ENHANCED ORAL BIOAVAILABILITY IN ALBINO RABBITS OF LOVASTATIN NANO PARTICLES

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ABSTRACT

The aim of the study was to compare the single dose oral bioavailability of lovastatin (LV) nanoparticles in albino rabbits. Plasma was analyzed for lovastatin using a sensitive, reproducible, accurate and validated RP-HPLC method. Pharmacokinetic parameters including AUC, Cmax, Tmax, t1/2, MRT and Kel were determined from plasma concentration of the formulations. The randomly divided into three treatment groups with six animals in each group, as standard I, Standard II and Test. Lovastatin pure drug and lovastatin lovastatin marketed formulation were administered to standard group. Lovastatin loaded nanoparticles suspension was administered to test group. The Cmax of LV nanoparticles was found to be 72.28 ±0.158 ng/ml, whereas Cmax value for the drug suspension and marketed tablet formulation was found to be 33.10 ± 0.176ng/ml and 40.96 ± 0.244ng/ml respectively. (P < 0.001) indicating facilitated absorption of LV by nanoparticles. Tmax of lovastatin nanoparticles was 2 hrs, whereas for the drug suspension and marketed tablet formulation were 1 h respectively. (P < 0.001). The AUC (0–∞h) value for the lovastatin nanoparticles, drug suspension and marketed tablet formulation were found to be 301.43 ±0.165 (ng/ml × h), 73.88 ± 0.210 (ng/ml × h) and 120.51 ± 0.338 (ng/ml × h) respectively. (p < 0.001) The mean residence time (MRT) values for the lovastatin nanoparticles, drug suspension and marketed tablet formulation were found to be 1.41 h, 1.35 h and 1.63 h respectively. (P < 0.001) The relative bioavailability was found to be significant improvement in bioavailability (1.5 fold) as compared with the conventional tablets.

Key Words: Bioavailability, Pharmacokinetics, HPLC, lovastatin, lipophilic drug

INTRODUCTION

Poor solubility is in most cases associated with poor bioavailability. The first approach was of limited success as clearly demonstrated by the low number of products on the market based on such technologies.1,2 A much more straight forward way is increasing the dissolution velocity by increasing the surface area of the drug powder, i.e. micronisation leading to mean particle sizes of approximately 3 - 5 μm. However, many of the new compounds show such a low solubility that micronisation does not lead to a sufficient increase in bioavailability after oral administration. Therefore the next step taken was nanonisation.3,4 The drug powder is transferred to drug nanocrystals or nanoparticles, typical sizes are around 200 - 600 nm. However, the most convenient dosage form for the patient is a dry product, e.g. tablet or capsule. The present work was describes the formulation of drug nanoparticles to tablets. Lovastatin is a potent, effective lipid lowering agent, insoluble in water and its the extensive first-pass effect, with a good tolerability profile has systemic bioavailability only 5%. For water insoluble drugs with high permeability, drug absorption by GIT is limited by drug dissolution rate solubility/dissolution are good pointer and major contributor to drug bioavailability.5,6

Nanoparticles have drawn greater attention because of their solubilisation and transport properties. Therefore, it is very important to devise effective methods to enhance the solubility and dissolution rate of drug, consequently increasing its bioavailability.10 Limited analytical methods have been developed for the determination of lovastatin in biological samples by high performance liquid chromatography methods. Therefore, the aim of the present investigation was to develop a new, sensitive HPLC method for the estimation of lovastatin in albino rabbits plasma.11-12 The outcome of a study depends upon the reliability, reproducibility and sensitivity of the analytical methodology employed. The objective of the study is to develop a nanoparticle of lovastatin and to assess the bioavailability in comparison to lovastatin tablets in albino rabbits.
MATERIALS AND METHODS

Lovastatin was a obtained from gift sample from Aurobindo Pharmaceutical Ltd., Hyderabad; Chitosan was procured from Marine chemicals, Cochin. Sodium tripolyphosphate was purchased from Loba chemie, Mumbai, dialysis bag (cellophane membrane, molecular weight cut off 10000-12000 Da, purchased from Hi-Media, Mumbai, India. All other reagents and chemicals used in this study were of analytical Grade.

Preparation of Chitosan Nanoparticles

The Chitosan nanoparticles containing lovastatin were prepared by ionotropic gelation method. 13-16 Chitosan was dissolved in 1% acetic acid solutions at various concentrations to obtain (0.1%, 0.2% & 0.3%) and adjusted the pH 5-6 with 0.1N sodium hydroxide solution. while STPP was dissolved in deionized water at various concentrations to obtain 0.1%, 0.15% and 0.20%. Lovastatin was dissolved in ethanol/ water mixture (1:1) to obtain clear solution. Lovastatin solution was added dropwise with syringe needle size 0.45 mm to 40ml chitosan solution. The 20ml of STPP solution was added dropwise 0.75ml/min under stirring (1000 rpm) at ambient temperature to the chitosan solution. The formulation was stirred for 30 minutes, so as to remove ethanol content. All the formulation was sonicated at fixed time for 30 minutes. Nanoparticles were collected by centrifugation at 15,000 rpm for a period of 1 h and supernatant were discarded. The resultant dispersion was dried using a freeze-drying method.17-21

Characterisation of Nanoparticles

Lovastatin nanoparticles was characterized for particle size, zeta potential and polydispersity index was determined by Zetasizer (Malvern, UK).22 percent entrapment efficiency, percent process yield, percent drug content, In-vitro drug release studies were performed in USP Type II dissolution apparatus at rotation speed of 50 rpm. The compatibility of drug and excipients determined by Fourier Transform Infrared Spectroscopy study. The % degree of crystallinity of LV. Physical mixture and various batches of nanoparticles were calculated by differential scanning calorimetry. Detect the crystallinity of the pure drug and the nanoparticles formulation, which was performed using a Philips PW 3710 x-ray diffractometer. The morphology of nanoparticles was examined by using scanning electron microscopy (SEM, JSM-6360LV scanning microscope Tokyo, Japan) and Transmission electron microscopy. The optimized batch of prepared nanoparticles was selected for oral bioavailability study on albino rabbits.

Saturation Solubility Studies

Accurately weighed 10mg of lovastatin and the nanoparticles equivalent to 10 mg of the drug were separately introduced into 25ml stoppered conical flasks containing 10ml phosphate buffer (pH 7.4). The sealed flasks were agitated on a rotary shaker for 24 h at 37°C and equilibrated for 2 days. An aliquot was passed through 0.45 μm membrane filter and the filtrate was suitably diluted and analyzed for drug content on a UV spectrophotometer at 243 nm wavelength.

Determination of lovastatin in albino rabbits blood by RP-HPLC

Experimental animals

Eighteen rabbits were used for the bioavailability study. All the animals having weight 1.5 to 3.0 kg and randomly divided into treatment three groups with six animals in each group. Albino rabbits were grouped standard I, Standard II and Test. LV suspension and LV marketed formulation were administered to standard group. LV loaded nanoparticles suspension was administered to test group. Animals had free access to food and water ad libitum. The albino rabbits were kept under standard conditions in animal house of Bharati Vidyapeeth College of Pharmacy, Kolhapur, as per guidelines of CPCSEA; approval letter no. BVCPK/ CPSESEA/ IAEC/ 01/ 17 dated Jan. 12, 2011. The formulations were provided orally using 23-gauge oral feeding needle. LV tablet suspended in purified water and 1 mg equivalent dose orally administered to reference group (RG) rabbits. LV nanoparticles formulation 1 mg equivalent dose orally administered to treatment group (TG) rabbits. Rabbits were anaesthetized using ether. Blood samples were withdrawn from marginal ear vein of rabbits at 0.5, 1, 2, 4, 8 and 12 h. The plasma was separated and drug content was estimated using RPHPLC.23 In this method acetonitrile and double distilled water (pH 3) (80:20) with 0.1% OPA as mobile phase and RP-HPLC (Jasco PU 2080 Pump, UV 2075 Detector).

Determination of lovastatin in albino rabbits blood by RP-HPLC

Linearity study of lovastatin

Standard working solutions of 1000μg/ml of lovastatin, were prepared using mobile phase as a solvent. Required volume of solution from standard working solution was taken to get final dilutions of required strength for calibration curves and made up the volume with mobile phase. The HPLC analysis of all aliquots was carried out and response factor for each analyte was calculated.

Preparation of Internal Standard Solution

Standard stock solution containing simvastatin was prepared by dissolving 50 mg of simvastatin in 20 ml of mobile phase. It was then sonicated for 10 minutes and the final volume of solution was made up to 50 ml with mobile phase to get 1000 μg/ml of simvastatin in a 50 ml volumetric flask.

Recovery Studies

Accuracy of analysis was determined by performing recovery studies by spiking different concentrations of pure drug in the pre-analyzed laboratory sample

System Suitability Parameters

System suitability parameters were analyzed on freshly prepared standard stock solutions of lovastatin. All these analytes were injected into the chromatographic system
under the optimized chromatographic conditions. Parameters that were studied to evaluate the suitability of the system were number of theoretical plates, calibration curve, capacity factor, resolution factor, retention time.

**Processing of Blood samples for HPLC analysis**

All frozen plasma samples were thawed at ambient temperature. 200 μl plasma sample was transferred to a 2 ml polypropylene test tube. The tube was vortexed and then liquid extraction was carried out with 1 ml of methyl tert-butyl ether. Tube was vortexing for 30 sec. and centrifuged for 15 min at 4°C at 3000 rpm. The supernatant was separated and transferred to a clean polypropylene test tube and air dried at 40°C. The residue was reconstituted with 100 μl of methanol and filtered through 0.22 μm syringe filter, then 20 μl volume was injected into RP-HPLC. The flow rate was 1 ml/min and UV detection was performed at 243 nm. The retention time and detection of lovastatin was determined.

**Estimation of Pharmacokinetic Parameters**

The pharmacokinetic parameters for lovastatin pure drug suspension, marketed formulation and nanoparticles dispersion following oral administration were determined from plasma concentration data. The total area under the concentration-time curve (AUC) from time zero to infinity was calculated by the trapezoidal rule method. The maximal concentration (Cmax) and the time to maximal concentration (tmax) were obtained directly by observation. The relative bioavailability is determined, when there are no i.v. data, by comparing different dosage forms. As with calculation of bioavailability, clearance is assumed to be constant. The relative bioavailability can be determined from AUC data. The pharmacokinetic (PK) parameters were performed by non-compartmental analysis. All values are expressed as the mean ± SD. All the analysis of data was performed using statistical software package Graph pad prism 5 version. (Graph Pad Software, San Diego,CA, USA), using one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. Difference between two parameters were considered stastically significant for P<0.001.

**RESULT AND DISCUSSION**

**Characterization of nanoparticles**

The lovastatin loaded nanoparticles by ionotropic gelation method found to white in color with characteristic odor. Analysis of results indicates that particle size range was 200-700 nm shown in figure 1. Polydispersity index (PI) of prepared nanoparticles batches was found to be in the range 0.5376 to 0.9188. Entrapment efficiency of batches under investigation was in the range of 94.03 ± 0.025 to 98.85 ± 0.035 %. Zeta potential for nanoparticles CL1– CL9 batches were in the range of +11.27 ± 0.020 to + 48.50 ± 0.072 shown in figure 2. The cumulative percentage drug release of lovastatin in Phosphate buffer pH 7.4 medium of CL1-CL9, 77.98 ± 0.026 % to 91.06 ±0.026% respectively, after 60 min. The drug release follows Hixon crowel and first order release kinetics mechanism. The drug content of the freeze dried nanoparticles batches were found to be in the range of 80.93±0.569 - 94.87 ± 0.495.

From FTIR and DSC spectra indicated that there was no chemical interaction between lovastatin and chitosan used in the formulation hence, can be used in the formulation of nanoparticles. The crystallinity of LV pure, physical mixture (1:1 lovastatin & CS), lyophilized optimized batch were 100%, 83.80% and 31.36% respectively. The solubility of lovastatin-loaded nanoparticles resulted increase in solubility after 48h (237.4 ± 0.041μg/ml) in comparison with lovastatin pure drug, i.e increase solubility approximately 5 fold. The revealed from the SEM of prepared lovastatin–loaded nanoparticles of optimized batch had a spherical shape with a relatively uniform size of about 292 nm in diameter and no drug crystals were present, depicted in figure 3. The morphology of nanoparticles was observed by Transmission electron microscopy (TEM) of optimized batch, spherical shape and uniform size as shown in figure 4.

![Intensity Distribution](image)

**Figure 1: Particle size of nanoparticles of optimized batch**
Validation of method development

The HPLC chromatogram of lovastatin, overlain spectra of HPLC chromatogram of lovastatin and calibration curve of lovastatin by HPLC are given in figure 5, 6 respectively. Different parameters of calibration curve such as slope, intercept and coefficient of correlation obtained are given in table 1.
Figure 5: HPLC chromatogram of lovastatin

Figure 6: Calibration curve of lovastatin by HPLC

Table 1: Various constant for calibration curves in HPLC

<table>
<thead>
<tr>
<th>Regression Equation Data</th>
<th>Simvastatin</th>
<th>Lovastatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y = A + B*C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope (B)</td>
<td>2037.28</td>
<td>68371.95</td>
</tr>
<tr>
<td>Intercept (A)</td>
<td>13076.77</td>
<td>13562.00</td>
</tr>
<tr>
<td>Correlation coefficient (R)</td>
<td>0.9910</td>
<td>0.98175</td>
</tr>
</tbody>
</table>

Where C is the concentration in µg/ml and Y is the unit of response factor.

Linearity study of lovastatin

The calibration curve for lovastatin was found to be linear in concentration range of 50µg/ml to 250µg/ml. The result of laboratory sample assay is reported in table 2. Results of recovery studies indicating that the method is rapid, accurate and reproducible are shown in table 3.

System Suitability Parameters All these analytes were injected into the chromatographic system under the optimized chromatographic conditions shown in table 4.

The limit of detection and limit of quantitation given in table 5.

Table 2: Results of Analysis of Laboratory Sample

<table>
<thead>
<tr>
<th>Analyte</th>
<th>% Concentration estimated* (Mean ± S.D.)</th>
<th>% R.S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simvastatin</td>
<td>99.13 ± 1.6517</td>
<td>0.8754</td>
</tr>
<tr>
<td>Lovastatin</td>
<td>99.17 ± 1.9255</td>
<td>0.8351</td>
</tr>
</tbody>
</table>

* Average of six determinations.
Table 3: Results of Recovery Studies

<table>
<thead>
<tr>
<th>Analyte</th>
<th>% Recovery estimated*(Mean ± S.D.)</th>
<th>% R.S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simvastatin</td>
<td>99.05 ± 1.13586</td>
<td>0.9632</td>
</tr>
<tr>
<td>Lovastatin</td>
<td>99.59 ± 1.2459</td>
<td>0.4173</td>
</tr>
</tbody>
</table>

* Average of six determinations.

Table 4: System Suitability Parameters

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Parameters</th>
<th>Simvastatin</th>
<th>Lovastatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Retention Time in minutes (α)</td>
<td>9.073</td>
<td>7.388</td>
</tr>
<tr>
<td>2.</td>
<td>Number of Theoretical plates</td>
<td>9021.26</td>
<td>7642.3</td>
</tr>
<tr>
<td>3.</td>
<td>Asymmetry</td>
<td>2.161</td>
<td>2.015</td>
</tr>
<tr>
<td>4.</td>
<td>Calibration Curve (μg/ml)</td>
<td>10-250</td>
<td>0.2-25.6</td>
</tr>
<tr>
<td>5.</td>
<td>Capacity factor (k')</td>
<td>902.35</td>
<td>738</td>
</tr>
<tr>
<td>6.</td>
<td>Resolution (R_s)</td>
<td>4.52</td>
<td>2.86</td>
</tr>
</tbody>
</table>

Table 5: Limit of Detection and Limit of Quantitation

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Analyte</th>
<th>Simvastatin</th>
<th>Lovastatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Limit of Detection (μg/ ml)</td>
<td>0.045</td>
<td>0.031</td>
</tr>
<tr>
<td>2.</td>
<td>Limit of Quantitation ( μg/ ml)</td>
<td>0.035</td>
<td>0.094</td>
</tr>
</tbody>
</table>

The retention time of HPLC chromatogram was 7.360 min. Pharmacokinetic parameters of LV nanoparticles, LV pure and LV marketed tablets were compared in albino rabbits. The bioavailability study was performed with objective of estimating lovastatin after oral administration. By comparison of standard and test group it was observed that, Cmax of LV nanoparticles was found to be 72.28 ± 0.158 ng/ml, whereas Cmax value for the drug suspension and marketed tablet formulation was found to be 33.10 ± 0.176ng/ml and 40.96 ± 0.244ng/ml respectively, indicating facilitated absorption of LV by nanoparticles shown in figure 7. After oral administration, lovastatin nanoparticles were absorbed much slower than lovastatin pure and marketed formulation. Tmax of lovastatin nanoparticles was 2 hrs, whereas for the drug suspension and marketed tablet formulation were 1 h respectively. (P < 0.001)

AUC (0–∞ h) value for the lovastatin nanoparticles, drug suspension and marketed tablet formulation were found to be 301.43±0.165 (ng/ml × h), 73.88 ± 0.210 (ng/ml × h) and 120.51 ± 0.338 (ng/ml × h) respectively. (P < 0.001) The elimination rate constant (Ke) value for the lovastatin nanoparticles, drug suspension and marketed tablet formulation were found to be 0.703, 0.735 and 0.610 respectively. (P < 0.001) The elimination half life (t1/2) for the lovastatin nanoparticles, drug suspension and marketed tablet formulation were found to be 0.98 h,
0.94 h and 1.136 h respectively. (P < 0.001) The mean residence time (MRT) values for the lovastatin nanoparticles, drug suspension and marketed tablet formulation were found to be 1.41 h, 1.35 h and 1.63 h respectively. (P < 0.001) The relative bioavailability was increased as compared to oral control group Standard I & II, it was found that relative bioavailability was 407.9 % and 250.12 % respectively. (P < 0.001) Pharmacokinetic data for pure drug, marketed formulation & optimized batch formulation in plasma given in table 6. The in vivo study results reveals that LV nanoparticles show better bioavailability than drug suspension and marketed formulation. The above results indicated that the LV nanoparticles have the potential to be used to increase the oral bioavailability of highly lipophilic drugs.

### Table 6: Pharmacokinetic data for Pure drug, Marketed formulation & optimized batch formulation in plasma

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Pharmacokinetic parameters</th>
<th>Pure Drug</th>
<th>Marketed formulation</th>
<th>Nanoparticles CL4 batch</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>C max (ng/ml)</td>
<td>33.10 ± 0.065</td>
<td>40.96 ± 0.075</td>
<td>72.28 ± 0.198</td>
</tr>
<tr>
<td>2.</td>
<td>T max (h)</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>3.</td>
<td>AUC (ng/ml × h) (0-8 h)</td>
<td>65.86±0.29</td>
<td>107.56 ± 0.337</td>
<td>252.85± 0.134</td>
</tr>
<tr>
<td>4.</td>
<td>AUC (ng/ml × h) (0-∞ h)</td>
<td>73.88±0.210</td>
<td>120.51±0.338</td>
<td>301.43±0.165</td>
</tr>
<tr>
<td>5.</td>
<td>Ke (h)</td>
<td>0.735</td>
<td>0.610</td>
<td>0.703</td>
</tr>
<tr>
<td>6.</td>
<td>t½ (h)</td>
<td>0.940</td>
<td>1.136</td>
<td>0.980</td>
</tr>
<tr>
<td>7.</td>
<td>MRT (h)</td>
<td>1.35</td>
<td>1.63</td>
<td>1.41</td>
</tr>
<tr>
<td>8.</td>
<td>Fr (%)</td>
<td>--</td>
<td>--</td>
<td>407.99 *</td>
</tr>
</tbody>
</table>

* Calculated on AUC (0-∞ h) with lovastatin suspension (Std. I) as reference
** Calculated on AUC(0-∞ h) with lovastatin marketed tablet (Std. II) as reference

### CONCLUSION

In vivo studies on rabbits revealed overall increase in bioavailability of the drug upon oral administration of nanoparticles formulation as compared with pure suspension and marketed formulation. The experimental findings collectively support that prepared nanoparticles had the potential to enhance solubility, dissolution rate correlates with faster oral absorption and bioavailability of poorly water soluble drugs. Lovastatin nanoparticles formulation showed a significant improvement in bioavailability as compared with the conventional tablets. As a result, nanoparticles could be a promising delivery system to enhance the oral bioavailability of highly lipophilic drug of lovastatin.

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### CONFLICTS OF INTEREST

The authors declare no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

### REFERENCES


