BRAIN TARGETING OF FLUNARIZINE HYDROCHLORIDE BY SOLID LIPID NANOPARTICLES FOR PROPHYLAXIS OF MIGRAINE

Mandal Surjyanarayan*, Shah Pratik, Shah Gunjan, Khatri Doli, K. S. Rajesh
Parul Institute of Pharmacy, PASM, Lindia, Waghadia-391760, Vadodara, Gujarat, India

INTRODUCTION

Nanoparticles, a delivery system with high surface area to volume ratio, have been successfully used for the targeted delivery of hydrophilic as well as hydrophobic drugs. It is a common knowledge that the use of physiological lipids, the avoidance of organic solvents, a hydrophilic as well as hydrophobic drugs, and biodegradable materials and capable of incorporating nanoparticles (SLNs) are simple submicron colloidal drug delivery systems having particle size 50-1000 nm made up of biocompatible and biodegradable materials and capable of incorporating hydrophilic as well as hydrophobic drugs. Advantages of SLN are the use of physiological lipids, the avoidance of organic solvents, a potential wide range of drug loading capacity, and the high pressure homogenization as an established production method. Additionally, improved bioavailability, protection of sensitive drug molecules from the outer environment (water, light), control the release characteristics and even relatively higher intracellular uptake were claimed by incorporation of poorly water soluble drugs in the solid lipid matrix. In the last decade, significant effort has been made to develop nanoparticles for drug delivery. Poloxamers are used in a variety of oral, parenteral, and topical pharmaceutical formulations, and are generally regarded as nontoxic and nonirritant materials. Poloxamers are not metabolized in the body. So it has been the choice of polymer for the preparation of the Nanoparticles.

Oral route still remains the favorite route of drug administration in many diseases and till today it is the first way investigated in the development of new dosage forms. The major problem in oral drug formulations is low and erratic bioavailability, which mainly results from poor aqueous solubility. This may lead to high inter- and intra subject variability, lack of dose proportionality and therapeutic failure. Successful oral delivery of drugs has always remained a challenge to the drug delivery field because almost 40-50% of new drugs candidates have poor water solubility thus oral delivery is frequently associated with implications of low bioavailability. To overcome these bioavailability problems, various formulations strategies have been reported including use of surfactants, cyclodextrine inclusion complexes, solid dispersions, nanoparticles and absorption enhancers, but the possible formulations and their metabolic products are still to worth to be further investigated. High aqueous solubility and low bioavailability lead Flunarizine hydrochloride to comparatively less brain access. Hence, the objective of this study was to improve the solubility of Flunarizine hydrochloride by formulating into SLNs for the accomplishment of the drug into brain for the prophylaxis of migraine.

MATERIALS & METHODS

Flunarizine hydrochloride was obtained as gift sample from Zydus Pharmaceutical, Ahmedabad. Isopropyl myristate, Isopropyl palmitate, PEG 400, PEG 600, Tween 60, Methanol, Chloroform were purchased from Baroda Chemicals, Vadodara, Gujarat. Glycerine was purchased from Metro Golden Laboratories, SLS from QFC Fine Chem Industries. Poloxamer 188 (F-68) from Ozone International, Mumbai. All the reagents used were of analytical reagent grade.

Preparation of Solid Lipid Nanoparticle

Flunarizine hydrochloride loaded SLN (FSLN) was prepared by high speed homogenization technique using Pluronic F-68 polymer followed by lyophilization. For Phase 1, 20mg of Flunarizine hydrochloride was dissolved in Isopropyl myristate (4 %, v/v) and Tween 80 (30%, v/v). Poloxamer F-68 (60%, w/v) was added to the mixture of PEG 400 (6%) and water at constant speed of 100 rpm for 20 minutes to prepare Phase 2. Then Phase 1 was added dropwise in Phase 2 at high speed in order to produce nanosuspension of Flunarizine hydrochloride. The developed nanosuspension was then lyophilized using mannitol as cryoprotectant (1:1 w/v) to obtain solid lipid nanoparticle.
Interference Study

Interference study between lipid and drug in solvent [MeOH: CHCl₃ (6:4)] was performed by first derivative spectroscopy method (UV-Vis Spectrophotometer, Shimadzu). Standard drug solution (10 µg/ml) and lipid solution (100 µg/ml) was prepared. Then absorbance of standard drug solution in absence and presence of lipid was taken at 236.4 nm using MeOH: CHCl₃ (6:4) as a blank. The percent yield of the product was calculated by the following equation:

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\text{Product Yield} = \frac{\text{Weight of product}}{\text{Weight of drug and polymer}} \times 100
\]

Droplet size and Zeta potential analysis

Droplet size and zeta potential measurement of developed Flunarizine hydrochloride nanoparticles were carried out by dynamic light scattering technique through Zetasizer HAS 3000 (Malvern Instruments Ltd., Malvern, UK).

DSC analysis

DSC thermogram of Flunarizine hydrochloride showed an endothermic peak at 120°C corresponding to its melting temperature, which was not detected in thermogram of FSLNs. Thermogram of physical mixture (drug + lipid) showed endothermic peak of melting at 71.64°C for lipid and 122°C for drug. No melting endotherm of drug in FSLNs and no shifting of melting endotherm of drug in the physical mixture of drug and lipid were observed which indicates the compatibility between the drug and lipid. Moreover in FSLNs, the drug was present in the amorphous phase and homogeneously dispersed in the lipid matrix.

Drug content analysis

% drug content from the developed nanoparticle was analyzed spectrophotometrically (UV-Visible Spectrophotometer 1800, Shimadzu, Japan) at 248 nm after reconstituting the nanoparticle in distilled water.

Drug loading capacity

Free Drug content was analyzed by adding 100ml of propylene glycol with 30 mg of nanoparticles (=2.5 mg Flunarizine hydrochloride) and kept at room temperature for 24 hours. The above solution was filtered through Whatmann filter paper (0.45µm) and the filtrate was analyzed using spectrophotometrically (UV-Visible Spectrophotometer 1800, Shimadzu, Japan) at 248 nm. The residue on filter paper was then collected and dissolved in 100ml of distilled water and analyzed to find drug loading capacity by spectrophotometrically (UV-Visible Spectrophotometer 1800, Shimadzu, Japan) at 248 nm. After that the nanoparticles were centrifuged at 16000 rpm and the drug loading capacity was analyzed in supernatant liquid by UV-Visible spectrophotometer at 248 nm.

IN-VITRO DRUG RELEASE STUDY

In-vitro drug release study was carried out using dialysis bag (12,000 D) in a Modified Dissolution Apparatus equilibrated at 37±2°C. The dialysis bag was soaked overnight in the dissolution medium i.e. distilled water. One end of the dialysis bag was tied and 30 mg of nanoparticles (=2.5 mg Flunarizine hydrochloride) was put into it followed by tying up of another end. The dissolution bag was then immersed in 250ml of dissolution medium and the process was carried out at 37±2°C and at 50 rpm. 5ml of sample was withdrawn at 0, 0.5, 1, 2, 3, 4, 6, and 8 hours, replaced with same volume of dissolution medium and was analyzed in triplicate by UV-Visible Spectrophotometer 1800 (Shimadzu, Japan) at 248 nm after required dilutions.

EX-VIVO DRUG RELEASE STUDY

Ex-vivo drug release study was carried out using rat ileum in a Modified Dissolution Apparatus equilibrated at 37±2°C. One end of the rat ileum was tied and 30 mg of nanoparticles (=2.5 mg Flunarizine hydrochloride) was put into it followed by tying up of another end. The rat ileum was immersed in 250ml of dissolution medium i.e., simulated intestinal fluid and the process was carried out at 37±2°C and at 50 rpm with constant aeration. 5ml of sample was withdrawn at 0, 0.5, 1, 2, 3, 4, 6, and 8 hours, replaced with same volume of dissolution medium and was analyzed spectrophotometrically in triplicates at 248 nm after required dilutions.

RESULTS AND DISCUSSION

The average particle size was found to be 282±50nm with Pdl=0.424±0.028 as shown in Figure 1, suggests that the particle size data is significant. Zeta potential of 29.4±1.24mV indicating the better stability of the developed formulation. DSC analysis clearly showed that there was no interaction between the lipid and the drug as shown in the Figure 2. That is the fact why Poloxamer F-68 was selected for the preparation of FSLN.

Drug content and drug loading capacity were found to be 98.9±0.86% and 62±0.87% respectively. In-vitro release results were found to be 73.7±1.9% in 8 hours as shown in Figure 3. Ex-vivo drug release result revealed that 65.26±1.1% of drug was released in 8 hrs and the release of Flunarizine hydrochloride from the formulation is more and sustained as compare to the Plain Drug Solution which released 52% of drug in 8 hrs as shown in Figure 4. Again more than 20% decrease in the percentage cumulative release in the ex-vivo study compared to in vitro may be due to the deposition of Flunarizine hydrochloride in the intestinal wall of the rat ileum.

CONCLUSION

The developed nanoparticles through high speed homogenization technique were prepared with a view to get the sustain release of the Flunarizine hydrochloride following oral administration which may help to improve the patient compliance. The developed nanosuspension found to be physically stable with particles in nano range. Results from the investigation suggested that the Flunarizine hydrochloride solid lipid nanoparticles represent a promising dosage form for oral drug delivery for the prophylaxis of migraine as compare to the conventional dosage form which might also reduce the side effects associated with the conventional dosage form. However, the findings of this investigation can only be settled after animal models experimentation followed by an extensive clinical evaluation.

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REFERENCES


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