Hypolipidemic activity of simvastatin solid dispersions in triton X-100-induced hyperlipidemic Wistar rats

Vidyadhara Suryadevara*, Sandeep Doppalapudi, Aruna Kumar Chadalawada, Mounika Mudda, Ravi Teja Potla
Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Chandramoulipuram, Chowdavaram, Guntur, Andhra Pradesh, India – 522019

ABSTRACT

The present research work was focussed on the preparation as well as the evaluation of Simvastatin solid dispersions for hypolipidemic activity. Solid dispersions were formulated using soybean powder as polymer in different ratios. The solid dispersions were prepared using solvent evaporation technique. The solid dispersions were found to release the drug faster than the pure drug in dissolution media of phosphate buffer pH 7.0. The formulation SS5 showed best drug release profile when compared to other formulations. This formulation was used for the evaluation of hypolipidemic activity against triton X-100 induced hyperlipidemic Wistar rats. When compared to the hyperlipidemic control group, the group of animals treated with SS5 formulation showed better results. From the test results, a decrease in the total cholesterol, total triglyceride, low density lipoproteins, very low density lipoproteins levels and an increase in the high density lipoproteins were observed. Thus from the present study it was concluded that Simvastatin solid dispersion could be a promising hypolipidemic agent which would be a boon for most of the patients suffering from heart diseases.

Keywords: Simvastatin, Solid dispersions, Hyperlipidemia, Triton X-100, Wistar rats

INTRODUCTION

Hyperlipidemia is a condition in which an elevation in the blood lipid concentration was observed. It often results from delayed or defective clearance, or overproduction of VLDL by the liver, which subsequently transforms into LDL [1]. Some of the food products like meat, whole milk dairy products such as high fat milk, cheese, cream, butter and tropical oils like palm and coconut oil contributes their part in the development of hyperlipidemia, as they are rich sources of saturated fats. Higher concentrations of LDL and low levels of high-density lipoprotein (HDL) cholesterol in blood predict the cardiovascular risk in both men and women. A group of naturally occurring fats present in the blood and tissues of the body are called as lipids. Some of the examples include cholesterol, cholester esters, triglycerides and phospholipids. Lipids are the major components which serve as the major structural component of the body and also act as energy source. The plasma contains lipoproteins like high density lipoproteins (HDL), low density lipoproteins (LDL), very low density lipoproteins (VLDL) and chylomicrons whose levels determine the human health [2]. Life style habits like lack of exercise, smoking, age, excess alcohol intake causes hyperlipidemia. Other conditions like obesity, diabetes mellitus and pregnancy also have a part in causing hyperlipidemia [3]. Hyperlipidemia develops as a consequence of abnormal lipoprotein metabolism or reduction in the LDL receptor expression or activity and consequently diminishing the hepatic LDL clearance from the plasma. This condition was mainly due to the end products of lipoprotein metabolism which involves an endogenous and an exogenous pathway. The end products of these pathways are VLDL, LDL and chylomicrons [4]. Various targets like HMG CoA reductase enzyme, bile acids and lipoprotein lipase have been selected in these pathways for the treatment of hyperlipidemia [5]. Among all the hypolipidemics, HMG CoA reductase inhibitors, “statins” were effectively used. Some of the examples of these agents are Lovastatin, Simvastatin, Atorvastatin, Pravastatin and Rosuvastatin. Statins mainly work by inhibiting the enzyme HMG CoA reductase, which is a key regulatory enzyme in the biosynthesis of cholesterol. Simvastatin is one such popular agent which reduces serum cholesterol levels by 30 – 40%, LDL by 35 – 45%.
triglycerides up to moderate level and HDL is elevated slightly. It is a well tolerated drug with minimal side effects [6]. Oral route of drug administration is the most common and preferred route of delivery due to convenience and ease of ingestion [7]. Although the oral route is much preferred, limited drug absorption resulting in poor bioavailability is a potential problem that can be encountered while delivering an active agent via the oral route. This may be due to various factors like poor aqueous solubility or membrane permeability of the drug molecule. In biopharmaceutical classification system (BCS), drugs with less aqueous solubility and high membrane permeability are categorized as class – II drugs. One of such drugs which fall under his category is Simvastatin. An approach which increases the dissolution rate of poorly water soluble drugs is “solid dispersions”. Solid dispersions are a group of solid products consisting of a hydrophilic matrix and a hydrophobic drug [8]. As they increase the dissolution rate of the drug at the absorption site, there is a gradual increase in bioavailability [9]. Although they are having many advantages like easy in production, prevention of pre-systemic metabolism, the stability issues were a bit complicated [10,11]. The solid dispersions were prepared using suitable technique. The hypolipidemic potency of a compound can be evaluated various methods, out of which the usage of surfactant like triton is mostly preferred. Although the traditional methods like feeding the experimental animals with cholesterol rich foods like butter gives good results, they are now-a-days subsided due to prolonged experimentation time. The main objectives of the current work are to enhance the solubility of poorly water soluble drug Simvastatin and to evaluate its hypolipidemic activity in Wistar rats. The solubility can be enhanced by making solid dispersions using solvent evaporation method. Whereas the hypolipidemic activity was evaluated using triton X-100 induced hyperlipidemia model in Wistar rats.

MATERIALS AND METHODS

Simvastatin was a gift sample from MYLAN LABORATORIES, HYDERABAD. Triton-X 100 was a gift sample from M/s. TRIMS LABORATORIES, VISAKHAPATNAM and soybean powder was obtained from the local market of Guntur. Methanol, disodium hydrogen phosphate and potassium dihydrogen phosphate were procured from S.D. FINE CHEMICALS, MUMBAI. All other materials used were of analytical grade and procured commercially.

Preparation of Simvastatin solid dispersions by solvent evaporation method: The solvent evaporation method was employed for the preparation of solid dispersions. Specified quantity of Simvastatin and soybean powder which acts as a hydrophilic carrier were taken in a china dish and to that few ml of methanol was added and slightly heated until both drug and polymer dissolves. Then it is subsequently allowed to evaporate. The obtained mixture was dried, passed through the sieve no.80, packed in a wide mouthed amber colored glass container and was hermetically sealed and stored [12].

In vitro drug release studies of Simvastatin solid dispersions: The dissolution test for the solid dispersions was carried out in USP Apparatus Type II (paddle) with 900ml of Phosphate buffer pH 7.0 as the dissolution medium which is maintained at 37 ± 0.5. The samples were drawn at 5, 10, 15, 30, 45, 60, 75, 90 minutes. Fresh volume of the medium was replaced with the withdrawn volume to maintain the sink conditions and constant volume throughout the experiment. Samples withdrawn were suitably diluted with same dissolution medium and the amount of drug dissolved was estimated by ELICO double beam spectrophotometer at 239nm and subsequently analyzed for the cumulative percentage of drug released.

Evaluation of Hypolipidemic activity on Wistar rats

Experimental animals: Healthy adult male albino rats (Wistar strain) weighing 250–300g, housed in polypropylene cages, maintained under standardized condition i.e., 12:12 hour light/dark cycle at 25 ± 2 with paddy husk bedding at the animal house, Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Guntur, India were provided with standard pellet food and had free access to purified drinking water. The guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India were followed and prior permission was soughted from Institutional Animal Ethics Committee (IAEC) for conducting the study.

Acute toxicity study: The acute oral toxicity study was performed according to the OECD toxicity guidelines [13]. The animals were fasted overnight prior to the experiment. Test doses of 2 and 5g/kg body weight were given orally to rats. During first four hours after drug administration, the animals were observed for gross behavioural changes such as hyperactivity, grooming, convulsions, sedation,
hypothermia, body weight and mortality were observed up to 14 days.

**Standardization of hyperlipidemic dose of Triton X-100:** To induce the hyperlipidaemia, rats were kept in fasting for 18 hours with access of water and subjected to Triton X 100 at the dose of 300, 400, 500, 600 and 700mg/kg p.o. and the levels of different lipoproteins was evaluated at 24, 48 and 72 hours [14].

**Induction of hyperlipidaemia:** Animals were divided into four groups of three rats each. All the animals were fasted overnight for 18 hours prior to the experiment. Group I was considered as control which received normal saline solution. Group II was considered as hyperlipidemic control which received Triton X-100 at a dose of 400mg/kg p.o. body weight which was prepared in physiological saline solution. Group III was considered as standard group which received the standard drug Simvastatin at a dose of 5mg/kg p.o body weight along with Triton X 100. Group IV was considered as test group and received the test formulation at a dose of 500mg/kg p.o. along with the Triton X 100. After the treatment, the animals were allowed to have standard pellet diet and water freely. According to the treatment protocol, the first dose of drug treatment was given immediately after triton administration to animals. Second and third doses were administered after 24 and 44 hours respectively [15]. After 4 hours of third dose, the animals were used for the study of various biochemical parameters.

**Collection of blood samples:** After 48 hours, the animals were subjected to euthanasia and the blood was collected through cardiac puncture. The collected samples were centrifuged at 2000 rpm for 30 minutes to get the serum. The samples thus collected were stored in well closed containers.

**Biochemical analysis:** The collected serum was analyzed for the presence of various biochemical parameters like total cholesterol (TC), triglycerides (TG), high density lipoproteins (HDL), low density lipoproteins (LDL) and very low density lipoproteins (VLDL) [16]. These samples were processed using a semi-auto analyser. The standard kits were used for the estimation of parameters in the test samples.

**RESULTS AND DISCUSSION**

**Preparation of Simvastatin solid dispersions by solvent evaporation method:** By using solvent evaporation method, the Simvastatin solid dispersions were prepared using soybean powder as a carrier, keeping the drug concentration constant and by increasing the carrier concentration. Different formulations with various drug to polymer ratios were placed in table-1.

**In vitro drug release studies of Simvastatin solid dispersions:** The dissolution studies were performed for different formulations of Simvastatin solid dispersions along with the Simvastatin pure drug with soybean powder as a carrier using U.S.P paddle method (apparatus II) with 7.0 pH phosphate buffer as a medium which is maintained at a temperature of 32. The absorbance values were noted at 239nm by using ELICO Double beam spectrophotometer. The results thus obtained were tabulated in table 2 and indicated in figure 1. The formulation SS5 with a composition of 10mg Simvastatin and 30mg soybean powder, prepared by solvent evaporation method showed best dissolution profile when compared to other formulations. The cumulative percentage drug release for this formulation is 63.538%. This gives a detail that as the carrier concentration increases; there is an increase in the dissolution rate which was due to increased wettability of the drug which there by leads to enhanced solubility, thus favouring the absorption of drug in the gastrointestinal tract. Hence this formulation was selected for the evaluation of hypolipidemic activity on Wistar rats.

**Acute toxicity studies:** The acute toxicity study revealed the non toxic nature of the formulation. The doses 2 and 5g/kg were found to be safe. Hence the oral test dose was selected in such a way that it lies in between 1/100th and 1/50th of the maximum safe dose i.e., in the range of 100 – 500mg/kg.

**Standardization of hyperlipidemic dose of Triton X-100:** It was observed that Triton X-100 in the dose of 400mg/kg p.o. can induce maximum hyperlipidemia after 48 hours. Hence 400mg/kg p.o. was considered as the ideal dose for induction of hyperlipidemia.

**Biochemical analysis:** After the induction of hyperlipidemia in rats, the blood was collected from rats and the samples were analysed for the estimation of various biochemical parameters in serum like total cholesterol, triglycerides, high density lipoproteins, low density lipoproteins and very low density lipoproteins. The effect of Simvastatin solid dispersions (SS5) on serum total cholesterol (TC) and triglyceride (TG) levels was shown in table 3 and figure 2, whereas the effect on serum HDL, LDL and VLDL levels was represented in table 4 and figure 3. From the obtained results, it was observed that there was an extremely significant increase in the serum TC.
TG, HDL, LDL and VLDL levels in hyperlipidemic control group when compared to that of normal control group. The test group which was treated with the SS5 formulation showed good results. A significant decrease in the serum TC, TG, LDL and VLDL levels were observed, whereas the HDL levels were increased when compared to that of the hyperlipidemic control group. Approximately 30% increase in the HDL levels, 45% decrease in LDL levels and 42% decrease in triglyceride levels were observed. When compared to the regular decrease in these levels, the SS5 formulation showed a promising result.

Triton X-100 has been widely used to block clearance of triglyceride-rich lipoproteins to induce acute hyperlipidemia in several animals [17]. This model is widely used for a number of different aims particularly, in rats it has been used for screening natural or chemical hypolipidemic drugs [18,19]. The large increase in plasma cholesterol and triglycerides due to Triton X-100 injection results mostly from an increase of VLDL secretion by the liver accompanied by a strong reduction of VLDL and LDL catabolism [20]. The reduction of total cholesterol by the Simvastatin solid dispersion, SS5 was associated with a decrease of its LDL fraction, which is the target of several hypolipidemic drugs. This result suggests that the cholesterol lowering activity of the test formulation could be a result from the rapid catabolism of LDL cholesterol through its hepatic receptors for final elimination in the form of bile acids [21]. Abnormalities in cellular cholesterol metabolism could partly be responsible for the changes in the plasma cholesterol levels in diabetes [22]. Diabetes is also associated with hyperlipidemia. Serum total cholesterol and triglycerides have been decreased significantly in rats. These effects may be due to low activity of cholesterol biosynthesis enzymes and/or low-level lipolysis.

CONCLUSION

As the polymer concentration increased, the Simvastatin solid dispersions prepared using solvent evaporation method showed an increase in the cumulative percentage drug release. The formulation SS5 which showed best drug release was further subjected to hypolipidemic evaluation in rats against triton X-100. From the obtained results, it was concluded that there was a significant increase in the serum TC, TG, HDL, LDL and VLDL levels in hyperlipidemic control group when compared to that of normal control group. The test group which was treated with the SS5 formulation showed good results. A significant decrease in the serum TC, TG, LDL and VLDL levels were observed, whereas the HDL levels were increased in SS5 treated group when compared to that of the hyperlipidemic control group. Approximately 30% increase in the HDL levels, 45% decrease in LDL levels and 42% decrease in triglyceride levels were observed when the rats were treated with the test formulation. Thus from the present study it was concluded that the Simvastatin solid dispersions prepared with soybean powder as a polymer (SS5) shows better hypolipidemic activity in rats which were treated against triton X-100.

ACKNOWLEDGEMENTS

The authors express their gratitude to Mylan Laboratories, Hyderabad for providing Simvastatin pure drug sample and M/S. Trims Laboratories, Visakhapatnam for providing Triton X-100 sample. The authors are thankful to the management of Chebrolu Hanumaiyah Institute of Pharmaceutical Sciences, Guntur for providing the facilities to carry out the research work.

Graph 1: Drug Release profiles of Simvastatin solid dispersions prepared by solvent evaporation method

SPD indicates Simvastatin Pure Drug
Graph 2: Effect of Simvastatin Solid Dispersion (SS5) on serum TC and TG levels
Here groups indicate – Group-1: Control; Group-2: Hyperlipidemic control; Group-3: Standard; Group-4: Test; TC – Total Cholesterol; TG – TriGlycerides.

Graph 3: Effect of Simvastatin Solid Dispersion (SS5) on serum HDL, LDL and VLDL levels
Here groups indicate – Group-1: Control; Group-2: Hyperlipidemic control; Group-3: Standard; Group-4: Test; HDL – High Density Lipoproteins; LDL – Low Density Lipoproteins; VLDL – Very Low Density Lipoproteins.

TABLE 1: COMPOSITION OF SIMVASTATIN SOLID DISPERSIONS

<table>
<thead>
<tr>
<th>S. No</th>
<th>Composition</th>
<th>Drug : Polymer ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SS1</td>
<td>1:1</td>
</tr>
<tr>
<td>2</td>
<td>SS2</td>
<td>1:1.5</td>
</tr>
<tr>
<td>3</td>
<td>SS3</td>
<td>1:2</td>
</tr>
<tr>
<td>4</td>
<td>SS4</td>
<td>1:2.5</td>
</tr>
<tr>
<td>5</td>
<td>SS5</td>
<td>1:3</td>
</tr>
</tbody>
</table>

*1 part = 10 mg of Simvastatin*
Table 2: Dissolution profiles of simvastatin solid dispersions prepared by solvent evaporation method

<table>
<thead>
<tr>
<th>S. No</th>
<th>Time (m)</th>
<th>Cumulative % Drug Released</th>
<th>SPD*</th>
<th>SS1</th>
<th>SS2</th>
<th>SS3</th>
<th>SS4</th>
<th>SS5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>3.224</td>
<td>5.762</td>
<td>13.192</td>
<td>16.983</td>
<td>17.893</td>
<td>20.522</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>5.104</td>
<td>7.152</td>
<td>16.554</td>
<td>20.269</td>
<td>23.656</td>
<td>26.689</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>10.059</td>
<td>14.911</td>
<td>18.171</td>
<td>24.768</td>
<td>37.935</td>
<td>45.442</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>12.768</td>
<td>16.023</td>
<td>21.533</td>
<td>27.700</td>
<td>47.287</td>
<td>50.092</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>14.002</td>
<td>17.919</td>
<td>22.013</td>
<td>37.935</td>
<td>51.128</td>
<td>60.732</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>45</td>
<td>18.475</td>
<td>20.269</td>
<td>24.212</td>
<td>47.287</td>
<td>54.995</td>
<td>63.538</td>
<td></td>
</tr>
</tbody>
</table>

*SPD indicates Simvastatin Pure Drug

Table 3: Effect of simvastatin solid dispersion (SS5) on serum TC and TG levels

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Total Cholesterol (TC) (mg/dl)</th>
<th>Triglyceride (TG) (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>82.3±3.32</td>
<td>17.08±1.38</td>
</tr>
<tr>
<td>II</td>
<td>Hyperlipidemic control</td>
<td>123.12±2.40</td>
<td>89.96±2.88</td>
</tr>
<tr>
<td>III</td>
<td>Standard</td>
<td>92.34±1.28</td>
<td>38.33±1.76</td>
</tr>
<tr>
<td>IV</td>
<td>Test</td>
<td>102.40±4.77</td>
<td>50.87±1.21</td>
</tr>
</tbody>
</table>

Table 4: Effect of simvastatin solid dispersion (SS5) on serum HDL, LDL and VLDL levels

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>VLDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>36.65±2.40</td>
<td>42.23±1.50</td>
<td>3.14±0.27</td>
</tr>
<tr>
<td>II</td>
<td>Hyperlipidemic control</td>
<td>16.11±0.58</td>
<td>89.02±3.04</td>
<td>19.98±0.58</td>
</tr>
<tr>
<td>III</td>
<td>Standard</td>
<td>48.48±1.62</td>
<td>35.20±2.71</td>
<td>7.66±0.36</td>
</tr>
<tr>
<td>IV</td>
<td>Test</td>
<td>41.12±1.22</td>
<td>49.24±4.64</td>
<td>10.04±0.25</td>
</tr>
</tbody>
</table>

REFERENCES