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Rutin-encapsulated chitosan nanoparticles targeted to the brain in the treatment of Cerebral Ischemia

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ABSTRACT

Objective: Rutin, a potent antioxidant, has been reported to reduce the risk of ischemic disease. Our study aims to prepare rutin-encapsulated-chitosan nanoparticles (RUT-CS-NPs) *via* ionic gelation method and determine its results, based on different parameters *i.e.* surface morphology characterization, *in-vitro* or *ex-vivo* release, dynamic light scattering and differential scanning calorimetry (DSC), for treating cerebral ischemia.

Methods: UPLC-ESI-Q-TOF-MS/MS was used to evaluate the optimized RT-CS-NPs1 for brain-drug uptake as well as to follow-up the pharmacokinetics, bio-distrbution, brain-targeting efficiency and potential after intranasal administration (i.n.).

Key findings: A particle size of <100 nm for the formulation, significantly affected by drug:CS ratio, and entrapment efficiency and loading capacity of 84.98% ± 4.18% and 39.48% ± 3.16%, respectively were observed for RUT. Pharmacokinetics, bio-distribution, brain-targeting efficiency (1443.48 ± 39.39%) and brain drug-targeting potential (93.00 ± 5.69%) showed enhanced bioavailability for RUT in brain as compared to intravenous administration. In addition; improved neurobehavioral activity, histopathology and reduced infarction volume effects were observed in middle cerebral artery occlusion (MCAO) induced cerebral ischemic rats model after i.n. administration of RUT-CS-NPs.

Conclusion: A significant role of mucoadhesive-RT-CS-NPs1 as observed after high targeting potential and efficiency of the formulation prove; RUT-CS-NPs are more effectively accessed and target easily the brain.

1. Introduction

The third largest neurological disease resulting long term disability worldwide and a cause of morbidity and mortality is considered to be Stroke [1–3,38]. Cerebral ischemia, a reason for stroke, generally causes permanent deterioration of the central nervous system (CNS) [4]. It is believed that once cerebral ischemia is developed, it's the determinant factor (released due to inflammatory process stimulation) which leads towards serious cerebral damage alongwith morbidity and mortality [3,5,38]. The literature cited here reports; alongwith aforementioned causes, oxidative stress also plays an important role in cerebral ischemia reperfusion injury. The strive for high amount of oxygen consumption after oxidative stress leads to production of free radicals and reactive oxygen species (ROS) acting as a source of injury [1,2,6]. Due to improper developed antioxidant defense mechanism in brain, oxidants or free radicals release from inflammatory cells intimidates tissue viability in the vicinity of ischemic core resulting a good source of pathogenesis for ischemic-reperfusion [1,7]. Hence it indicates that pharmacological modification for treating oxidative damage may be an important part of management.

Neuroglobin (Ngb), newly discovered globin, have been reported with protection as offered against hypoxic/ischemic cell

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injury and thus plays a role in brain protection [57–63]. According to research hypoxic/ischemic injury results in accumulation of reactive oxygen (ROS) and nitrogen (RNS) species which were effectively encountered by Ngb hence improve neuronal cell survival [57]. Ubiquilin-1 (biomarker) as applied in RNS and ROS can oxidize different proteins which lead to misfolding as well as changes in three dimensional structures of protein after MCAO insult brain [64].

Numerous antioxidant drugs *i.e.* Thymoquinone, Curcumin, Ropinirole, Thioperamide with well-established mechanisms *i.e.* reducing ROS-mediated reactions and rescue the neurons from reperfusion-induced neuronal loss in animal models of cerebral ischemia have been reported [1,8–10]. Recently, research studies have reported Rutin (found in *Carpobrotus edulis* and *Ruta graveolens*) a lipophilic drug to be a potent source of treatment in cerebral ischemia [11–15] but still different factors exists as hindrance while using Rutin *i.e.* low water solubility and hence less bioavailability, chemical and enzymatic degradation in gastrointestinal tract due to lipophilic nature thus posing permeability complications and extensive hepatic first pass metabolism [16–20]. Likewise, another important factor posing problems related to drug discovery and development of an effective formulation in order to treat and manage cerebral ischemia is blood brain barrier (BBB) *i.e.* a protective natural barrier surrounding the central nervous system (CNS) that hinders the delivery of drugs to the brain. Even the most powerful drug-delivery systems (DDS) *i.e.* Intravenous, buccal and transdermal route are unable to affect BBB [21,22]. Hence different magnetic drug targeting and drug carrier systems approaches *i.e.* liposomes, antibodies, nanoemulsions, nanoparticles (NPs) or nanomicelles have been adopted to overcome the aforementioned problems. Amongst aforementioned approaches, Nanoparticles preparation may be best utilized to prepare controlled and targeted delivery system for drugs with different nature *i.e.* hydrophilic, hydrophobic natural and synthetic drugs, vaccines, proteins as well as biological macromolecules [23,24]. Similarly, out of the available route of drug administrations for brain targeting, Intranasal route (i.n) showed numerous advantages *i.e.* significant amount of drug transportation into cerebrospinal fluid (CSF) and olfactory bulb *via* olfactory sensory neurons, noninvasiveness, effective, safe and painless route, less skilled required for drug delivery as well as property of localized therapeutic effect with less side effects observed [1,2,25].

In our study we utilized the same approach of i.n drug delivery system and brain targeting in order to achieve therapeutic goals *via* eliminating different factors which can lead to less amount of Rutin access in brain *i.e.* avoiding first pass metabolism and distribution to non-targeted sites and hence reducing the peripheral side effects. Thus, Rutin loaded Mucoadhesive polymeric nanoparticles (NPs) were prepared in order to enhance the nasal residence, slow release of drug supply to brain at a constant rate (problems encountered with convention i.n route of drug delivery) [2,26,27]. The other major objective for this study is; determination of Rutin loaded NPs pharmacokinetics in brain and plasma after i.n and i.v. administration in Wistar rats and hence evaluate direct nose-to-brain transport pathway.

2. Materials and methods

Chitosan (medium mol. wt. chemical with degree of acetylation of 85%), Sodium tripolyphosphate (TPP) and cellophane tube with specifications; cut off mol. wt., 12,000 Da, flat (25 mm), diameter (16 mm) and capacity (60 mL/ft) alongwith LC–MS grade solvents *i.e.* Acetonitrile, Methanol and Formic acid were obtained from Sigma-Aldrich (St Louis, MO). Acetic acid (glacial) was obtained from IOL Chemical Ltd. (Mumbai, India) whereas Methanol, Ethanol, sodium hydroxide (NaOH), Potassium dihydrogen phosphate and 1-octanol were provided by SD Fine Chemicals, Ltd. (Mumbai, India).

2.1. Nanoformulation development of chitosan nanoparticles (ionic gelation method)

Chitosan nanoparticles (CS-NPs) were prepared *via* ionic gelation techniques as reported, Calvo et al., Aktas et al. [28,29] Placebo NPs were prepared initially which was attained as; dropwise addition of aqueous solution of TTP (0.15%) with a solution of CS (0.15%) while continuously stirring at room temperature. The mechanism behind formulation of placebo CS-NPs was ionic interaction between positively charged amino group form CS and negative groups of TTP, the final ratio for which was established on the basis of preliminary studies. The same procedure was utilized for the preparation of RUT-loaded CS-NPs, keeping the ratio for CS/TTP unchanged while the ratio for RUT was varied in order to observe the effect of initial RUT concentration upon the characteristics as well as the *in-vitro* release profile of NPs. Followed by; centrifugation for 30 min at 4000 rpm and 4 °C and collection of CS-NPs after supernatant is discarded.

2.2. Characterization of nanoparticles

2.2.1. Particle size and zeta-potential measurements

For the determination of particle size a Nano-series Zetasizer (Nano-ZS, HAS 3000, Malvern Instruments Ltd., Worcestershire, UK), based on the principle of photon correlation spectroscopy, was used. In detail, for the determination of zeta potential; all the samples (NPs) were diluted properly with Milli-Q water (the dispersant dielectric constant value for water set as 78.5) and the electrophoretic mobility was obtained at 25 °C which was calculated finally with the help of DTS (version 4.1) software from (Malvern, Worcestershire, UK).

2.2.2. Transmission electron microscopy (TEM)

The surface morphology for prepared CS-NPs was determined with the help of TEM (Morgagni 268D; FEI Company, Hillsboro, OR). In detail; one drop of nanosuspension was put on a paraffin sheet successively followed by covering with a copper grid, keeping for a time period of one (01) minute in order for the NPs to stick and at the end keeping the grid for a time of >5 s on one drop of phosphotungstate. The samples, after clearing the remaining solution with the help of filter paper, were air dried and observed again with TEM.

2.2.3. Scanning electron microscopy (SEM)

The surface texture for the optimized RUT-CS-NPs was confirmed with the help of SEM (Zeiss EVO40; Carl Zeiss, Cambridge, UK). In detail; the sample was spread on a double-sided conductive tape successively followed with stucking, under high vacuum with gold, in SCD020 Blazers sputter coater unit (BAL-TEC GmbH, Witten, Germany) where the environment was already maintained with argon gas (50 mA) for 100 s.

2.2.4. Determination of the loading capacity, encapsulation efficiency and process yield of CS-NPs

Ultracentrifugation, 30 min at 4 °C and 15,000 rpm, is used to estimate the entrapment efficiency (EE) and loading capacity (LC) of NPs. The developed UPLC–MS/MS method; acetonitrile (85%): 2 mM ammonium formate (15%): formic acid (0.1%) v/v/v with flow rate, 0.25 mL/min, was used to estimate and validate the free amount of rutin in supernatant. The following equation, after triplicate measurements, is used to calculate EE and LC for developed NPs:

$$EE(\%) = (\text{totaldrug-freedrug})/\text{totaldrug} \times 100$$