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Improved anti-hyperlipidemic activity of Rosuvastatin Calcium via lipid nanoparticles: Pharmacokinetic and pharmacodynamic evaluation

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ABSTRACT

The intent of this investigation was to improve pharmacokinetic (PK) and pharmacodynamic (PD) effects of Rosuvastatin calcium (RC) by solid lipid nanoparticles (SLNs). RC is anti-hyperlipidemic drug with low oral bioavailability (20%) due to first-pass metabolism. Hot homogenization followed by ultrasonication method was used to prepare RC-SLNs with stearic acid, glyceryl behenate and glyceryl trilaurate as lipid matrices, egg lecithin and poloxamer 188 as surfactants. The prepared SLNs were tested for particle size, PDI, zeta potential (ZP), entrapment efficiency (EE), drug content and in vitro release. Further, PK and PD studies were conducted on selected SLNs. No changes in physical stability of the optimized SLN were observed at refrigerated and room temperature for 90 days. SLNs prepared with glyceryl trilaurate having average size of 67.21 ± 1.71 nm, PDI of 0.25 ± 0.01, ZP of −28.93 ± 0.84 mV with 93.51 ± 0.34% EE was considered as optimized. DSC and XRD studies revealed that no interaction occurred between the drug and lipid. SEM and TEM studies revealed that SLNs were nearly spherical in shape. PK studies showed improvement in the oral bioavailability (extent of absorption) of SLNs by 4.6-fold when compared to that of suspension. PD study of SLNs in hyperlipidemic rats exhibited a decrease in lipid profile for 36 h, while a suspension exhibited for 24 h.

1. Introduction

In general, most of the drugs can be delivered by the oral route, but several factors like pH of GIT, residence time and solubility of drug can affect delivery of drugs by this route [1]. Lipid based delivery systems reduce the hepatic first-pass metabolism and improve the oral bioavailability, due to transport of drugs through the intestinal lymph vessels, which drain directly into thoracic duct, further into the venous blood, thus bypassing the portal circulation [2]. The lymphatic system plays a major role in the absorption of long chain fatty acids via chylomicron formation. Various approaches are known for enhancement of lymphatic transport of drugs, among them include: construction of a highly lipophilic prodrug [3] and incorporation of drug in a lipid carrier [4].

Solid lipid nanoparticles (SLN) are colloidal carrier systems, having a solid lipid core, which is composed of a solid lipid material for targeting of drugs to intestine associated systems such as lymph and lymph nodes [5].

SLNs have been studied since 1991 as an alternative to delivery systems like lipid emulsions, liposomes, and polymeric nanoparticles, because of the associated advantages such as nontoxicity, biocompatibility and small scale production [6,7]. SLNs are also useful for the improvement of bioavailability of poorly water soluble drugs, like cyclosporine A [8], and to prolong the release of lipophilic drugs, like camptothecin [9]. It is known that drug delivery through SLNs improved the pharmacokinetic behavior. But, not many studies were reported on the follow up effects on pharmacodynamics along with pharmacokinetics.

Rosuvastatin calcium (RC), is a synthetic lipid-lowering agent, chemically known as (3R, 5S, 6E)-7-{4-(4-fluorophenyl) -6-(1-methylethyl)-2-[methyl (methylsulfonyl) amino] pyrimidin-5-yl}-3, 5-dihydroxyhept-6-enoic acid calcium salt (2:1) [10]. RC is available as "Crestor®", a successful brand in USA. RC inhibits hydroxyl methyl glutaryl-coenzyme A (HMG-CoA) reductase competitively. It exhibits low aqueous solubility because of its crystalline nature [11–13] and poor oral bioavailability of about 20% due to first-pass hepatic metabolism. It is extensively metabolized by liver via oxidation, lactonisation, and glucuronidation. Predominantly, the metabolites are eliminated by biliary secretion, further by direct secretion from the blood into the intestine. Krishnamoorthy et al. [14] reported self nanoemulsifying drug delivery system (SNEDDS) of RC, which resulted in improved solubility and oral bioavailability by overcoming the hepatic first-pass metabolism. The objectives of this investigation were to develop RC loaded solid

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lipid nanoparticles (RC- SLN) to enhance the oral bioavailability and to understand the pharmacodynamic effect of RC-SLNs in rat models. Accordingly, RC-SLNs were prepared by hot homogenization followed by ultrasonication method. Prepared SLNs were characterized and an optimal system was evaluated for pharmacokinetic (PK) and pharmacodynamic (PD) effects in comparison to a suspension of RC in induced hyperlipidemic wistar rats.

2. Materials

RC and Poloxamer-188 were obtained as gift samples from M/s. Aurobindo Labs, Hyderabad, India. Trilaurate (Dynasan-112), stearic acid, glyceryl behenate (Compritol ATO 888) were purchased from M/s. Sigma-Aldrich Chemicals, Hyderabad, India. Egg Lecithin E-80 was a gift sample from M/s. Lipoid, Germany. Methanol, acetonitrile, chloroform and formic acid were of HPLC grade (Merck, India). Centrisart filters (molecular weight cut off 20,000) were purchased from M/s. Sartorius, Goettingen, Germany.

3. Methods

3.1. Preparation of SLNs and drug suspension

Hot homogenization followed by the ultrasonication method was used for preparation of RC loaded SLNs [15]. Table 1 shows the composition of RC-SLNs. In 10 mL of 1:1 mixture of methanol and chloroform RC, solid lipid and egg lecithin were dissolved to get oil phase. The aqueous phase was prepared with 150 mg of poloxamer 188 by dissolving in distilled water to get 1.5%w/v solution. Organic solvents were completely removed by a rotary flash evaporator. The drug embedded lipid layer was molten by heating to 5 °C above the melting point of the lipid to get a clear homogenous lipid phase. The aqueous phase was heated to the same temperature as that of the oil phase. To the hot homogenous lipid phase, the hot aqueous surfactant solution was added and homogenized at 12,000 rpm, for 4 min, with DIAX 900 high-speed homogenizer (Heidolph, Germany). The obtained coarse oil in water emulsion was subjected to sonication using a probe soincator (Vibracell, USA; 12T-probe) for 20 min. To prevent the precipitation during homogenization and ultrasonication, the temperature was maintained at least 5 °C above the lipid melting point. RC loaded SLNs were allowed to form by cooling the hot nanoemulsion to room temperature. A coarse suspension of RC was prepared by adding10 mg of RC to 50 mg of sodium carboxy methyl cellulose in a mortar and triturated together for 3 min. Further, 10 mL of distilled water was added and triturated for 5 min to get the suspension of RC.

3.2. Characterization of SLNs

3.2.1. Determination of particle size, PDI and zeta potential of SLNs

Mean diameter of the formulation and polydispersity index (PDI) were determined by photon correlation spectroscopy (PCS) using a Zetasizer Nano ZS90 (Malvern Instruments, UK). The prepared solid lipid nanoparticle preparations were diluted in 1:50 ratio with double distilled water and size was measured at 90° angles [16]. All measurements were done in triplicate. The surface charge was determined by measuring the zeta potential (ZP) of SLN based on the Smoluchowski equation and ZP measurements were made at 25 °C.

3.2.2. Measurement of entrapment efficiency

To determine the entrapment efficiency (EE), about 2.5 mL of SLN formulation was subjected to centrifugation using Centrisart separators (Sartorius, Germany) at 4000 rpm for 15 min, which had a separating membrane with 20,000 Da molecular weight cut off to separate the ultra filtrate [17]. This ultra filtrate contained the un-entrapped drug, which was estimated by high performance liquid chromatography (HPLC) method [18]. The concentration of free drug in aqueous medium was estimated and the EE was calculated.

3.2.3. Determination of total drug content

About 0.1 mL of the SLN formulation was dissolved in 0. 9 mL of a 1:1 mixture of chloroform and methanol and then further diluted with mobile phase [19]. The drug in diluted samples was estimated by HPLC.

3.2.4. In vitro drug release studies

In vitro release studies of RC-SLN formulations were done in 0.1 N HCl (pH1.2) for 2 h followed by pH 6.8 phosphate buffer for 22 h, by using dialysis technique [20]. During in vitro release studies, dialysis membrane (Himedia, India) with a molecular weight cut-off ranging from 12,000 to 14,000 Da was used. The dialysis membrane was kept in double distilled water for overnight. The apparatus for release study had a donor and a receptor compartment. About 2 mL of SLN dispersion was taken for release study in donor compartment, which consisted of a boiling tube with opening at one end and tied with dialysis membrane at the other end. A 250 mL beaker was used as receptor compartment with 100 mL release medium and the temperature was maintained at 37 ± 0.5 °C. About 2 mL samples were withdrawn from receiver compartment and were replaced with fresh medium periodically at intervals of 0.25, 0.5, 1, 2 h in 0.1 N HCl medium and followed by 2, 3, 4, 6, 8, 10, 12 and 24 h in pH 6.8 phosphate buffer medium. The collected samples were suitably diluted and analyzed by UV–

Table 1
Composition of Rosuvastatin calcium loaded solid lipid nanoparticles and suspension.

Ingredients	Formulation code						
	RD1	RD2	RS1	RS2	RC1	RC2	RCS[a]
Organic phase							
RC (mg)	10	10	10	10	10	10	–
Dynasan-112 (mg)	100	200	–	–	–	–	–
Stearic acid (mg)	–	–	100	200	–	–	–
Compritol ATO 888 (mg)	–	–	–	–	100	200	–
Egg lecithin (E-80)(mg)	100	100	100	100	100	100	–
Chloroform:Methanol (1:1) (ml)	10	10	10	10	10	10	–
Aqueous phase							
RC (mg)	–	–	–	–	–	–	10
Sodium carboxy methyl cellulose (mg)	–	–	–	–	–	–	50
Poloxamer-188 (mg)	150	150	150	150	150	150	–
Double distilled water (ml)	10	10	10	10	10	10	10

[a] RCS-Suspension formulation.