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Thermo-sensitive gels containing lorazepam microspheres for intranasal brain targeting

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ABSTRACT

Thermo-sensitive gels containing lorazepam microspheres were developed and characterized for intranasal brain targeting. Pluronics (PF-127 and PF-68) have been selected since they are thermo-reversible polymers with the property of forming a solution at low temperatures (4–5 °C), and a gel at body temperature (37 °C). This property makes them an interesting material to work with, especially in case of controlled release formulations. The present study focuses on the development of an intranasal formulation for lorazepam, as an alternative route of drug delivery to the brain. Direct transport of drugs to the brain circumventing the brain barrier, following intranasal administration, provides a unique feature and better option to target brain. The presence of mucoadhesive microspheres in the gel vehicle via nasal route can achieve a dual purpose of prolonged drug release and enhanced bioavailability. To optimise the microsphere formulation, Box Behnken design was employed by investigating the effect of three factors, polymer concentration (chitosan), emulsifier concentration (Span 80) and cross-linking agent (glutaraldehyde) on the response variable which is the mean particle size. The concentration of 21% PF-127 and 1% PF-68 were found to be promising gel vehicles. The results showed that the release rate followed a prolonged profile dispersion of the microspheres in the viscous media, in comparison to the microspheres alone. Histopathological studies proved that the optimised formulation does not produce any toxic effect on the microscopic structure of nasal mucosa.

1. Introduction

Lorazepam, a benzodiazepine derivative is the specific drug of choice used widely in the treatment of status epileptics. *Status epilepticus* is a neurological disorder, which is defined as prolonged seizures or a period of repeated seizures without restoration of normal consciousness in between, lasting for more than 30 min. It requires quick management of seizures to avoid the risk of permanent brain damage (Shafique et al., 2006). Intravenous and rectal administration of diazepam is commonly used as first-line anticonvulsant agents in the emergency treatment of seizures in children (Cock and Scaphira, 2002). The problem associated with diazepam treatment is seizure recurrence, requiring repeated doses. This can lead to saturation of peripheral compartment and unpredictable rise in serum levels.

Lorazepam is preferred over diazepam as it combines improved seizure outcome, less respiratory depression, rapid onset with prolonged duration of antiepileptic action (Cock and Scaphira, 2002) Currently, lorazepam is available as tablet formulations and injectable dosage forms. These formulations release lorazepam into the peripheral circulation resulting in limited drug uptake across the BBB and drug distribution to non targeted sites (Tushar et al., 2005). Although intravenous administration provides rapid seizure suppression, an alternative route of drug delivery is needed, since oral and intravenous routes for delivering drugs are sometimes impractical and/or inconvenient.

The present paper aimed to assess a drug and delivery system that is potentially more effective, safer, and easier to administer than those presently in use. Intranasal lorazepam is effective, safe, and provides a less invasive alternative to intramuscular paraldehyde in children. The ease of use of this drug makes it an attractive and preferable pre-hospital treatment option. By intranasal route a wide variety of therapeutic agents including both small molecules and macromolecules can be successfully delivered to the central

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nervous system (CNS). When a nasal drug formulation is delivered deep and high enough into the nasal cavity, the olfactory mucosa may be reached and drug transport into the brain and/or cerebrospinal fluid (CSF) occurs via the olfactory receptor neurons (Lisbeth, 2003, 2012).

Microsphere technology is one of the specialized systems becoming popular for designing nasal products, as it may provide prolonged contact with the nasal mucosa and thus enhances absorption and bioavailability (Vidgren et al., 1992). This is particularly relevant to overcome certain limitations of the nasal route, especially in the delivery of drugs with high molecular weight and hydrophilic properties (Ozsoy et al., 2009; Ali et al., 2010). The present study focuses on the deposition of particles on the olfactory epithelium. To be safe, the particle size should be controlled and optimised in the range of 6–15 μm, so as to minimize the deposition of the particles in the anterior part of nasal cavity and also to the lungs (Grassin-Delyle et al., 2012). The rationale behind the use of microspheres is that, the application of bioadhesive microspheres (in the powder form) with good bioadhesive properties would allow such systems to swell in contact with nasal mucosa to form a gel and control the rate of clearance from the nasal cavity, thereby giving poorly absorbed drugs, a longer time to be available at the absorptive surface. The remaining issue is the prolongation of the duration of activity of lorazepam unfortunately, has a narrow therapeutic index. Many ideas have been proposed to prolong the activity of drug delivery systems, e.g. surface extraction, surface coating, and non-uniform drug loading, surface modification (Frederic et al., 2005). Unfortunately, the solutions proposed will involve costly additional steps or are not simple to implement. Instead of modifying the formulation, the idea proposed in the present study was to use thermo-sensitive gels to disperse the microspheres prior to instillation.

The suspension media was chosen for their biocompatibility and their ability to become highly viscous at 37 °C, but not too viscous at 20 °C, to allow a reliable suspension of the microspheres to instil into the intranasal space. Microspheres embedded hydrogel showed a sustained release of drug, as well as additional properties such as thermo-sensitivity and biocompatibility due to the presence of both chitosan and Pluronics moieties.

A Box Behnken design (BB design) was used to investigate the effect of three factors (polymer concentration as X_1, emulsifier concentration as X_2 and cross linking agent as X_3) on the response variables Y_1 (particle size in micron). The dependant variable was the mean particle size of the chitosan microspheres. Response surface methodology using a Box Behnken design was chosen because it allows the determination of influence of the factors on particle size of the microspheres with a minimum number of experiments.

2. Materials and methods

2.1. Materials

Lorazepam was a gift sample from Strides Acro Labs, Bangalore. Chitosan was a gift sample from CIFT–Cochin. Pluronics (PF-127 and PF-68) were purchased from Sigma Chemical Co., (St. Louis, MO, USA). Glacial Acetic acid, light liquid paraffin, and Span 80 were purchased from Central Drug House Laboratory, New Delhi. Gluteraldehyde (25%, v/v), petroleum ether, acetone, ethanol, sodium hydroxide were from Nice Chemicals, Cochin. All other chemicals and solvents were of analytical reagent grade and were used without further purification.

2.2. Compatibility studies

The compatibility studies were carried out at room temperature by Fourier transform infrared (FTIR) spectroscopy to determine the interaction of lorazepam with other excipients used in the formulation. The IR spectra of drug alone and in combination with pluronics (Pluronics F 127 and Pluronics F 68) and chitosan were taken. Physical mixtures of the drug with excipients in the ratio of 1:1 were prepared and the samples were analysed by Shimadzu IR Spectra Analyzer (Shimadzer, Japan).

2.3. Preparation and evaluation of chitosan microparticles

2.3.1. Preparation of chitosan microspheres

The chitosan microspheres were prepared by emulsion cross-linking method (Hetal and Murthy, 2008). Chitosan solution (2%, w/v) was prepared in 4% aqueous glacial acetic acid by overnight stirring in a magnetic stirrer. The drug was dissolved in ethanol and mixed well in the polymer solution. 6 ml of the above resultant mixture was then injected through a syringe (no. 23) into 40 ml of oil phase containing span 80 (7%, v/v) and stirring was performed by mechanical stirrer at 1500 rpm to form w/o emulsion. Oil phase was light liquid paraffin. After 30 min of homogenization period 1.0 ml of glutaraldehyde 25% (v/v) was added to it stage by stage. It was then left for stabilization and cross-linking for a period of 3 h. Microspheres obtained were centrifuged at 4000 rpm. The sediment was then washed with petroleum ether and acetone thrice, and then dried in a hot air oven at 50 °C.

2.4. Evaluation of chitosan microspheres

2.4.1.1. Shape and surface morphological analysis

The shape and surface morphology of the chitosan microspheres were studied with the aid of scanning electron microscope. Chitosan microspheres were fixed with carbon tape and mounted on metal stubs and then coated with platinum, keeping the acceleration voltage at 10 kV. Photographs were taken using Jeol JSM-6390 (Jeol, Japan) scanning electron microscope.

2.5. Characterization of prepared microspheres

2.5.1. Fourier transform infrared

FTIR spectra of prepared cross-linked and non-cross linked microspheres were recorded on Perkins-Elmer AX-1 spectrophotometer (Perkin-Elmer, Singapore) in KBr disc and reported as wave number (cm^{-1}).

2.5.2. Differential scanning calorimetry (DSC)

The thermo analytical examinations were carried out with a differential scanning calorimeter equipped with a thermal analysis data system (Perkin-Elmer DSC7 calorimeter, Perkin-Elmer Inc. Wellesley, MA, USA). Samples weighing 3–5 mg were heated in flat-bottomed sealed aluminium pans over a temperature range of 25–300 °C at a constant rate of 10 °C/min under nitrogen purge of (50 ml/min) using empty aluminium pan as reference (Hekmatara et al., 2006; Raghavendra et al., 2009).

2.5.3. Powder X-ray diffraction analysis

XRD powder method was applied to characterize the drug substance and the microspheres (Zhuzhu et al., 2007; Barbara, 2008; Tapan and Biswanath, 2010). The diffraction patterns of the lorazepam powder, blank microspheres and drug loaded microspheres were conducted with a X-ray powder diffractometer (Brucker D Advance, Germany), using a copper Kα target with a nickel filter at 40 kV voltage, 30 mA current and at scanning speed of 1°/min over a 2θ range of 0–50°.