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Optimization of brain targeted chitosan nanoparticles of Rivastigmine for improved efficacy and safety

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ABSTRACT

The study aims at formulation and optimization brain targeted nanoparticles (NP) of Rivastigmine (RT) to improve its therapeutic potential and to verify its safety profile. The NP were optimized using a two factor three level (3^2) central composite design aiming to minimize particle size; maximize zeta potential and drug entrapment efficiency of NP. The optimized formulation (cRTNP) was evaluated using *in vitro* drug release study; *in vivo* behavioral, and biochemical and maximum tolerated dose (MTD) study. The optimized formulation evidenced a significant reversal of scopolamine-induced amnesia by Tween 80® coated nanoparticles as compared to both pure RT as well as uncoated nanoparticles. The MTD of RT was increased by 10% by formulating them as cRTNP. Thus, formulation of RT as cRTNP improved the therapeutic and safety profile of RT.

1. Introduction

Drug delivery to brain is a challenge for formulation scientists. Two physiological barriers, *i.e.*, blood–brain barrier (BBB) and blood–cerebrospinal fluid barrier, restrict the transport of bioactive compounds to brain [1] and render them therapeutically ineffective. The efficacy of a central nervous system (CNS) acting drug depends on the ability of the drug to cross such physiological barriers so as to show its therapeutic effect [2]. Many CNS drugs are administered at high doses for their therapeutic efficacy, causing peripheral side effects [3]. Approximately 98% of small molecules and nearly all large molecules with molecular weight > 1000 Da do not cross BBB in pharmacologically active concentration [1].

Rivastigmine (RT), a reversible cholinesterase inhibitor, is used in management of Alzheimer's disease. Absolute bioavailability of RT after a 3-mg dose is approximately 36% [4]. It has limited entry to brain owing to its hydrophilic nature. This restricted entry into brain necessitates frequent dosing and cholinergic side effects such as severe bradycardia, nausea, dyspepsia, vomiting and anorexia [6,7]. The targeted delivery of RT to brain may improve its therapeutic efficacy.

Nanoparticulate drug delivery is a promising strategy to improve the entry of drugs across physiological barriers such as BBB. The nanoparticles have the ability to interact cellular functions in new way owing to their tiny size, tailored surface, better solubility, multi-functionality, ability to persist in the circulatory system and accumulate at the target site [1,2].

Chitosan is a promising polymer for preparation of biodegradable and biocompatible nanoparticles [8]. Some workers have formulated RT loaded chitosan nanoparticles. Wilson et al. formulated chitosan NP containing RT by spontaneous emulsification method [5] using toluene and glutaraldehyde. According to the Occupational and Health Safety Administration (OSHA), exposure to toluene may adversely affect the health specially, CNS. The exposure to glutaraldehyde has been reported to cause respiratory irritation, corneal opacity, corneal injury, conjunctivitis, irritation and necrosis in eye irritation test. Necropsy findings depicted adverse effects of glutaraldehyde on stomach, intestine, lungs, liver, spleen, kidney and adrenal glands when tested on mice and rats [9]. In human, glutaraldehyde may irritate skin, eye, and respiratory tract. Glutaraldehyde is reported as mutagenic in several short-term genotoxicity assays [9].

Fazil et al. formulated RT encapsulated chitosan NP for intranasal delivery [6]. Using intranasal brain drug delivery, the concentration achievable in different regions of the brain and spinal cord, may vary with each agent. Moreover, some therapeutic agents may be susceptible to partial degradation in the nasal mucosa or may cause irritation to the mucosa. Nasal congestion may hamper this method of delivery and its frequent use results in mucosal damage.

Keeping in view of the above factors, the present investigation was designed to develop biodegradable and biocompatible chitosan NP avoiding use of hazardous organic solvents. The selected route

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of administration was intravenous drug delivery. Such nanoparticles may improve brain uptake, provide sustained drug release, reduce its frequent dosing, avoid distribution to non-targeted sites and thus minimizes peripheral side effects. The nanoparticles were optimized systematically using design expert software (DES) for optimum concentration of polymer and ligand Tween 80® (T80) with an aim to achieve desired particle size, zeta potential (ZP) and entrapment efficiency. Further, the pre-clinical efficacy and safety of ligand coated nanoparticulate drug delivery system was studied. The cumulative maximum tolerated dose (MTD) was determined for nanoparticles loaded with RT and compared with MTD of free RT. According to IUPAC Compendium of Chemical Terminology, MTD is the highest dose used in chronic toxicity testing that is expected on the basis of an adequate sub-chronic study to produce limited toxicity when administered for the duration of the test period. It should not induce overt toxicity or 10% or greater retardation of body weight gain as compared with control animals.

2. Materials

RT and chitosan were obtained as a gift sample from Sun Pharmaceutical Industry Ltd. (Mumbai), and Central Institute of Fisheries Technology, Kochi, India, respectively. Other chemicals were of suitable analytical grade and were used as received.

Young Swiss albino mice (25–30 g, 6 week old) and young male Wistar rats (230–250 g, 8 week old) were procured from Disease Free Small Animal House, LLRUVAS, Hisar (Haryana), India. Animals were acclimatized to laboratory conditions before experimentation. All the experiments were carried out between 08:00 am and 04:00 pm. Only male mice were used in the study because estrogens (female sex hormones) have been reported to affect memory [10]. Efforts were made throughout to minimize animal discomfort and to use the minimum number of animals ($n = 6$) required for statistical significance. The experimental protocol was approved by Institutional Animals Ethics Committee (IAEC) and animal care was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India (Registration no. 0436).

3. Methods

3.1. Preparation of NP

The chitosan NP were prepared by modified ionotropic gelation method [11]. Limits and ranges for chitosan and T80 for the optimization studies were selected based on our previous findings [12,13] using a two factor three level central composite design (CCD) and are summarized in Table 1. To produce high yields of stable and solid nanometric structures, the CS/TPP weight ratio should be within the range 3:1–6:1 [14]. The particle size of CS nanoparticles increases with increase in CS concentration [15]. In order to get the minimum particle size with maximum yield, CS:TPP::3:1 was selected. The chitosan (CS) and tri-polyphosphate pentasodium (TPP) were combined electro-statically entrapping the drug followed by coating with T80. Briefly, to specified concentration of CS solution in 2% acetic acid solution (pH 5.5–5.7) containing drug (100 mg), an aqueous solution of TPP (CS:TPP::3:1) was added drop wise and stirred for 1 h using magnetic stirrer. Centrifugation of the dispersion was carried out at 15,000 rpm for 60 min at 10 °C to get pellets containing required NP [6,12,13]. For coating, all the pellets were washed, re-dispersed in phosphate-buffered saline pH 7.4 at a concentration of 10 mg/ml under constant stirring and sonicated. Then the required volume of Tween 80® was added to obtain a final solution of requisite concentration of Tween 80®, and the mixture was incubated for 30 min. 2 ml of this suspension was further diluted 10 times, sonicated and used for the determination of particle size, polydispersity index (PDI) and ZP. The undiluted NPs were lyophilized using lyophilizer (Alpha 2–4 LD Plus CHRIST, Germany) after adding D-mannitol.

Table 1
Composition of various chitosan–TPP nanoparticle formulations prepared as per experimental design.

Formulation code	Trial no.	Coded factor levels	
		X_1	Tween 80® (%)
7	1	0	−1
12	2	0	0
5	3	−1	0
1	4	−1	−1
10	5	0	0
13	6	0	0
6	7	1	0
11	8	0	0
8	9	0	1
3	10	−1	1
2	11	1	−1
4	12	1	1
9	13	0	0
Translation of coded levels in actual units			
Coded level	−1	0	+1
X_1: Chitosan (%)	0.05	0.15	0.25
X_2: Tween 80® (%)	1	1.5	2

3.2. Experimental design

A CCD (with $\alpha = 1$) using three levels each of the two factors factor X_1 (*i.e.* percent CS concentration; w/v) and X_2 (*i.e.* percent T80 concentration; w/v), were adopted for further investigations as required by the design, and the factor levels were suitably coded. Table 1 summarizes the 13 experimental runs studied employing different levels of the two factors.

3.3. Determination of particle size, PDI and ZP

The average hydrodynamic diameter (particle size), PDI and ZP of the formulated NP were determined by dynamic light scattering (DLS) analysis using ZetaSizer Nano ZS90 (Malvern Instruments Limited, UK) equipped with a 4.0 mW He–Ne laser operating at 633 nm. All measurements were carried out after dispersing the NP in appropriate volume of HPLC grade water, at detection angle of 90° at 25 °C (for size and PDI) and 120° at 25 °C (for ZP).

3.4. Percent drug entrapment efficiency (%DEE)

The supernatant after centrifugation was collected, filtered through 0.45 μm filter, and amount of drug present was determined by UV spectrophotometer ($\lambda_{max} = 205$ nm). A standard calibration curve of concentration *versus* absorbance was plotted for this purpose. The amount of drug in supernatant (w) was then subtracted from the total amount of drug added (W, 100 mg in this case) and %DEE was calculated [12,13].

3.5. Optimization data analysis

DES ver.8.0.7.1 (Stat-Ease, Minneapolis, MN, USA) was employed to fit full second order polynomial equations with added interaction terms to correlate the studied responses with the examined variables. The response variables for systematic optimization were particles size, ZP and %DEE. The optimum formulations' prognosis was conducted by locating feasible space and secondly, an exhaustive grid search was conducted to obtain the