IMPROVED AMLODIPINE BIOAVAILABILITY USING NASAL CHITOSAN MICROSPHERES

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ABSTRACT

The aim of this study was to formulate nasal amlodipine chitosan microspheres with enhanced bioavailability. The microspheres were prepared by spray-drying method and characterized regarding particle size, morphology, drug entrapment efficiency and in-vitro drug release. Bioavailability of amlodipine was studied in rabbits and compared to intravenous and oral solutions. Results showed that the prepared microspheres characters were dependent on polymer: drug ratio. Microspheres were spherical and had particle size in the range of 2-10 µm. The prepared microspheres were able to sustain amlodipine for 6 h. Nasal chitosan microspheres revealed superior bioavailability over oral solution. Therefore, nasal mucoadhesive chitosan microsphere is promising strategy to improve amlodipine bioavailability via circumventing first-pass metabolism, prolonging nasal residence time and enhancing nasal absorption.

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INTRODUCTION

Intranasal administration is a non-invasive route for drug delivery which possesses many benefits as rapid onset of action, high total blood flow and forestalling first-pass metabolism [1]. Several strategies have been applied to enhance nasal bioavailability as permeation enhancers or using mucoadhesive delivery systems to overcome mucociliary clearance [2]. Mucoadhesive polymers as chitosan, cellulose derivatives and starch are widely used in nasal drug delivery systems [3]. Chitosan is a linear polysaccharide derived from the partial deacetylation of chitin, exhibits favorable biological behavior such as bioadhesion, permeation enhancing properties with interesting physicochemical characteristics making it a unique material for the design of ocular, nasal, buccal, vaginal and injectable drug delivery vehicles [4]. Chitosan can also be used for the preparation of micro and nano-particulates carriers for drug delivery.

Microspheres are free flowing powders consisting of polymers with ideal particle size less than 200μm. It is an ideal approach for targeted, sustained and controlled delivery systems [5].

Amlodipine, a dihydropyridine calcium channel blocker used for the treatment of hypertension, myocardial ischemia, and vasospasm in surgical patients [6]. Since amlodipine suffers from extensive first pass metabolism [7], the present study aims to formulate it into chitosan microspheres suitable for nasal administration. In this study the prepared microspheres were characterized regarding particle size, shape, \textit{in-vitro} release, \textit{in-vivo} bioavailability study and nasal integrity test.

MATERIALS AND METHODS:

Materials:

Amlodipine besylate was kindly supplied by Novartis, Egypt. High molecular weight chitosan was supplied by Sigma–Aldrich Company, St. Louis USA. Acetonitrile (HPLC grade) and Methanol (HPLC grade) were supplied by Riedel-de Haen GmbH, Germany. All other chemicals and reagents were of an analytical grade.

Preparation of amlodipine loaded chitosan microspheres by spray drying technique:

Different concentrations of amlodipine were dissolved in 1% w/v chitosan solution dissolve in 1%v/v acetic acid (table 1). Spray drying process was performed using Mini Spray Dryer with a standard 0.5 mm nozzle (BÜCHI B-290, Switzerland) by feeding drug polymer solutions (100 mL) at the following conditions; inlet temperature 120 °C, outlet temperature 60 °C, aspiration speed 70% and pump rate 5mL/min. The dried microspheres was then collected and stored in desiccator for further evaluations.

Characterization of microspheres:

Spray Drying Production Yield:

The production yield was determined as a percentage of the obtained microspheres to the total weight of chitosan and amlodipine [8].

Drug content and entrapment efficiency:

The amount of amlodipine incorporated into chitosan microspheres was determined by dissolving 10 mg drug loaded formulae into 100 mL methanol. Amlodipine concentration was quantified spectrophotometrically (Schimadzu 2450, Japan) at 360 nm using the pre-constructed calibration curve. Entrapment efficiency was calculated using the following equation [9]:

\[
\text{Entrapment Efficiency} = \left( \frac{\text{Determined amount of amlodipine besylate in microspheres}}{\text{Theoretical amount of amlodipine besylate in microspheres}} \right) \times 100
\]

Particle size analysis:

The particle size parameters including mean particle size and polydispersity index (PDI) of the prepared microspheres were measured using Horiba laser scattering particle size distribution analyzer model (LA-920- Japan).

Scanning electron microscopy (SEM):

The obtained microspheres were coated with gold under vacuum and examined by scanning electron microscope (SEM) (JXA-840A, Japan).

\textit{In-vitro} drug release:

The \textit{in-vitro} amlodipine release from the prepared microspheres was determined using USP apparatus I [10]. A quantity of 100 mg microspheres was immersed in 500 mL of phosphate buffer saline (pH 7.4) adjusted at 35± 0.5°C the reported nasal mucosa temperature [1] and the shafts rotated at 50 ±0.1 rpm. Drug concentration was determined spectrophotometrically at 360 nm. Blank experiment using plain formulae was carried out at the same times and conditions. The release data were analyzed to determine the kinetic modeling of the drug release. The results obtained from \textit{in-vitro} release studies were plotted in four kinetics models (zero, first, Higuchi’s classical diffusion and Korsemeyer Peppa’s). Comparing the $R^2$ values obtained the best fit model was selected.
In-vivo pharmacokinetic and bioavailability study:

In-vivo study was approved by the ethics committee of Faculty of Pharmacy, Ain Shams University, Cairo, Egypt. Six male New Zealand rabbits weighing 2.5 ± 0.25 kg were used by using crossover study with 1-week as wash-out period. Rabbits were housed in stainless steel cages at ambient temperature with 12 h light/dark cycle. The animals were quarantined under these conditions for two weeks before starting the experiment to allow for adjustment to the experiment environment. The animals were fasted for 12 h prior to the pharmacokinetic study. A volume of 50 μl of the selected microsphere suspension in PBS (pH 7.4) was administered in each nostril using 10-100 μl high performance micropipette (Robfield-GBtt Kobenicker, Strabe 320 Deutsch Land) to which tips, of 0.1 mm in diameter at the delivery site, were fixed. The administrated formula was equivalent to 2.5 mg/kg body weight amlodipine [11]. For comparison, intravenous (IV) amlodipine solution was injected through the ear vein of the rabbits as well as oral solution with was administered by an oral tube (both solutions had the same dose). In-vivo studies of different amlodipine formulae were performed over 6 h. Blood samples were collected from the ear vein of the rabbits into EDTA containing test tubes and centrifuged at 3500 rpm for 15 min. Plasma samples were immediately stored at -40°C until analysis. Each sample (500 μl), mixed with 500 μl acetonitrile was vortex-mixed for 30 s and centrifuged at 9000 rpm for 15 min. The drug content in the supernatant was analyzed by HPLC.

Chromatographic conditions:

The samples were assayed using HPLC system (Agilent 1100, Germany) equipped with G 1311A quaternary pump and UV detector (VWD-G1314 A). A reverse phase C18 column (Thermo® BDS, 250X4.6 mm, 5μ) was used at 25°C. The mobile phase was consisted of mixture of acetonitrile: phosphate buffer pH 3(40:60 v/v). The wavelength of the UV detector was set at 360 nm with a flow rate 1 mL/min [11].

Calculations:

Drug concentration data in plasma samples were dose- and weight normalized and analyzed using Wagner-Nelson Method for pharmacokinetics determination [12].

Nasal integrity assessment:

Briefly, anesthetized male albino rats (250–300 g) was intraperitoneally injected by thiopental (45 mg/kg), received once daily nasal administration of 10 μl of amlodipine loaded chitosan microsphere suspension for 14 days [1]. The rats were then sacrificed and the nasal septum with the epithelial cell membrane on each side was carefully separated from the bone. The septum was fixed with 10% formalin, washing was done under tap water, and then dehydrated with alcohol. Specimens were cleared in xylene and embedded in paraffin at 56 °C in hot air oven for 24 h. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns by slide microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin and eosin stain and then examined through the electric light microscope (Kyowa Optical, Japan).

Statistical Analysis:

Data are presented as mean of three replicates ±SD for in-vitro experiments and six replicates ±SD for in-vivo study. For comparing variable between different groups One Way Analysis of Variance (ANOVA) was applied followed by Tukey HSD test and P value <0.05 was considered significant.

RESULTS AND DISCUSSION:

Characterization of amlodipine loaded chitosan microspheres:

Composition and characterization of the prepared microspheres are shown in table (1). Spray drying strategy produce microspheres with production yield more than 50% for all formulae. Table (1) shows that all the prepared formulae possess a relatively high drug content and entrapment efficiency (>90%). On the contrary to particle size, the entrapment efficiency decreased as the polymer: drug ratio increased [2].

The particle size and particle size distribution of the prepared formulae are displayed in table 1. All the prepared microspheres had particle sizes in the range 2-10 μm. Increasing polymer to drug ratio increased feed solution viscosity led to a significant increase (P<0.05) in particle size [13]. The low PDI values (less than 0.5) indicate a narrow size distribution.

Table (1): Composition and characterization of different amlodipine loaded chitosan microspheres.

<table>
<thead>
<tr>
<th>Formula</th>
<th>Chitosan: amlodipine ratio</th>
<th>Production Yield</th>
<th>Drug Content (mg)</th>
<th>Entrapment Efficiency (%)</th>
<th>Mean Particle (µm)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>1:1</td>
<td>62.96±7.25</td>
<td>976.00±8.18</td>
<td>97.83±0.58</td>
<td>2.93±0.66</td>
<td>0.48±0.07</td>
</tr>
<tr>
<td>F2</td>
<td>2:1</td>
<td>56.70±5.69</td>
<td>483.00±7.21</td>
<td>96.20±1.05</td>
<td>6.50±0.62</td>
<td>0.42±0.07</td>
</tr>
<tr>
<td>F3</td>
<td>3:1</td>
<td>52.06±2.35</td>
<td>313.66±5.68</td>
<td>94.56±1.26</td>
<td>9.53±1.00</td>
<td>0.45±0.14</td>
</tr>
<tr>
<td>F4</td>
<td>4:1</td>
<td>51.93±2.30</td>
<td>232.33±10.96</td>
<td>94.57±1.74</td>
<td>12.16±0.73</td>
<td>0.46±0.05</td>
</tr>
<tr>
<td>F5</td>
<td>5:1</td>
<td>50.70±4.95</td>
<td>181.33±3.78</td>
<td>90.22±1.54</td>
<td>16.03±2.10</td>
<td>0.49±0.04</td>
</tr>
</tbody>
</table>
The morphology of microsphere surface is shown in figure (1). All the prepared microspheres were smooth and spherical in shape with size in good agreement with that determined in particle size analysis.

Amlodipine release from different prepared microspheres is displayed in figure (2). The release of amlodipine was sustained for 6 h. The release of amlodipine was affected by polymer: drug ratio and microsphere particle size. Increasing polymer: drug ratio decreased amount of amlodipine release. This may be due to low amount of drug presents close to the surface when the polymer concentration increases [10]. Furthermore, polymer matrix density is increased at higher concentrations and therefore increase diffusional pathlength required for drug release [14]. Similar results were previously obtained in other studies with different drugs as verapamil [2], glipizide [10] and captopril [14]. In addition, particles with small size have larger surface area which allowed the drug to be in contact with dissolution media and facilitate drug release [15]. Kinetic analysis of dissolution data revealed that all the prepared microspheres showed diffusion release mechanism with $R^2 \approx 0.99$.

![Figure (1): Scanning electron micrograph of different amlodipine loaded chitosan microspheres.](image1)

![Figure (2): In-vitro release of amlodipine different loaded chitosan microspheres.](image2)

From the above results, F2 was chosen for in-vivo study since it has high entrapment efficiency (96.2%) and relatively high amlodipine release with particle size with suitable range for nasal administration since particles with size less than 5 µm is expected to migrate to lungs [2].
**In-vivo pharmacokinetic and bioavailability study:**

Figure (3) shows plasma amlodipine concentration data after intranasal administration of amlodipine loaded chitosan microsphere, IV and oral solution. Pharmacokinetic parameters for different formulae were calculated and recorded in table (2). From data in table (2), intranasal amlodipine loaded chitosan microspheres showed significantly higher (P<0.05) Cmax than oral solution with respective values of 3.36µg/mL±0.57 and 2.27±0.44. Furthermore, AUC0-∞ increased significantly (P<0.05) from 3.25±0.25 µg/mL.h in oral solution to 5.3±0.62 µg/mL.h in intranasal amlodipine loaded chitosan microspheres. On the contrary, intranasal amlodipine loaded chitosan microspheres showed significantly lower (P<0.05) Tmax (0.25h) than oral amlodipine solution (1h). By inspecting these data, it could be concluded that intranasal chitosan microspheres enhance both extent and rate of absorption over oral solution with relative bioavailability equal 162.88%. Furthermore, AUC0-∞ for IV amlodipine solution was 7.05 µg/mL.h ±0.84 which corresponds to 75.16% and 46.14% absolute bioavailability for intranasal amlodipine chitosan microspheres and amlodipine oral solution respectively.

Amlodipine is rapidly absorbed and is extensively metabolized in the liver. It is a substrate of cytochrome P450 (CYP) 3A subfamily and also to P-glycoprotein [6]. Nasal microspheres showed significantly higher bioavailability of amlodipine than oral solution due to averting of metabolism and increase residence time accompanied with mucoadhesive chitosan microspheres [2]. Furthermore, chitosan which is penetration enhancer has the ability to transiently open the tight junctions by interaction with a protein kinase C pathway allowing for the penetration of the released drug [16]. In addition, positive charge of chitosan allows its interaction with negatively charged cell membranes [2]. Therefore amlodipine loaded chitosan microsphere allowed superiority over oral amlodipine solution.

Table (2): Plasma pharmacokinetic parameters.

<table>
<thead>
<tr>
<th>Formula</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (µg/ml)</th>
<th>T&lt;sub&gt;max&lt;/sub&gt; (hr)</th>
<th>AUC&lt;sub&gt;0-6&lt;/sub&gt; (µg/mL.h)</th>
<th>AUC&lt;sub&gt;0-∞&lt;/sub&gt; (µg/mL.h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intranasal Amlodipine Microspheres</td>
<td>3.36±0.57</td>
<td>0.25</td>
<td>4.48±0.41</td>
<td>5.30±0.62</td>
</tr>
<tr>
<td>Oral Amlodipine Solution</td>
<td>2.27±0.44</td>
<td>1</td>
<td>3.23±0.42</td>
<td>3.25±0.25</td>
</tr>
<tr>
<td>Intravenous Amlodipine Solution</td>
<td>--------</td>
<td>--------</td>
<td>5.47±0.62</td>
<td>7.05±0.84</td>
</tr>
</tbody>
</table>

Figure (3): Plasma concentration curves of amlodipine from nasal chitosan microspheres (F2) compared to IV and oral solution.

**Nasal integrity assessment:**

No histopathological alternations as vascular congestion, edema, necrosis, or hemorrhage could be observed after daily intranasal administration of amlodipine chitosan microspheres (F2) for 14 days (figure 4). These results confirm the tolerability and safety of chitosan microspheres on nasal epithelial tissue.
CONCLUSION

Nasal amlodipine loaded chitosan microspheres could be prepared by spray drying technique with high entrapment efficiency (>90%). The prepared microspheres characters were affected by polymer: drug ratio. The prepared microspheres were able to sustain amlodipine release for 6 h. Nasal microspheres showed superior bioavailability over oral solution with no histopathological changes to the nasal mucosa after 14 days administration.

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REFERENCES


