsive polymer exhibits hydrophobic – hydrophilic phase transition at its Lower Critical Solution Temperature (LCST). It has significant characteristic of water retaining capacity and permeability properties (Zhang et al., 2014). These smart polymers are successfully used in drug delivery applications for many years (Shao et al., 2011) and they are widely accepted for its biocompatibility (Gandhi et al., 2014) and cytocompatiblity in multiple cell lines (Tang et al., 2010; Tekin et al., 2011). Nanogels are reported to be nontoxic materials with greater drug loading capacity and higher BBB permeability (Jain, 2012). Hence, in this study we had synthesized and functionalized the donepezil conjugated nanogel (DCN) with polysorbate 80 by chemical route to overcome the BBB (Kreuter et al., 2005).

Zebrafishes are mainly used due to its genetic similarity with human, as an *in vivo* animal model for drug development, as well the physiological and toxicological studies to assess the drug efficacy and biocompatibility (Kari et al., 2007). In the present study, we had developed a thermo-responsive nanogel conjugated with donepezil for its sustained drug delivery in brain and studied for its biocompatibility in adult and embryonic zebrafish.

Materials and methods

Fabrication of PNIPAM nanogel

PNIPAM hydrogel was prepared based on the method described by Xia et al. (2013). NIPAM (0.5 g), methyl bis acrylamide (MBA) (0.03 g), potassium per sulphate (0.01 g), sodium dodecyl sulphate (0.1 g) were added and dissolved in Milli-Q water, followed by nitrogen gas bubbling and the polymerization was allowed for 10 min with 300 rpm at 60 °C. The sealed flask was kept on ice to cease the polymerization and dialyzed against Milli-Q water for 10 days. Dialyzed mixture and distilled water in the ratio of 9:1 was bubbled with nitrogen gas. NIPAM (1 M) monomer was added to the above mixture and dissolved well. To accelerate the polymerization, 10 μl of TEMED was added and kept sealed on ice for 2 h, followed by 2 days incubation at room temperature. Further, the samples were freeze dried (Christ Alpha 1–2 LD) under vacuum for 48 h and stored at −80 °C. The freeze dried nanogel was gold sputtered and observed under Field Emission Scanning Electron Microscopy (FESEM) (Supra-55, Carl Zeiss, Germany) for studying their structural morphology. The parameters such as average size and size distribution of the suspension were calculated by dynamic light scattering studies in particle size analyzer (Microtrac Bluewave, Turbotrac).

Functionalization of nanogel

The nanogel was functionalized with polysorbate 80 and donepezil (Sigma Aldrich) for the effective and targeted drug delivery. Functionalization of hydrogel was done by adding 10 mg of nanogel in 100 mM of 1 ml sodium carbonate buffer, treated with 20% of polysorbate 80 and 1 μg/ml of donepezil by continuous stirring for 4 h at room temperature (Vinogradov et al., 2004). The conjugation was confirmed by analyzing 100 μl of functionalized nanogel in liquid Fourier transform infra-red spectroscopy (FTIR) analysis (Perkin Elmer, Spectrum one, USA).

Analysis of thermo responsiveness

Thermoresponsiveness of the PNIPAM nanogel was measured by cloud point measurement method as described by Jafari et al. (2011), using multimode plate reader (Perkin Elmer, EnSpire, USA). PNIPAM nanogel was measured at 500 nm absorbance in series of increasing temperatures (25–45 °C) with 10 min incubation time and 5 s shaking at each temperature. The drug release studies were performed using Janus green dye to ensure the measurement of dye release at 660 nm.

Oral drug delivery in adult zebrafish

Zebrafishes were maintained in a recirculating stand-alone system (Aquaneering, USA) at 28 °C and 10:14 h dark: light cycle. All protocols were reviewed and approved by Institutional Ethical Committee (Approval number for animal usage IBSC/2013/DBT-IDB/RRK-009) of Sathyabama University. Adult zebrafishes with 340 mg average weight and 2.5 cm body length were taken as 5 fishes in each batch for the administration of donepezil, nanogel, DCN and control. Venflon syringe of 24G was used to administer 5 μl volume of the drug containing various concentrations to adult zebrafish by inserting the plastic needle 1 cm below the gills to reach the end of esophagus (Collymore et al., 2013). Control fishes were treated with system water orally instead of drug. As soon as the drug was given orally, these fishes were put in warm water above LCST ~37 °C for 1 min for the release of donepezil from DCN, until regaining their original physiological function from the anesthetic condition. The drug concentration on each set of experiment was determined after effective dosage calculations. The oral delivery was continued for a week as one time dosage and the changes were observed.

Acetylcholine esterase inhibition assay for the donepezil conjugated nanogel

Acetylcholine esterase inhibition assay was performed using 75 mM acetylthiocholine iodide and 10 mM DTNB chromogen in a 96 well microtitre plate containing homogenized zebrafish brain extract. This change in absorbance was recorded at 412 nm for every 1 min for 10 min using the multimode plate reader (Kannan and Vincent, 2012b).