JYP

Preparation, Characterization and *in vivo* Evaluation of Parenteral Sustained Release Microsphere Formulation of Zopiclone

Swapna N, Jithan AV

Department of Pharmaceutics, Vaagdevi College of Pharmacy, Warangal -506 001, Andhra Pradesh, India

Address for correspondence: *Dr. A.V. Jithan; E-mail: aukunjv@gmail.com*

ABSTRACT

The aim of this study was to prepare zopiclone-loaded polycaprolactone microspheres by emulsion solvent evaporation technique with different drug-to-carrier ratios {MP 1 (1:1), MP 2 (1:2), MP 3 (1:3), and MP 4 (1:4)}, characterize and evaluate the *in vivo* performance. The microspheres were characterized for particle size, surface morphology, drug excipient compatibility, percentage yield, drug entrapment, and *in vitro* release kinetics. Pharmacokinetics and pharmacodynamics were evaluated after parenteral administration so as to determine the sustained action of the drug after one-time administration of the formulation in a rat model. Of four formulations prepared, MP 2, i.e., 1:2 (drug–polymer) ratio was selected as the optimized formulation based on particle size, particle shape, and the release behavior. The size of microspheres was found to be ranging from 5.4 to 12.1 µm. The shape of microspheres was found to be spherical by SEM. Among the four formulations, MP 2 (1:2) showed maximum percentage yield of 75% ± 2.68%. There was no interaction between drug and polymer by FT-IR study. In the *in vitro* release study, formulation MP 2 (1:2) showed 86.5% drug release and was found to be sustained for 10 days. The microsphere formulations were able to sustain the release of drug both *in vitro* and *in vivo*. Pharmacodynamic study (Maze apparatus) indicated that the anxiolytic activity shown by zopiclone microspheres was significant when compared to the zopiclone solution given daily.

Key words: *In vitro* release, microspheres, pharmacodynamics, pharmacokinetics, polycaprolactone, zopiclone

DOI: 10.4103/0975-1483.66792

INTRODUCTION

Zopiclone is a non-benzodiazepine, cyclopyrrolone derivative[1] with a chemical name [6-(5-chloropyridin-2-yl)-5-oxo-7*H*-pyrrolo[3,4-b]pyrazin-7-yl]4-methylpiperazine-1-carboxylate. Zopiclone exerts its action by binding on the benzodiazepine receptor complex[1] and modulation of the $GABA_BZ$ receptor chloride channel macromolecular complex. As it is non-benzodiazepine,[2] it is nonaddicting, less sedating with no physical, or physiological dependence. Most commonly seen side effects are taste alteration or dysgeusia (bitter, metallic taste, which is usually fleeting in most users), dry mouth, and headache. The therapeutic and pharmacological properties of zopiclone include anxiolytic, anticonvulsant, hypnotic, and myorelaxant properties. For these purposes, it is administered daily via the oral route. Such an administration lacks patient compliance and is very often associated with side effects because of crests and troughs in the plasma levels. On the other hand, a parenteral sustained release dosage form like biodegradable microspheres encapsulating zopiclone can lead to effective therapeutic levels and reduced side effects for this drug and thereby can enhance its therapeutic utility. Thus, in this study we aimed to develop a sustained release microsphere formulation for zopiclone using polycaprolactone as the biodegradable polymer. After preparation of microsphere

formulations using different drug–polymer ratios, the formulations were characterized for *in vitro* drug release, drug–polymer compatibility, size, and surface morphology.

The main objective of any drug therapy is to achieve a desired concentration of the drug in blood or tissue which is therapeutically effective and nontoxic for extended period of time, and this goal can be achieved by proper design of sustain release dosage regimen.[3,4] Thus, once we developed and characterized sustained release dosage forms using *in vitro* methods, we aimed to investigate its performance in an animal model. For this purpose, the developed microsphere formulations were administered via intraperitonial route, the pharmacokinetics and pharmacodynamics of the drug were investigated. The results are discussed.

MATERIALS AND METHODS

Materials

Polycaprolactone (average M_n: 60000; average M_w: 80,000; MP: 60 °C) was purchased from Sigma-Aldrich, Germany. Zopiclone was a gift sample from Natco Pharma, Hyderabad. Polyvinylalcohol was purchased from Qualigens Fine Chemicals (New Delhi, India). Dichloromethane was purchased from Finar reagents. To conduct *in vitro* drug release studies, magnetic stirrer and cyclo mixer from Remi Equipments Pvt. Limited were used. A SL 164 Elico Double Beam UV–Vis spectrophotometer was used to analyze the samples. For separation of plasma from blood, centrifuge (Remi industries) was used. HPLC of Cyberlabs was used to analyze the drug. Diethyl ether was obtained from Finar Chemicals, Ahmedabad. Maze apparatus was used to evaluate the anxiolytic activity. The protocol of all experiments was approved by the institutional animal ethical committee. Male Wistar rats (100–150 g, 5 to 6 weeks old) purchased from animal center of Mahaveera enterprises whose Registration No. is 146per1999perCPSCEA, Hyderabad were used in this study.

Preparation of microspheres

Microspheres of zopiclone using biodegradable polycaprolactone as the polymer were prepared by emulsion–solvent evaporation method. Four different formulations MP 1, MP 2, MP 3, MP 4 containing drug–polymer in the ratio of 1:1, 1:2, 1:3, and 1:4, respectively, were prepared. Dichloromethane (10 mL) was taken as organic phase in which polymer and drug were dissolved.[2] The drug content was always 100 mg in all the batches. The aqueous phase contained polyvinylalcohol PVA solutions in water at 2% w/v (30 mL). The organic phase was added to the aqueous phase drop by drop while the aqueous phase was kept for stirring on a magnetic stirrer. Stirring was continued till complete evaporation of dichloromethane occurred. As the organic phase evaporates, precipitation of the polymer and drug occurs due to which drug gets entrapped in the polymer and stirring results in size reduction as well as spherical particle formation. The microspheres, thus obtained were filtered through Wattmann filter paper number I, and the free unentrapped drug was also removed. Microspheres were air dried and used for further studies.

Physico-chemical evaluation of the microspheres

The microspheres prepared by emulsion solvent evaporation method[5] were evaluated for the following characteristics. The surface morphology and the internal textures of the microspheres were observed under scanning electron microscope. Particle size analysis was carried out using optical microscope ×40. About 50 particles were selected randomly, and their size was determined. Drug-excipients compatibility study[6] was carried out by the FTIR analysis of pure drug (zopiclone), pure polymer (polycaprolactone), microparticular system (zopiclone MS), and placebo microparticles (polycaprolactone MS). High-performance liquid chromatographic analysis was used for estimation of drug content.

In vitro release studies

The dialysis bag diffusion technique is widely used to evaluate drug release from micro- and nanosized carriers.[7] This technique with the help of a dialysis membrane was used in this study. This membrane was then carefully clamped to one end of the hollow glass tube and considered as the donor compartment. The dissolution medium phosphate buffer pH (7.4)—50 mL was taken into the receiver compartment. The donor compartment was immersed into the receiver compartment so that the edge just touches the receiver compartment. An aliquot of 100 mg of the microparticles were dispersed in 1% NaCMC in phosphate buffered saline (PBS) solution and placed in the donor compartment. The rpm of the system was maintained at 50 by using magnetic stirrer and bead. Samples (5 mL) were removed from the receptor compartment and replaced with fresh medium immediately. These were analyzed by spectrophotometric method at 303 nm wavelength.

Kinetics of drug release[3]

In order to understand the mechanism and kinetics of drug release, the result of the *in vitro* dissolution study of