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Research Paper

Effective in-vivo utilization of lipid-based nanoparticles as drug carrier for carvedilol phosphate

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Abstract

Objectives Lipid nanoparticles as carrier for oral drug administration improve gastrointestinal solubility of poorly soluble drugs and thus enhance bioavailability. However, basic drugs may undergo rapid dissolution from such solid dispersions in the stomach and precipitate in the intestine due to their higher solubility in acidic medium. Therefore, the objective of this work was to study the enhancement in bioavailability of carvedilol phosphate (basic drug) by providing an alkaline gastric environment to drug-loaded solid lipid nanoparticles.

Methods An alkaline gastric environment in rats was created and maintained with oral administration of an antacid suspension 5 min before and 30 min post dosing.

Key findings The formulation administered orally exhibited enhanced bioavailability (~27%) when compared with drug suspension and sustained release behaviour when compared with formulation under ideal gastric conditions. The enhanced bioavailability is due to the presence of lipid nanoparticles as drug carrier while the sustained-release characteristic may be attributed to the presence of antacid, which resulted in elevation of gastric pH and reduced the drug's solubility.

Conclusions It may be concluded that although lipid nanoparticles can be instrumental in improving bioavailability, additional sustained release may be achieved by targeting intestinal release of basic drugs from lipid vehicles, which is possible by incorporating them into suitable enteric-coated formulations.

Keywords alkaline gastric environment; basic drug; bioavailability; lipid nanoparticles; sustained release

Introduction

Lipid-based nanoparticles for oral drug administration are specifically used to target the uptake of drugs by the lymphatic system, which prevents their first-pass metabolism and thus helps to improve bioavailability.[1] Lymphatic uptake of drugs administered orally through nanoparticulate drug delivery systems follows two routes of transportation: the first being the transcellular transport through the enterocyte and the other being phagocytosis of drugs by M cells of Peyer's patches lining the intestinal mucosa. Biodegradable nanoparticles, such as lipid nanoparticles, are also naturally taken up by these lymphoid tissues in Peyer's patches based on the size of the particles. It has been observed that particles in the size range of 0.3–1.0 μm are preferably absorbed by Peyer's patches over those of 3.0 μm particle size.[2]

The basic mechanism by which lipid-based solid dispersions improve bioavailability of lipophilic drugs is by preventing their precipitation in the aqueous media of gastrointestinal tract.[3] Therefore, the functionality of lipids can be effectively utilized if the drug remains associated with, or entrapped within, the carrier and is gradually released during its gastrointestinal transit such that the drug concentration is within the saturation solubility of the intestinal fluid. As a rule of thumb, the solubility of a typical basic drug is relatively higher in acidic medium. This would result in the rapid dissolution and dissociation of the drug from the solid dispersion well before its gastric transit, finally causing its precipitation in the alkaline condition of the intestine. It is proposed that under the present circumstances an alkaline environment may be advantageous in harvesting maximum benefit of the lipid as a carrier by decreasing its solubility in the gastric environment. In one of our previous studies, carvedilol phosphate (non-selective β-adrenergic blocking agent) was found to exhibit a

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similar pH-dependent solubility profile (i.e. higher solubility in gastric medium (0.958 ± 0.018 mg/ml at pH 1.2) and lower in intestinal medium (0.160 ± 0.024 mg/ml at pH 6.8)).[4]

Carvedilol phosphate is frequently prescribed for the post management of cardiovascular disorders in western countries. It is a non-selective β-blocker that has recently drawn attention because of its therapeutic benefits over other prescribed analogues for the treatment of cardiovascular diseases. However, its extremely low oral bioavailability (~25%) due to extensive first-pass metabolism restricts its use. The drug is lipophilic in nature and is affected by food.[5] There is a need to improve its oral bioavailability, and development of lipid-based nanoparticulate formulations is therefore highly desirable. In the above context, this work was designed to study the enhancement in bioavailability of carvedilol phosphate (a basic drug) by using a novel nanoparticulate formulation approach and an alkaline environment in the stomach was created to study the effect of enteric-coated solid lipid nanoparticles.

Materials and Methods

Materials

Carvedilol phosphate was a kind gift from Lupin Ltd (Pune, India). Polyvinyl alcohol (PVA) was purchased from Himedia (Mumbai, India). Other chemicals and solvents were of analytical grade, purchased locally and used as received.

Preparation of lipid nanoparticles of carvedilol phosphate

The test formulation, consisting of carvedilol phosphate-loaded lipid-based nanoparticles, was prepared and evaluated using the fabrication and analytical methods as per our earlier report.[6] Briefly, the stearic acid-based nanodispersion of carvedilol phosphate was prepared by solvent emulsification evaporation technique and adsorbed on Neusilin US2 (pharmaceutical adsorbent) to convert the nanodispersion into a dry powder form. The particle size and drug loading efficiency of the nanodispersion and the drug content of the final dry product were evaluated. The batch composition and analytical results of the test formulation are presented in Table 1 and the morphology by scanning electron microscopy is presented in Figure 1.

In-vitro drug release study

The drug release study was performed in two different media (0.1 N HCl and pH 6.8 sodium phosphate buffer) by a dialysis bag method using presoaked dialysis membrane (thickness 0.025 mm, mol. wt. cutoff 6000–8000 Da; Sigma Aldrich, St Louis, MO, USA). Test formulation or suspension containing 2.0-mg equivalent of drug along with 2.0 ml of the media was placed into the bag and the two ends were tied and fixed by clamps. The bag was inserted into a beaker containing 250 ml of dissolution medium (temperature: 37°C, stirring rate: 100 rpm) to maintain sink condition.[4] Samples (2 ml) were withdrawn at fixed time intervals and were immediately replaced with equal volumes of the same medium. The samples were filtered through 0.2-µm syringe filters and the drug content was estimated spectrophotometrically at 240.2 nm (λ_{max}) using a Shimadzu UV-1205 UV–Vis spectrophotometer (Japan). All the operations were carried out in triplicate.

Table 1 Formulation details of the test sample used for in-vivo study

Batch composition	
Carvedilol phosphate	20 mg
Stearic acid	100 mg
Polyvinyl alcohol	1%
Sodium taurocholate	10 mM
Mixed phosphate buffer	75 ml
Neusilin US2	746 mg

Evaluation results	Initial data	3-month data
Drug loading efficiency of nanodispersion	93.89 ± 2.63%	–
Particle size of nanodispersion (T_{90})	292.8 ± 9.33 nm	309.4 ± 7.5 nm
Zeta potential of nanodispersion	−10.4 ± 0.49	–
Adsorption efficiency of dry product	71.8 ± 1.42%	–
Drug content of final dry product	97.21 ± 2.08%	96.87 ± 1.48%

Data are expressed as mean ± SD, $n = 6$.

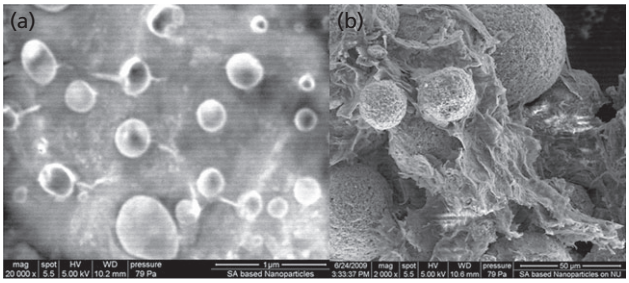

Figure 1 Scanning electron photomicrographs of (a) nanoparticulate suspension and (b) adsorbed lipid nanoparticles (with permission from Chakraborty *et al*.).[6]

In-vivo study

In-vivo studies were carried out as per the guidelines of the Council for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Government of India. The study protocol was approved by the Animal Ethical Committee of Banaras Hindu University (Approval letter no. Dean/2009–2010/573, Dated: 08.08.2009).

Animal experimental protocol

Male albino rats, 250 ± 20 g, were divided into three groups comprising of six rats each. Rats were fasted for 12 h before the experiment with only free access to water. After light anaesthetization with ether, either the drug or formulation (both as 2.0 ml suspension prepared in 0.5% aqueous polyvinyl alcohol solution) containing drug equivalent to 40 mg/kg body weight was orally administered.[7] For one group receiving the formulation, an alkaline gastric environment was created by oral administration of 1 ml Gelusil MPS (antacid

suspension containing 250-mg aluminium and 250-mg magnesium hydroxide per 5 ml) (Pfizer Ltd, Mumbai, India) 5 min before and 30 min post dosing (to maintain an alkaline environment).[8,9] The design of the in-vivo study is given in Table 2. Blood (0.5 ml) was collected via the caudal vein at 0, 0.25, 0.50, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 h during the study. Food was withheld until collection of the 12-h blood samples, but free access to drinking water was provided during the entire study period. Blood samples were placed into heparinized tubes and plasma was separated immediately by centrifugation. Finally, the plasma obtained was stored at −20°C until further analysis.

Quantification of plasma drug concentration

A liquid–liquid extraction method was employed for extraction of drug from the blood and was analysed using a validated high-performance liquid chromatographic (HPLC) method.[7] Briefly, 60 µl of 0.1 N sodium hydroxide solution was added to 200 µl of plasma and vortexed for 1 min. Then, 5 ml of the solvent mixture (7 : 3 ratio of n-hexane, dichloromethane) was added and mixed for 5 min and centrifuged (Remi, Mumbai, India) at 3500 rpm for 10 min. The organic phase was separated and the residues were re-extracted with 3 ml of the solvent mixture by centrifugation. The separated portions were pooled and evaporated in a vacuum oven. The residues were reconstituted in 100 µl of mobile phase and 20 µl of this solution was spiked onto the HPLC column. The HPLC system consisted of two delivery pumps (Waters Corp., Milford, MA, USA), a diode array detector (Waters 2998) and an operating software (Empower Node 2054). A rheodyne manual injector (USA) attached with 20-µl sample loop was used for loading the sample. A C18 reverse-phase 250 × 4.6 mm 5 µm ODS2 column (Waters Corp., Milford, MA, USA) and a C18 guard column were utilized for drug separation. The mobile phase consisted of acetonitrile and 15 mM orthophosphoric acid (37 : 63), and 0.25% v/v triethylamine (pH 2.5 adjusted with orthophosphoric acid) with a flow rate of 1.0 ml/min. The retention time of the drug was approximately 12 min and was detected at a wavelength of 240 nm. Standard calibration curves, constructed over the concentration range of interest, were used to determine plasma drug concentrations.

Pharmacokinetic parameters, such as peak plasma concentration (C_{max}), time to reach peak concentration (T_{max}), area under the curve from time zero to last measured concentration ($AUC_{0\text{-}t}$) and the time span during which the plasma concentrations were at least 50% of the C_{max} value ($HVD_{t50\%\ Cmax}$), were obtained for each subject by non-compartmental pharmacokinetic models using WinNonlin software. The ratio between the $HVD_{t50\%\ Cmax}$ values of the test formulation and the drug suspension expressed as RΔ was also calculated to check any possible sustained-release effect. A ratio of 1.5, 2 and >3 indicated a low, intermediate and strong sustained release effect, respectively.[10]

Table 2 Design of pharmacokinetic study

Group	1	2	3
Sample (aqueous suspension)	Drug	Formulation	Formulation
Gastric condition	Normal	Normal	Alkaline
$n = 6$			

Plasma cholesterol estimation

The plasma cholesterol level was checked at 0, 15, 30, 60, 120 and 240 min on the first day (acute) and last day (chronic) after once-a-day administration of the formulation for a period of 30 days. The estimation was carried out photometrically using the assay kit (Ecoline) procured from Merck, Ambernath, India.

Statistical analysis

All values are expressed as mean ± standard deviation (SD). Statistical analysis of the data was undertaken using one-way analysis of variance test followed by Tukey's multiple comparison test with Graphpad Prism statistical software program (version 5.03).

Results

In-vitro drug release study

The in-vitro dissolution profiles of the drug suspension and the formulation are shown in Figure 2. Both the samples showed rapid drug release in 0.1 N HCl as compared with their dissolution in pH 6.8 buffer. Compared with the drug suspension, the dissolution profile of the formulation in alkaline medium was found to be significantly retarded for over a period of 6 h.

Plasma concentration–time profiles

The mean plasma concentration–time profiles obtained after oral administration of the drug suspension and the nanoparticulate formulation are shown in Figure 3. The pharmacokinetic parameters are listed in Table 3. From statistical analysis of the $AUC_{0\text{–}24\ h}$ values of group 1 and group 2, it was observed that there was an increase of ~27% in $AUC_{0\text{–}24\ h}$ in group 2, which was significantly ($P < 0.05$) higher than for group 1. The differences between C_{max} and T_{max} values of group 1 and group 2 were found to be statistically insignificant ($P < 0.05$ for C_{max} and $P < 0.01$ for T_{max}).

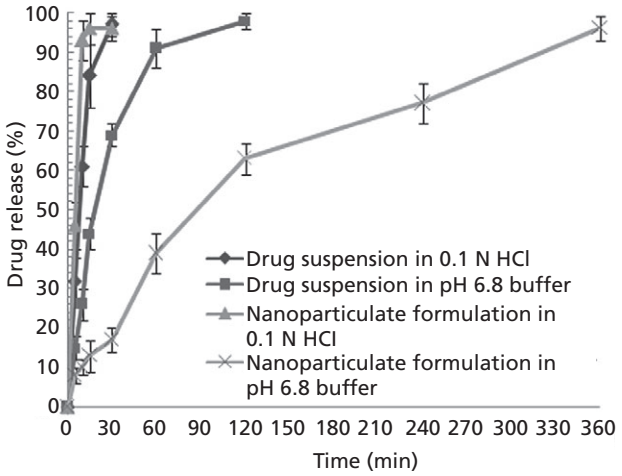

Figure 2 Dissolution profiles of carvedilol phosphate from drug suspension and nanoparticulate formulation in acidic and alkaline media.

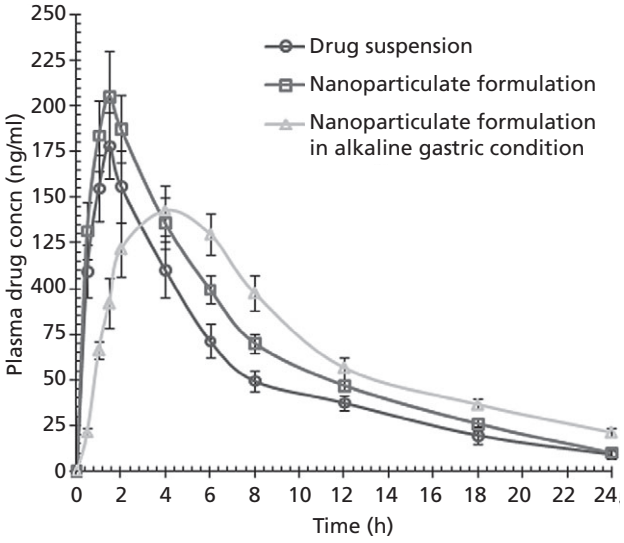

Figure 3 Mean plasma drug concentration–time profiles of carvedilol phosphate from drug suspension and nanoparticulate formulation under different gastric conditions in rats.

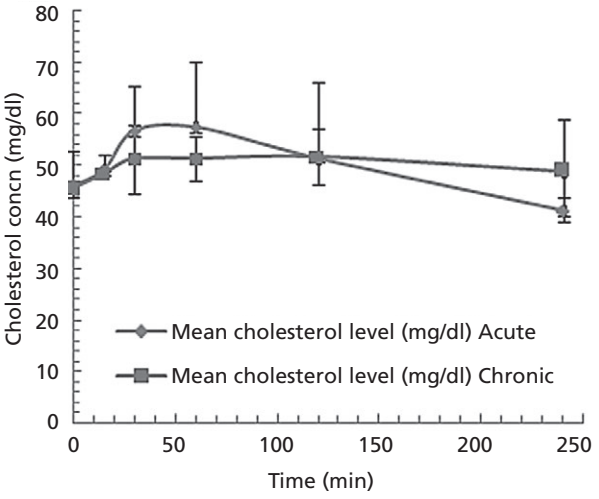

Figure 4 Mean plasma cholesterol concentration–time profiles in rats.

Table 3 Pharmacokinetic parameters of carvedilol phosphate following single oral administration

Pharmacokinetic parameters	Group 1	Group 2	Group 3
C_{max} (ng/ml)	178 ± 10.3	205.1 ± 14.9	142.7 ± 13.8
T_{max} (h)	1.33 ± 0.26	1.25 ± 0.27	4.3 ± 0.82
AUC $_{last}$ (ng/ml*h)	1237.08 ± 69.5	1566.24 ± 99.7	1621.83 ± 80.4
$HVD_{t50\%\ Cmax}$ (h)	4.57 ± 0.21	5.4 ± 0.15	9.2 ± 0.13
RΔ	–	1.18	2.01

C_{max}, peak plasma concentration; T_{max}, time to reach peak concentration; $AUC_{0\text{-}t}$, area under the curve from time zero to last measured concentration; $HVD_{t50\%\ Cmax}$, the time span during which the plasma concentrations were at least 50% of the C_{max} value. Data are expressed as mean ± SD, $n = 6$.

$AUC_{0–24\ h}$ values of group 3 was slightly higher than that of group 2; however, this difference was not statistically significant ($P < 0.001$). The differences between C_{max} and T_{max} values of group 2 and group 3 were found to be statistically significant ($P < 0.05$ for C_{max} and $P < 0.01$ for T_{max}).

The increase in the $HVD_{t50\%\ Cmax}$ values of group 2 compared with group 1 was insignificant in terms of sustained-release behaviour as indicated by its low R_Δ value of 1.18. However, a high value of $HVD_{t50\%\ Cmax}$ with R_Δ value of 2.01 of group 3 indicates an intermediate sustained release or retardation.

Plasma cholesterol profiles

The plasma cholesterol profiles after acute and chronic dosing of the formulation to rats is presented in Figure 4. Statistical analysis of the data obtained indicated no significant change in the plasma cholesterol level either in acute or chronic administration of the formulation as compared with plasma cholesterol level at time zero.

Discussion

It is generally stated that nanoparticulate lipid-based vehicles for oral delivery help in improving the bioavailability and even in sustaining the release of drugs by virtue of their insoluble nature in aqueous environment. This may not always be true as the in-vivo performance of a formulation is also significantly governed by the solubility of the drug in external media and the particle size of the formulation. This matter is of importance for basic drugs (e.g. carvedilol phosphate) delivered orally in lipid-based nanoparticles, which exhibit relatively higher solubility in acidic environment. In such cases, where the lipid matrix is not surrounded by any release retarding layer, most of the drug would dissolve out instantly as it comes into contact with the gastric fluid. The faster rate of drug release from nanoparticulate formulations can also be attributed to the extremely small size and enormous surface area of the nanoparticles, which is in contact with the dissolution medium. This was observed in the case of carvedilol phosphate where the lipid nanoparticulate formulation behaves like an immediate-release formulation in acidic medium but like a sustained-release product in alkaline medium as can be seen in Figure 2. Thus, due to dissociation of drug from lipid, the carrier lipid might fail to prevent the precipitation of the independent drug upon its transit into the alkaline intestinal environment, which would severely affect the bioavailability of the drug. In this in-vivo study, an

alkaline gastric environment was created to retard the release of basic drug, carvedilol phosphate, in the stomach and observe any possible effect on the pharmacokinetic profile of the drug.

A comparison of the blood plasma profiles obtained upon oral administration of drug suspension and the nanoparticulate formulation in rats without antacid treatment (Figure 3) indicated a significant improvement in bioavailability of the drug. It is important to note that despite the drug's high solubility in acidic medium, the lipid-based formulation was able to enhance the bioavailability of the drug. The increased bioavailability may be attributed to the presence of lipid in the gastrointestinal tract, which stimulates the secretion of bile salts and biliary lipids in the intestine and increases the gastric residence time. These two factors together are responsible for increasing the solubilization capacity and time for dissolution by improving micellar solubilization and vesicle formation which prevents the precipitation of the drug in the intestinal fluid.[3]

As compared with the ideal gastric condition, the sustained-release characteristics exhibited by the formulation in alkaline gastric condition clearly indicate that the presence of antacid resulted in elevation of gastric pH which reduced the drug's solubility. As a result, release of drug from the formulation was significantly retarded (as seen in the in-vitro dissolution study) and thus the integrity of the drug-loaded nanoparticles was maintained. These nanoparticles in an alkaline environment then can easily travel to the ileum, which is an ideal site for extensive nanoparticulate uptake (with hydrophobic surfaces such as lipid nanoparticles), due to the availability of abundant Peyer's patches and the existence of less proteolytic enzyme.[11–13] Further, lipid nanoparticles in circulation are also prone to physiological degradation, due to which drug is released into the blood stream. Moreover, the preferable tendency of nanoparticles to adhere to the mucous membranes also leads to delayed gastrointestinal transit, which may also be an added factor responsible for the controlled release of the drug from the formulation.[14] This study demonstrates that if a basic drug being delivered as lipid nanoparticles is allowed to bypass the gastric environment, it would exhibit a controlled-release plasma profile. From the above pharmacokinetic results, it may be concluded that the mere presence of lipid in the gastrointestinal tract would improve the bioavailability of basic drugs, but their association during the gastrointestinal transit would additionally improve its efficacy by providing a prolonged plasma drug concentration.

The pharmacokinetic results also indicate that the method adopted to create an alkaline gastric condition was effective. This method can be used as an alternative to the commonly employed intraduodenal administration of lipid nanoparticles to target lymphatic uptake, which requires anaesthetization of animals and precise surgical procedure.[15–17]

The insignificant changes in the blood cholesterol level, even upon continuous oral administration of stearic acid over a period of 30 days (Figure 4), are in agreement with previous findings that stearic acid is poorly absorbed as a free fatty acid.[18–20] Based on this in-vivo performance of the lipid, oral administration of such formulations can be considered safe for hyperlipidaemic patients.

Compounds that require a high-fat diet to achieve effective plasma drug concentration may attain sub-therapeutic drug levels in patients taking the drug without food. This may be of serious concern for compounds with a narrow therapeutic index. The increased plasma drug concentration is due to the enhanced bile secretion in the presence of food. In such a case, food intake in relation to dosing needs to be closely monitored. Lipids also demonstrate a similar in-vivo effect on bile secretion and therefore the above issue can be addressed by designing lipid-based formulations for drugs like carvedilol phosphate which exhibit ~20% increase in bioavailability when administered with a high-fat diet.[21] In this study, the enhanced bioavailability of carvedilol phosphate by approximately 27% in a lipid based formulation offers a prospective approach to reduce its food-dependent bioavailability.

Conclusion

Based on the above findings, it may be concluded that the bioavailability of carvedilol phosphate can be enhanced by its delivery in the form of lipid-based nanoparticulate formulations. Furthermore, the potential of the carvedilol phosphate-loaded lipid nanoparticulate system was improved by co-administration of an alkalinizing agent which also imparted sustained-release characteristics. Thus, our recent accomplishment implies the possibility of an improved utilization of lipid by intestinal release of basic drugs from a lipid vehicle which is made possible by enteric coating of the prepared formulations. This would in turn improve patient compliance, reduce the dosage requirement and finally make the therapy safer and more efficacious. Moreover, the use of simple in-vivo investigational models can help in faster evaluation of lipid-based formulations of basic drugs for their sustained release ability.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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