Valsartan Loaded Solid Lipid Nanoparticles: Development, Characterization, and \textit{In vitro} and \textit{Ex vivo} Evaluation

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Received May 05, 2011; accepted November 1, 2011

ABSTRACT

Valsartan is an antihypertensive drug with poor oral bioavailability ranging from 10-35\% because of poor solubility, dissolution and most importantly, extensive first pass hepatic metabolism. The present study deals with the development and characterization of Valsartan-loaded solid lipid nanoparticles (VSLNs) to enhance the solubility, bypass the first pass hepatic metabolism, and enhance the lymphatic absorption leading to improved bioavailability. VSLNs were developed using glyceryl behenate (Compritol 888 ATO\textsuperscript{®}) as the lipid and Poloxamer 407 (Pluronic F 127) as the surfactant by the solvent injection method. VSLNs were characterized for mean particle size (MPS), zeta potential, percentage drug entrapment (PDE), DSC Scans, XRD and TEM analysis. In vitro drug release studies were performed in 0.067 M phosphate buffer of pH 6.8 using dialysis diffusion bag method. \textit{Ex vivo} drug release studies were also performed for both VSLNs and valsartan suspension in stomach and intestine. The optimized formulation of having the 80 mg lipid, 10 mg drug and 250 mg surfactant was found to have particle size distribution of 142.5 ± 1.859 nm, zeta potential of – 14.3 ± 0.384 mV, and 84.59 ± 0.328\% drug entrapment. Based on these results, it is concluded that SLNs show promise for improving the oral bioavailability of valsartan.

KEYWORDS: Valsartan; poor solubility; oral bioavailability; first-pass metabolism; lymphatic absorption; solvent injection method.

Introduction

Valsartan is a nonpeptide, orally active, and specific angiotensin II antagonist acting on the AT1 receptor subtype. It is categorized as angiotensin-II receptor blocker (Cerulli et al., 2005). Valsartan is poorly soluble and the aqueous solubility is reported to be less than 1 mg/ml. Valsartan gets rapidly absorbed following oral administration, with a bioavailability of about 10-35\% because of extensive hepatic first-pass metabolism. Peak plasma concentrations of valsartan achieve at 2-4 hours after an oral dose (Brookman et al., 1997). A rapid onset of action is desirable to provide fast relief in the treatment of heart failure. Therefore, it is necessary to enhance the aqueous solubility and dissolution rate of valsartan to obtain a faster onset of action, minimize the absorption and improve its overall oral bioavailability bypassing first-pass hepatic metabolism.

According to the Biopharmaceutical Classification Scheme (Amidon and Lodenberg, 2000), valsartan is considered as a class-II compound having less water solubility and high permeability (Brunella et al., 2006). Various techniques for the improvement of solubility and dissolution rates of poorly water-soluble drugs are reported.

Some of them include the formation of the inclusion complexes with cyclodextrins, the formation of solid dispersions with hydrophilic carriers, micellar drug solubilization, dendrimers, self microemulsifying drug delivery systems, spray drying, nanoparticle formulations, prodrug approach, and salt synthesis (Kapadia et al., 2009). Apart from these approaches, possible methods to avoid first pass metabolism include administration of drugs via transdermal, buccal, rectal and parenteral routes, but the oral route is the most commonly used and a route of choice for the delivery of drugs, although several factors like pH of GIT, residence time and solubility can affect this route. Lymphatic delivery is an alternative choice to avoid first pass metabolism in peroral drug delivery (Driscoll 2002). Enhanced lymphatic transport of drugs reduces the hepatic first-pass metabolism and improves bioavailability, because intestinal lymph vessels drain directly into thoracic duct, further in to the venous blood, thus bypassing the portal circulation (Charman and Porter, 2001). The main function of the lymphatic system

ABBREVIATIONS: VSLNs : Valsartan loaded solid lipid nanoparticles; MPS: Mean particle size; PDE: Percent drug entrapment.
is to facilitate absorption of long chain fatty acids via chylomicron formation. Two different lipid based approaches are known to enhance the lymphatic transport, a highly lipophilic prodrug and incorporation of drug in a lipid carrier (Charman and Porter, 1996).

Solid lipid nanoparticles (SLNs) are a simple colloidal drug delivery system. They are submicron size (50-1000 nm) carriers made up of biocompatible and biodegradable materials and capable of incorporating hydrophilic as well as hydrophobic drugs (Manjunath et al., 2005). It is reported that the SLNs are a promising sustained release system for lipophilic drugs after oral administration to increase bioavailability. SLNs exhibits various advantages like enhancement of oral bioavailability, good physical stability, good tolerability, drug targeting, improved therapeutic effect, protection of labile drugs, possession of solid matrix-controlled release properties, lower cytotoxicity, possible sterilization and having the best production scalability (Mader and Mehnert, 2001).

In the present study, valsartan-loaded solid lipid nanoparticles (VSLNs) are prepared to enhance the oral bioavailability of valsartan. It is hypothesized that SLNs can avoid first pass hepatic metabolism and enhance the aqueous solubility and dissolution rate of valsartan.

**Materials and Methods**

**Drugs and Chemicals**

Valsartan was a gift sample from Torrent Pharmaceuticals, Ahmedabad, India. Glycerol benenate (Compritol 888 ATO®) was a gift sample from Colorcon Asia Pvt. Ltd (Goa, India). Pluronic F 127 was a gift sample from BASF, Germany. Pluronic F 68 and Trehalose was purchased from Himedia, India. Methanol, isopropyl alcohol and chloroform were purchased from SD Fine Chem. Ltd., Vadodara, India. All other reagents and chemicals used in this study were of analytical grade.

**Development and Characterization Studies**

**Method for the Preparation of VSLNs.** Solid lipid nanoparticles loaded with valsartan were prepared by Solvent Injection method (Muller-Goymann and Schubert., 2003). Lipid (80 mg) and drug (10 mg) were dissolved in 5 ml organic solvent (IPA) at 5 °C above the melting point of the lipid. Simultaneously, surfactant (250 mg) solution in an aqueous phase (25 ml) was prepared at the same temperature. When both systems reached to the same temperature, the organic phase was quickly injected into the aqueous phase with continuous stirring. Organic solvent was allowed to evaporate by continuous stirring on Euro Star High Speed Stirrer (Ika®, Werke, Germany). After complete evaporation of organic solvent, SLNs dispersion was cooled in ice bath with continuous stirring. Then the SLNs were subjected to centrifugation at 9000 rpm for 30 minutes using a Remi ultra centrifuge. Dispersion was decanted and drug pellets were separated. To the dispersion, trehalose [3 times of total drug content (10 mg)] 30 mg was added as a cryoprotectant and the nanoparticulate suspension was kept in deep freezer at 4 °C for 24 hours. Samples were lyophilized and used for further studies.

**Interference Study.** Interference studies of the lipid (Compritol 888 ATO®), surfactant (Pluronic F127 and Pluronic F68) and the drug in solvent [MeOH:CHCl3 (6:4)] (Chandrasekhar et al., 2007) were performed. Using the first derivative spectroscopy we can nullify the interference.

**Lipid Interference.** Standard drug solution (10 µg/ml) and lipid solution (100 µg/ml) were prepared. Then the absorbance of standard drug solution in the absence and presence of the lipid was taken at 236.4 nm using MeOH:CHCl3 (6:4) as a blank.

**Surfactant Interference.** Surfactant interference was studied using the standard drug solution (10 µg/ml) and surfactant solution of 250 µg/ml. Absorbance of standard drug solution in the absence and presence of the surfactant was taken at 236.4 nm using MeOH:CHCl3 (6:4) as a blank.

**Determination of Particle Size.** The particle size and size distribution of the VSLNs were characterized by laser light scattering using a Particle Size Analyzer (Malvern Mastersizer Hydro-2000 SM, UK). Each sample was suitably diluted with filtered distilled water (10 times) to avoid multiscattering phenomena and placed in a small disposable zeta cell and particle size was measured in triplicate manner.

**Determination of Zeta Potential.** Zeta potential is highly useful for assessment of the physical stability of colloidal dispersions. Zeta potential can be measured by determination of the movement velocity of the particles in an electric field (electrophoresis measurements). Zeta limits ranged from – 200 mV to + 200 mV. In the present work, the VSLNs dispersion was diluted 10 times and analyzed for the zeta potential by the Malvern Zetasizer Nanoseries-ZS apparatus (Malvern Instruments, UK). Each sample was suitably diluted with filtered distilled water (10 times) and placed in a small disposable zeta cell and zeta potential was measured in triplicate manner.

**Percent Drug Entrapment Study.** VSLN dispersion was centrifuged at 9000 RPM for 30 minutes in Remi centrifuge and SLN dispersion, was removed without disturbing the drug pellet. Free drug content was estimated in Methanol by a UV spectrophotometer (UV-1700, Shimadzu) at 249.5 nm (Babasaheb and Pravin, 2009). Entrapped drug content was estimated by dissolving 1 ml of SLNs dispersion from supernatant in 49 ml of MeOH:CHCl3 (6:4) using the First Derivative UV spectroscopy at 266.6 nm (Satana et al., 2001). This method has been followed to nullify the interference between the drug and lipid.

\[
\% \text{ Drug Entrapment} = \frac{\text{Entrapped Drug}}{(\text{Entrapped Drug} + \text{Free Drug})} \times 100
\]
Differential Scanning Calorimetry (DSC) Analysis. DSC analysis was carried out using a Differential Scanning Calorimeter (DSC-60, Shimadzu, Japan) at a heating rate of 20°C per minute in the range of 30°C-300°C under an inert nitrogen atmosphere at a flow rate of 40 ml/minute. DSC thermograms were recorded for valsartan, Compritol 888 ATO®, Pluronic F-127®, Physical mixture (of Compritol 888 ATO® and valsartan) and VSLNs.

X-ray Diffractometry (XRD). XRD study was carried out for valsartan, Compritol 888 ATO®, Pluronic F-127 and drug loaded SLNs using an X-Ray Diffractometer with a horizontal goniometer (Siemens® D-5000, Germany). The samples were placed in the sample holder and scanned at a rate of 1° per minute in scanning range of 2θ from 0° to 90°. XRD studies were carried out at Electrical Research and Development Association (ERDA), Baroda.

Transmission Electron Microscopy (TEM). TEM is useful since it allows measurement of particles much smaller than 1 µm. Samples were air dried on copper grid and made free of water. The sample given for the TEM study was having the actual particle size at 148 nm. The TEM study was carried out at Punjab University, Chandigarh using the JEOL 2010 200 kV TEM instrument.

Drug Release Studies

In Vitro Drug Release Study. A modified dialysis method (Hu et al., 2003, Ge et al., 2002) was used to evaluate the in vitro release of valsartan-loaded SLNs. One end of the dialysis sac (Molecular weight cut off 10000-12000) was tied with the thread and examined for any leaks. Later it was filled with 2.5 ml of VSLNs (equivalent to 1 mg/ml) and remaining open end was tied up using thread. The packed sac acted as a donor compartment. The sac was then immersed in glass beaker containing 25 ml of 0.067 M methanolic phosphate buffer pH 6.8 as the receptor compartment at 37°C ± 2°C. The content of beaker was stirred using magnetic bead stirrer and the beaker was covered with an aluminum foil to prevent loss of solvent during the experiment. At selected time intervals, 3 ml samples were withdrawn from the release medium and replaced with the same amount of freshly prepared phosphate buffer maintained at the same condition. The sample was assayed spectro-photometrically for valsartan at 250 nm and the percent drug release was calculated. Similar studies were also carried out for the production of SLNs and during two months (after 15, 30 and 60 days) of storage at different temperature conditions.

Ex Vivo Drug Release Study. Male wistar rats (250-300 g) were sacrificed and the stomach and intestine were isolated carefully. These tissues were thoroughly washed with cold Ringer’s solution to remove the mucous and lumen contents. Later, they were filled with 2.5 ml of VSLNs (equivalent to 1 mg/ml) and ex vivo studies were carried out for 2 hours in 25 ml of 0.1 N HCl as receptor phase (Smith., 1996). After the completion of 2 hours study, the contents of the stomach were transferred into the intestine and the further studies were carried out in 25 ml of 0.067 M Phosphate buffer pH-6.8 containing 10% methanol. 3 ml samples were collected at different time intervals, replaced the same volume with diffusion media and analyzed the samples at 250 nm by UV-visible spectrophotometer. Similar study was also carried out for VS. The whole study was carried out with a continuous carbogen supply and were gentle stirring condition at 37°C ± 2°C.

Stability Study

For both SLNs dispersion and lyophilized SLNs, short term stability studies were conducted. It is known that the particle size distribution and zeta potential of the colloidal system are most important characteristics for the evaluation of stability. Both SLNs dispersion and lyophilized SLNs batches were divided into two equal portions and one was stored at a refrigerated condition (2°C-8°C) and the other at room temperature, 25°C ± 2°C (ambient). Parameters such as particle size distribution and percentage assay were evaluated immediately after the production of SLNs and during two months (after 15, 30 and 60 days) of storage at different temperature conditions.

Results and Discussion

Interference Study

The results of interference studies are given in Table 1. In the case of lipid interference, the absorbance of the drug in the presence of lipid was found to be increased while there was no significant change in the absorbance of drug in the surfactant interference study. So it was found that there was a clear interference of drug with lipid but not with surfactant. The results of the studies were shown in Figure 1(b).

Table 1

| Interference Study between drug, lipid and surfactant. |
|-----------------|-----------------|

<table>
<thead>
<tr>
<th>Lipid Interference</th>
<th>Surfactant Interference</th>
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<tr>
<td><strong>Materials</strong></td>
<td><strong>Absorbance</strong></td>
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<td>Drug</td>
<td>0.458 ± 0.0009</td>
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<tr>
<td>Lipid</td>
<td>0.111 ± 0.079</td>
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<tr>
<td>Drug + Lipid</td>
<td>0.526 ± 0.0014</td>
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</table>

Particle Size and Zeta potential

The mean particle size of optimized VSLSN dispersion and lyophilized VSLSNs was found to be 142.5 ± 1.859 nm (PdI = 0.134) and 228.4 ± 2.021 nm (PdI = 0.253) respectively.
The zeta potential of optimized batch was found to be $-14.3 \pm 0.384$ mV. The obtained mean particle size and zeta potential of the optimum batch was shown in Figure 2 and Figure 3 respectively. Particle size was found to be relatively small with a PdI value well below 1. Result of zeta potential indicated the stability of the formulation.

**% Drug Entrapment**

The PDE of the optimized VSLNs was found to be $84.59 \pm 0.328$. High entrapment efficiency can be attributed to the lipophilic nature of drug having higher affinity for the selected lipid matrix.

**DSC analysis**

In the present study, DSC thermograms of valsartan (Figure 4a) showed an endothermic peak at $120^\circ$C corresponding to its melting temperature, which was not detected in the thermogram of VSLNs (Figure 4e). Thermogram of physical mixture (drug + lipid) (Figure 4c) showed an endothermic peak at $71.64^\circ$C for the lipid and $122^\circ$C for drug. No melting endotherm of drug in VSLNs and no shifting of melting endotherm of drug in the physical mixtures of drug and lipid were observed, which indicates the compatibility between the drug and lipid. Moreover, in VSLNs, the drug was present in the amorphous phase and homogeneously dispersed in the lipid matrix.
X-ray Diffractometry (XRD)

Valsartan (Figure 5(a)) showed peaks at 2θ = 13.659 (100% intensity), 13.904 (99.8% intensity). However, the characteristic peaks of valsartan were not shown in the XRD patterns of valsartan loaded SLNs, as VSLNs showed peaks at 2θ = 23.16 (100% intensity), 19.09 (75.2% intensity) and 21.23 (61.2% intensity) (Figure 5(d)).

Compritol 888 ATO® (Figure 5(b)) showed intense peak at 2θ = 21.226 (100% intensity). However, the intensity of the lipid peak was decreased 2θ = 21.231 (61.2% intensity) in the VSLNs. This reduced intensity indicates the decreased crystallinity of lipid in the SLNs formulation.

The relative reduction in the diffraction intensities in the VSLNs thus can be predicted due to the change in the orientation of crystals or reduction in the quality of crystals of valsartan and this change in diffraction pattern support conversion of crystalline drug to amorphous form and contributes in the enhancement of the solubility of the drug (Kahirsagar et al., 2009).
Transmission Electron Microscopy (TEM)

A TEM image of VSLN dispersion is shown in Figure 6. TEM image shows that the VSLNs are discrete, spherical, and regular in shape. The sample given for the TEM study was having the actual particle size at 148 nm. And size bar in the TEM image show the particle size at 140.48 nm. This reflects that the size of VSLNs derived from TEM might be considerably smaller than their real diameter (Song et al., 2008).

In Vitro drug release study

From the comparative in vitro study of VS and VSLNs, we can find that valsartan suspension followed the Peppas-Korsemeyer model with the $R^2$ value of 0.9909 which was nearer to 1 and for the Hixson-Crowell model $R^2$ value was found to be 0.9776 for VSLNs. The Correlation Co-efficient ($R^2$) of VS and VSLNs for in vitro study was given in Table 2. According to Peppas-Korsemeyer model there was linear correlation between Log % drug release and log time, hence it can be suggested that the mechanism led to the release of drug was an anomalous transport with constant release rate adequate for a sustained release dosage form from a lipid matrix. Hixson-Crowell model indicate that the release of valsartan from SLNs followed drug release mechanism that drug get released by constantly changing surface area. The graph of in vitro release studies is shown in Figure 7. In both the formulations, ‘n’ value was found to be between 0.5 < n <1 indicating the anomalous drug transport from the lipid matrix followed by non-fickian release mechanism.

Ex Vivo drug release study

From the comparative ex vivo study of VS and VSLNs, we can conclude that valsartan suspension followed the Higuchi model because $R^2$ value (0.9621) was nearer to 1. VSLNs followed the Peppas-Korsemeyer model because the $R^2$ value (0.9707) was nearer to 1. The Correlation Co-efficient ($R^2$) of VS and VSLNs for ex vivo study was given in Table 3. The Higuchi model suggest
that in case of VS, linear correlation exists between percentage drug release and square root of time. The Peppas-Korsemeyer model indicates linear correlation exists between the Log percentage drug release vs. Log time. The graph of ex vivo release study was shown in Figure 8. In both formulations, 'n' value was found to be between 0.5 < n < 1 indicating that this type of drug release is controlled by combination of lipid swelling, erosion and diffusion through the hydrated lipid matrix.

**TABLE 3**

Correlation co-efficient (R²) of valsartan suspension and valsartan loaded solid lipid nanoparticles for ex vivo study.

<table>
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<tr>
<th>Formulation</th>
<th>Linear Correlation Coefficient (R²)</th>
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<tr>
<td></td>
<td>Zero order</td>
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<td>First order</td>
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<tr>
<td></td>
<td>Higuchi model</td>
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<td></td>
<td>Peppas-Korsemeyer model</td>
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<tr>
<td></td>
<td>Hixson-Crowell model</td>
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<tr>
<td>Valsartan suspension</td>
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<tr>
<td></td>
<td>0.8914</td>
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<tr>
<td></td>
<td><strong>0.9621</strong></td>
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<td></td>
<td>0.9421</td>
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<tr>
<td></td>
<td>0.9341</td>
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<tr>
<td>Valsartan SLNs</td>
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<tr>
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<td>0.9371</td>
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<tr>
<td></td>
<td>0.8876</td>
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<tr>
<td></td>
<td><strong>0.9707</strong></td>
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<td></td>
<td>0.8456</td>
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</table>

**Stability Study**

Initially SLNs dispersion showed MPS of 139.2 ± 1.859 nm. After 60 days, SLNs dispersion showed MPS of 224.5 ± 3.208 nm at refrigerated condition and 276.3 ± 1.791 nm at room temperature in Figure 9(a). Initially lyophilized VSLNs showed MPS of 183.9 ± 2.854 nm. After 60 days, it showed MPS of 242.4 ± 4.221 nm at refrigerated condition and 288.3 ± 3.256 nm at room temperature in Figure 9 (b). After 60 days, SLNs dispersion showed % Assay of 94.72 ± 1.808 at refrigerated condition and 92.6 ± 3.227 at room temperature in Figure 9 (c) and lyophilized VSLNs showed percentage assay of 97.59 ± 2.206 at refrigerated condition and 95.89 ± 3.227 at room temperature in Figure 9(d). So it was found that lyophilized VSLNs were more stable when stored at refrigerated conditions than at room temperature. Thus to avoid increase in MPS and reduction in percentage assay, SLNs dispersion should be lyophilized and lyophilized SLNs should be stored in a refrigerated condition.
Conclusions

Valsartan loaded solid lipid nanoparticles prepared by the solvent injection method had high entrapment efficiency, spherical shape, regular surface and a particle size less than 200 nm. DSC scans indicated that valsartan was dispersed in an amorphous state in the SLNs. From the in vitro and ex vivo drug release studies, we can conclude that release of valsartan from SLNs followed the Hixson-Crowell kinetic model and Peppas–Korsemeyer kinetic model, respectively with a non-Fickian diffusion mechanism followed by combination of lipid swelling, erosion and diffusion through the hydrated lipid matrix. These preliminary results indicate that loaded SLNs could be effective in sustaining release for a prolonged period. Further studies are needed to confirm its performance in vivo. From the stability study, we can conclude that to avoid increase in MPS and reduction in percentage assay, SLNs dispersion should be lyophilized and lyophilized SLNs should be stored in a refrigerated condition.

Acknowledgement

Authors are grateful to Electrical Research and Development Association (ERDA), Baroda for providing the facilities to accomplish XRD analysis and also to Development Association (ERDA), Baroda for providing the conveniences to accomplish TEM analysis.

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