DEVELOPMENT OF MUCOADHESIVE MICROSPHERES FOR NASAL DELIVERY OF SUMATRIPTAN

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Accepted on: 10-02-2011; Finalized on: 10-04-2011.

ABSTRACT

In the present work mucoadhesive nasal microspheres containing sumatriptan were formulated and evaluated with the aim of increasing its bioavailability. Microspheres composed of Chitosan were obtained using emulsification cross linking method with different polymer: drug ratios. The prepared microspheres were evaluated for physical characteristics such as particle size, particle shape, surface morphology, drug entrapment efficiency, in vitro mucoadhesion, ex-vivo drug permeation studies and in vivo bioavailability. The particle size of the prepared microspheres was in the range of 46-68 μm, which was suitable for nasal administration. SEM analysis of the microspheres revealed that all the prepared microspheres possess similar surface morphology. The ex-vivo permeation of sumatriptan succinate from chitosan microspheres exhibited a biphasic diffusion mechanism. Initially the microspheres showed immediate permeation of drug due to the diffusion of non-encapsulated drug from the surface of microspheres followed by much slower diffusion of entrapped drug. In-vivo studies showed increased relative bioavailability with nasal route as compared to the oral route.

Keywords: Sumatriptan, Bioadhesion, Chitosan, Bioavailability.

INTRODUCTION

Nasal drug delivery has frequently been proposed as the most feasible alternative to parenteral injections. This is due to the high permeability of the nasal epithelium, allowing a higher molecular mass cut-off at approximately 1000 Da, and rapid drug absorption rate with plasma drug profiles sometimes almost identical to those from intravenous injections1. Conventionally, the nasal route has been used for delivery of drugs for treatment of local diseases such as nasal allergy, nasal congestion and nasal infections. Recent years have shown that the nasal route can be exploited for the systemic delivery of polar drugs having low molecular weight peptides and proteins that are not easily administered via other routes than by injection. From the pharmacokinetic standpoint, absorption is rapid which provides a faster onset of action compared to oral and intramuscular administration. Hepatic first-pass metabolism is also avoided, allowing increased, reliable bioavailability2. Sumatriptan is an agonist for a vascular 5-hydroxytryptamine receptor subtype (probably a member of the 5-HT1D family)3. The vascular 5-HT1-receptor subtype that sumatriptan activates is present on the human basilar artery, and in the vasculature of human dura mater and mediates vasoconstriction, this action in humans correlates with the relief of migraine headache. Sumatriptan has previously been shown to have a low oral bioavailability in human volunteers (15%), the intranasal route may be a viable alternative for self-administration where this limitation could be overcome. The problem associated with nasal delivery of sumatriptan solution is lower retention of solution in nasal cavity (15min) resulting in lower bioavailability. To overcome the rapid clearance and to facilitate the absorption, in the present study mucoadhesive polymer chitosan is used as a carrier to formulate microspheres. Chitosan being positively charged is able to interact strongly with the nasal epithelial cells and the overlying mucous thereby providing a longer contact time for drug transport across the nasal membrane, in addition it increases the Paracellular transport of polar drugs by transiently opening the tight junctions between the epithelial cells.

MATERIALS AND METHODS

Chemicals

Sumatriptan succinate was kindly given by Natco Pharma Ltd, Hyderabad, Chitosan was provided by India sea foods, Cochin, heavy liquid paraffin, light liquid paraffin, hexane, glutaraldehyde, acetic acid, dioctoyl sodium sulphosuccinate was provided by SD fine chemicals, Mumbai. All other solvents and chemicals used were of analytical grade.

Animals

New Zealand white rabbits weighing around 2-2.5 kg were used. They were maintained in a clean room. Animal ethical committee of K.L.E.S's College of Pharmacy Belgaum approved the protocol of this animal study.

Method

A series of batches of microspheres were prepared by simple emulsification cross linking method to optimize the parameters like stirring rate, surfactant concentration (dioctoyl sodium sulphosuccinate) and volume of crosslinking agent, glutaraldehyde. After optimization of these parameters, six batches were prepared namely SM-1, SM-2, SM-3, SM-4, SM-5 and SM-6 using drug polymer
ratio of 1:1, 1:1.5, 1:2, 1:2.5, 1:3 and 1:3.5 respectively. The detailed procedure follows: Chitosan solution was prepared in 5% acetic acid in which drug was dispersed, this was then dispersed in 125 ml (1:1) heavy and light liquid paraffin containing dioctyl sodium sulphosuccinate (0.05%w/v) under constant overhead stirring at 2000 rpm. Initially 4ml of glutaraldehyde saturated toluene (GST) was added, further after 30mins 4ml of GST was added drop wise. 4ml of aqueous 25% glutaraldehyde was added after 1hr to it with continued stirring up to 5hrs. The microspheres were filtered, washed with n-hexane and ice cold water and then air-dried.

Characterization of prepared microspheres

**Percentage yield:** Total amount of microspheres obtained were weighed and the percentage yield was calculated, taking into consideration the weight of drug and polymer.

**Particle size determination:** Particle size was determined by optical microscopy with the help of calibrated eyepiece micrometer. The size of around 100 microspheres was measured and their average particle size determined.

**Surface morphology:** Surface morphology of microspheres was studied using scanning electron microscopy (SEM) JEOL SEM, Model-6360 Japan. Microspheres were sprinkled on double side tape, sputter-coated with platinum and examined under the microscope at 15KV.

**Percentage drug encapsulation:** Encapsulation was determined by taking a known quantity of microspheres (approximately 25 mg) in a 25 ml volumetric flask, sufficient quantity of phosphate buffer pH 6.8 was added to make up the volume to 25 ml. The suspension was shaken vigorously and then kept aside for 24 hrs at room temperature with intermittent shaking. Supernatant was collected by centrifugation and drug content in supernatant was determined by using UV spectrophotometer at 265nm.

**Equilibrium swelling degree:** The equilibrium swelling degree (ESD) of microspheres was determined by swelling 5gms of dried microspheres in 5 ml of phosphate buffer pH 6.8 overnight in a measuring cylinder.

**Mucoadhesion studies (Falling film technique):** 100mg of microsphere sample was placed over a nasal mucosal segment mounted on a tilted slide at an angle of 45° as shown (Figure 1). The effluent was run over the segment. The effluent was collected on a whatmann filter paper and weight of detached particles were determined. Percentage of mucoadhesion was determined using following formula.

\[
\% \text{ Mucoadhesion} = \frac{\text{Wt. of sample} - \text{Wt. of detached sample}}{\text{Wt. of Sample}} \times 100
\]
In vivo bioavailability studies

In vivo bioavailability studies were conducted in healthy male rabbits weighing 2-2.5 kg. Twelve rabbits were divided into two groups of six each and were fasted for 24 hours\(^{9-11}\). One group was fed with Oral tablet preparation, the nasal formulation (microspheres) was administered to the second group of unanesthetized rabbits equally between both nostrils with polyethylene tubes. The formulation was released from the tube using a syringe containing compressed air. 2 ml of blood sample was collected from the marginal ear vein of the rabbits into heparinized centrifuge tubes at 1, 1.5, 2, 2.5, 3, 4, 5 and 6 hours after the drug administration during the study. Blood samples were centrifuged at 1500 rpm and plasma was separated. A five-point calibration standard, ranging from 20 to 100 ng/ml was prepared by spiking the drug free rabbit plasma containing EDTA with an appropriate amount of sumatriptan and internal standard respectively.

Extraction procedure for plasma samples

1ml aliquot of rabbit plasma sample was placed in a screw cap glass tube, 40µl of internal standard working solution (150 µg/ml ofloxacin), 1 ml of 2M sodium hydroxide solution was added and the mixture was vortexed for 30s\(^{12}\). The plasma samples containing sumatriptan were then extracted with 4 ml of ethyl acetate. The mixture was shaken for 5 min and centrifuged for 15 min at 10°C. Extraction procedure with 4 ml ethyl acetate was repeated twice for each sample; both the extracts were mixed together in a culture tube, and then evaporated to dryness. The extracted residue was reconstituted in 0.3 ml of the mobile phase and injected into the HPLC system.

Data analysis

The area under the curve (AUC) was obtained using the trapezoidal rule, the data from time zero to last experimental data point of 6h were used in calculating the bioavailability of both nasal and the oral formulation. The Cmax and Tmax were also obtained from plasma-time profile curve. The bioavailability was calculated using the following equation:

\[
F = \frac{(AUC)_{IN}}{(AUC)_{Oral}} \times \frac{(Dose (mg))_{Oral}}{(Dose (mg))_{IN}}
\]

RESULTS AND DISCUSSION

The percentage yield obtained in all the batches was in the range of 57-84%. The loss of material during preparation of microspheres was due to process parameters as well as during filtration of microspheres. Spherical particles were obtained for all the batches. All the microspheres obtained were in the size range of 46-68µm, which was suitable for intranasal administration. It was observed that with the increase in the concentration of polymer (Chitosan) there was an increase in the particle size of the obtained microspheres. This was due to increase in viscosity and hence difficulty in dispersion and subdivision of droplets. The scanning electron microscopy shows that microspheres obtained were fairly smooth and spherical in shape. The Sumatriptan Succinate loaded microspheres revealed drug crystals in microspheres confirming the dispersion of drug throughout the matrix. The SEM micrographs of drug free and drug-loaded microspheres are shown in the fig. No: 3 and 4.

Figure 3: SEM Micrograph of Sumatriptan-Free Chitosan Microspheres

Figure 4: SEM Micrograph of Sumatriptan-loaded Chitosan Microspheres.
turnover of the mucous. The physicochemical characteristics of SM1-SM6 batches is shown in table no 1

The ex vivo permeation of sumatriptan succinate from chitosan microspheres exhibited a biphasic pattern, initially showing a rapid permeation due to diffusion of nonecapsulated drug from the surface of microspheres followed by much slower diffusion of entrapped drug. Increase in polymer concentration attributed to higher density of polymer matrix with resultant increase in diffusion path length that the drug molecule has to travel, thereby significantly decreased the rate and extent of drug permeation [Figure 5].

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>Polymer: Drug</th>
<th>% Yield ±SD</th>
<th>Particle Size in µm ±SD</th>
<th>% Drug Encapsulation ±SD</th>
<th>Swelling Index in mg/ml ±SD</th>
<th>% Mucoadhesion ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>SM1</td>
<td>1:1</td>
<td>60.70 ± 2.41</td>
<td>49.76 ± 3.15</td>
<td>54.8 ± 4.32</td>
<td>3.73 ± 1.46</td>
<td>82.24 ± 0.23</td>
</tr>
<tr>
<td>SM2</td>
<td>1:1.5</td>
<td>75.73 ± 3.05</td>
<td>55.20 ± 4.28</td>
<td>66.6 ± 5.67</td>
<td>4.08 ± 0.79</td>
<td>88.92 ± 0.48</td>
</tr>
<tr>
<td>SM3</td>
<td>1:2</td>
<td>62.84 ± 4.86</td>
<td>56.04 ± 6.08</td>
<td>69.3 ± 3.43</td>
<td>4.12 ± 2.86</td>
<td>92.12 ± 0.43</td>
</tr>
<tr>
<td>SM4</td>
<td>1:2.5</td>
<td>66.66 ± 4.21</td>
<td>57.80 ± 3.46</td>
<td>73.3 ± 6.84</td>
<td>4.36 ± 0.35</td>
<td>93.67 ± 0.56</td>
</tr>
<tr>
<td>SM5</td>
<td>1:3</td>
<td>84.37 ± 5.15</td>
<td>60.28 ± 5.82</td>
<td>76.5 ± 5.72</td>
<td>4.48 ± 0.92</td>
<td>95.54 ± 0.34</td>
</tr>
<tr>
<td>SM6</td>
<td>1:3.5</td>
<td>72.16 ± 2.89</td>
<td>62.40 ± 7.04</td>
<td>79.6 ± 5.89</td>
<td>5.00 ± 1.28</td>
<td>96.18 ± 0.64</td>
</tr>
</tbody>
</table>

SM1 to SM6 represents the various nasal formulations of sumatriptan succinate, # Each value represents a mean of 3 determinations (n=3)

Table 2: Pharmacokinetic parameters of tablet and nasal microspheres of sumatriptan succinate.

<table>
<thead>
<tr>
<th>Dosage form</th>
<th>Tmax (hrs)</th>
<th>Cmax ng/ml*</th>
<th>Kel</th>
<th>AUC* (ng.hr/ml)</th>
<th>Elimination t1/2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral (tablet)</td>
<td>2</td>
<td>35 ±0.6</td>
<td>-0.147</td>
<td>126.25±5.8</td>
<td>4.71</td>
</tr>
<tr>
<td>Intranasal (microspheres)</td>
<td>2</td>
<td>40 ±0.4</td>
<td>-0.115</td>
<td>270±2.4</td>
<td>6.02</td>
</tr>
</tbody>
</table>

* Mean±SD (n=3)

Figure 5: Percentage of drug permeated for formulations SM-1 to SM-6

Chitosan being insoluble, the release of drug has been controlled by swelling control release mechanism. The results showed that concentration of Chitosan can vary the release profile of drug from the microspheres. The batches SM-1, SM-2, SM-3 showed a release of 98.6%, 96%, and 93.21% within 8 hrs where as SM-4, SM-5, SM-6 showed a release of 88.1, 84.4, and 81.5% respectively within 8 hr.

The HPLC analysis of the plasma showed a peak plasma concentration (Cmax) of 35ng/ml after oral dosing and the time to attain the peak plasma concentration i.e., Tmax was around 2 hrs while in the case of the nasal microspheres the Cmax was 40ng/ml and the Tmax was 2 hours. The mean pharmacokinetic parameters of the oral and the nasal formulation were estimated and are given in table No 2

Assessment of the AUCo-t from the plasma concentration-time curve showed that the relative bioavailability was increased to about 2.68 times with the nasal formulation as compared to the tablet formulation fig 6.

Figure 6: Plot for plasma concentration of Sumatriptan concentration Vs Time for oral and nasal formulation.
Acknowledgement: The authors are grateful to Natco Pharma Ltd, Hyderabad for providing gift sample of sumatriptan succinate and also to India Sea Foods, Cochin.

REFERENCES