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Triphenyl phosphonium coated nano-quercetin for oral delivery: Neuroprotective effects in attenuating age related global moderate cerebral ischemia reperfusion injury in rats

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Abstract

Cerebral ischemia–reperfusion is a classic example of reactive oxygen species (ROS) mediated acute damage to brain. Post-ischemic reperfusion induced oxygen free radicals production causes damage to brain cell mitochondria. Antioxidants like quercetin (Qc) have potentials to manage oxidative stress related pathophysiology. However low oral bioavailability and poor cell membrane permeability restrict its therapeutic efficacy. To overcome these hurdles mitochondria specific delivery of Qc nanocapsules was designed to efficiently counteract cerebral ischemia–reperfusion induced cell death and neurodegeneration in young and aged rats. The orally deliverable quercetin loaded polymeric nanocapsules (N1QC) were made mitochondria specific by using triphenylphosphonium cation as one of the matrix components. N1QC demonstrated higher brain uptake and remarkable mitochondrial localization post cerebral ischemia–reperfusion. This unique controlled mitochondrial delivery of quercetin ameliorated histopathological severity by preserving mitochondrial structural and functional integrity through sequestering ROS thus modulating mitochondrial ROS mediated apoptotic cell death in young and aged rats.

Key words: Mitochondria-targeting; Neurodegeneration; Nanoparticles; BCCAO; ROS

Cerebral ischemia is one of the leading causes of adult death and long term debility worldwide.[1] Among different mechanisms oxidative damage and inflammation play a critical role in the pathogenesis and progression of ischemic brain damage inducing neuronal death and neurological dysfunction.[2,3] Post ischemic oxidative burst during reperfusion further amplifies cerebral damage by activating free radical reaction cascades, particularly affecting the vital organelle mitochondria.

Mitochondria are the primary organelles that generate reactive oxygen species (ROS).[3] Elevated ROS level alters and damages the configuration of most of the enzymes in the mitochondrial matrix and those involved in the electron transport ultimately resulting in a shortage of energy currency i.e. ATP.[4] Thus mitochondria themselves being a major source of oxidants are also the target for their damaging effects, and, therefore, oxidative insult to mitochondria is a cause, rather than an outcome of cell death. During cerebral ischemia–reperfusion and other neurodegenerative disorders, oxidative stress alters the homeostasis between antioxidant defense and ROS generation in mitochondria.[5] Oxidative damage also upgrades with aging where the coupled mitochondria are attributed with decreased oxidative phosphorylation.[6] The condition becomes so severe

Abbreviation: Qc, quercetin; PLGA, poly(lactide-co-glycolide); DMAB, didodecyldimethylammonium bromide; TPP, dodecyl triphenylphosphonium bromide; ROS, reactive oxygen species; BBB, blood brain barrier; cyt c, cytochrome c; PARP, poly (ADP-ribose) polymerase; BCCAO, bilateral clamping of the common carotid arteries; AFM, atomic force microscope; FTIR, Fourier transform infrared spectroscopy; LC–MS, liquid chromatography-mass spectrometry; RFI, relative fluorescence intensity; ETC, electron transport chain; BrdU, 5-bromo-2′-deoxyuridine; CPCSEA, The Committee for the Purpose of Control and Supervision of Experimental Animal, India; HE, hematoxylin and eosin; GSH, reduced glutathione; SOD, superoxide dismutase.

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that the neuronal antioxidant system gets either inactivated or completely used up so that they fail to reduce the ROS pool in the cerebral tissue.

These circumstances demand engagement of a strong exogenous antioxidant to counter cerebral ischemia–reperfusion induced oxidative insult particularly in protecting mitochondria of aged population. A number of studies have shown that exogenously applied antioxidants fall short to attenuate cerebral ischemia–reperfusion injury.[7–10]

Quercetin (Qc), a plant flavonoidal antioxidant, has shown evidence of its antioxidant property against neurodegenerative diseases.[11] In addition to its manifold medicinal benefits, this compound is water insoluble and has a low oral bioavailability in blood. Moreover, this water insoluble polyphenolic compound cannot extravasate the blood brain barrier (BBB), a tight junction between blood capillaries and interstitial fluid and hence poses a major impediment in central nervous system (CNS) therapy.[1,12]

Biodegradable polymeric nanoparticles are a promising modality to deliver therapeutically active components in a more effective and nontoxic form. The polymer matrix of these nanoparticles can be modified in numerous ways to ensure proficient "cargo unloading" at target sites which are otherwise not so easily accessible by free drugs. Triphenylphosphonium (TPP^+) is a lipophilic cation which can preferentially accumulate in the mitochondria.[13] Previous reports of attaching this mitochondria specific cation to antioxidants like vitamin E, coenzyme Q, and quercetin have shown higher mitochondrial accumulation *in vitro*. But performance of such compounds in a nanoparticulated system against a pathophysiological condition *in vivo* is not yet investigated.[14] Nanocapsulated quercetin demonstrated neuroprotective properties and safeguarded brain mitochondria against a pathophysiological condition of cerebral ischemia–reperfusion.[15,16] We hypothesized quercetin loaded poly(lactide-co-glycolide) (PLGA) nanocapsules containing TPP^+ as one of the matrix components (N1QC) that would enable mitochondria specific delivery of quercetin and enhance neuroprotective and mito-protective properties of quercetin in cases of cerebral ischemia–reperfusion induced oxidative insult. The brain and brain mitochondria uptake efficacy of N1QC was determined. The protective effects of N1QC on the ischemia–reperfusion injury were assessed by cerebral edema formation, histological analysis, mitochondrial structural and functional analysis. In order to elucidate the mechanism by which N1QC ameliorates ischemia–reperfusion mediated neuronal cell death, DNA fragmentation and the expressions of some of the proteins including cytochrome c (cyt c), caspase 9,3 and poly (ADP-ribose) polymerase (PARP) involved in executing apoptotic cell death, were evaluated.

Methods

Description of materials, preparation of N1QC (quercetin loaded PLGA nanocapsules containing TPP^+) and N2QC (quercetin loaded PLGA nanocapsules) and other methods are described in Supplementary Materials.

Animals for in vivo administration

All *in vivo* experiments were done in animals with a prior approval of the Animal Ethics Committee, Registration No. 147/99/CPCSEA. All the animals used in this experiment received proper care in compliance with the Animal Ethics Committee, India. A minimum possible number of animal experiments were designed with reduced number of rats and shorter time period and handled with professional care to reduce probable sufferings of animals.

Wister rats, male, belonging to two age groups (2 months and 20 months) weighing 160-180 g and 415-440 g, respectively, were used. The animals were housed in a temperature and humidity-controlled rooms with 12-h light and dark cycles. The animals were allowed free access to food and water and were acclimatized for 3-5 days to the new environment before any experiment.

Induction of cerebral ischemia and assessment of nanocapsule treatment impact

Rats were categorized into young (2 months old) and old (20 months) groups. Animals from each group were further randomly subdivided into following six subgroups for respective treatment 24 h prior to cerebral ischemia insult.

Group A: normal group, untreated controls, fed with 0.3 ml of normal saline.
Group B: sham operated control, fed with 0.3 ml of normal saline.
Group C: untreated cerebral ischemia–reperfused control, fed with 0.3 ml of normal saline.
Group D: oral gavage of 2 mg/kg b.wt free Qc suspended in neutral oil.
Group E: oral gavage of 2 mg/kg b.wt Qc in N1QC.
Group F: oral gavage of 2 mg/kg b.wt Qc in N2QC.

Young and aged rats from all groups (leaving out normal groups) were anesthetized by a single dose of urethan (17 mg/kg), i.p. Cerebral ischemia was induced by bilateral common carotid artery occlusion (BCCAO) and 30 min of reperfusion following a previously described process.[17] Post BCCAO–reperfusion rats were killed by decapitation. The brain was isolated immediately and its surface was rinsed with ice-cold saline. The subarachnoid membrane and vessels were gently removed and the brain was dissected into several coronal sections. All following experiments to assess the neuroprotective and mitoprotective impact of nanocapsule treatment were done with these brain samples.

Statistical analysis

Mean and standard error of mean (SEM) were calculated for all experimental data. Significant differences between means were evaluated by an analysis of variances. A difference was considered significant when $P < 0.05$.

Results

Nanocapsule characterization

Nanocapsules were observed under atomic force microscope (AFM) (5500 Pico Plus AFM system, Agilent Technologies Santa Clara, California, USA) for size, shape and surface