Research Article

Development and Targeting Efficiency of Irinotecan Engineered Proniosomes

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Abstract

Purpose: This study is aimed at achieving improvement in the efficacy, reduced toxicity and enhancement of therapeutic index of irinotecan.
Methods: Proniosomes of irinotecan hydrochloride trihydrate were prepared by slurry method using different surfactants, cholesterol and dicetyl phosphate. The formulations were then characterized with respect to shape, surface morphology, entrapment efficiency, in vitro drug release, in vivo drug targeting and stability.
Results: The proniosomes were smooth in texture indicating, thin and uniform coating over maltodextrin powder. The highest entrapment efficiency was found for formulation F2 (74.9 ± 2.7 %). The highest cumulative drug release in 24 h was achieved with formulation F3 (98.2 %) In vivo results for the proniosomes reveal that the drug was preferentially targeted to liver followed by lung and spleen. Stability studies indicate that 4 ºC was the most suitable condition for the storage of formulation F2.
Conclusion: Proniosomes offer a suitable alternative colloidal carrier approach to achieving drug targeting. Proniosomes containing irinotecan are retained at targeted sites and are capable of releasing drug for an extended period of time.

Keywords: Irinotecan, proniosomes, Drug targeting, In vivo tissue distribution, Stability studies.

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INTRODUCTION

Research in the field of drug delivery system, which continues to progress rapidly, aims at the development of drug delivery systems (DDS) with optimum therapeutic benefits including safe and effective management of disease [1].

The concept of drug delivery to a specific site for the treatment of localized disease in the body, thereby decreasing drug adverse effects and improving its therapeutic index, is often considered a challenge [2]. The idea of a drug carrier with targeted specificity has always fascinated scientists for decades and in the last decade, limited success have been achieved in this regard. One such approach involves the use of vesicular drug carrier that can provide site specificity combined with optimal drug release profile [3]. Amongst various carriers utilized for target-oriented drug delivery, vesicular drug delivery systems in the form of liposome and niosomes have been most extensively investigated. Liposomal formulations have the limitation of poor stability and low drug entrapment efficiency while niosomes exhibit physical instability, aggregation, fusion, and leakage of entrapped drug, thus limiting the shelf-life of the dispersion [4,5]. Proniosomes [6] circumvent all the inherent drawbacks of niosomes and they offer a versatile vesicle delivery concept with the potential for targeted drug delivery. They are dry formulations of surfactant-coated carrier which can be hydrated before their use to obtain a suspension of niosomes. The additional convenience of transportation, distribution, storage and dosing make proniosomes a promising industrial product [7].

Irinotecan, chemically, is a synthetic analogue of the natural alkaloid, campto-thecin. It is a chemotherapeutic agent, a topoisomerase 1 inhibitor and mainly used as a drug of choice in colon cancer [8]. The most significant adverse effects of irinotecan are severe diarrhea and extreme suppression of the immune systems. Irinotecan-associated diarrhea is severe and clinically significant, sometimes leading to severe dehydration requiring hospitalization. Also, the immune system is adversely impacted which is reflected in dramatically lowered white blood cell counts in blood [9]. Hence, the objective of the present work is to develop an alternative vesicular drug delivery system for irinotecan in the form of proniosomes which will have advantages of controlled drug release and site specificity, increased drug stability, high drug pay load and absence of bio-toxicity of carrier [10].

EXPERIMENTAL

Materials

Irinotecan hydrochloride trihydrate was obtained as a gift sample from M/S Cipla Limited, Bangalore, India while maltodextrin was also received as a gift from Riddhi Siddhi Glucobials Ltd, Gokak, Karnataka, India. Surfactants, cholesterol and dicetyl phosphate were purchased from Loba Chem Pvt Ltd, Mumbai, India. All other reagents used were of analytical grade.

Experimental animals

Healthy Albino Wistar rats of either sex weighing 200 – 250 g were selected for the study. The animals were housed under standard conditions and fed with standard pellet diet (Lipton India Ltd., Mumbai) and drinking water. The experimental protocol was approved by the Institutional Animal Ethics Committee of N.E.T. Pharmacy College Raichur, Karnataka, India (Reg no. 576/02/bc/CPCSEA). The animals were handled as per Recommended Animal Handling, Guidelines and Audit Guide [11].

Formulation of proniosomes

Optimized proportions of Surfactants, cholesterol, and dicetylphosphate (molar ratio 47.5:47.5:5, respectively), were used in this work [7]. A stock solution of surfactants in chloroform was prepared with 164 mmol/L

surfactant, 164 mmol/L cholesterol, and 17.2 mmol/L dicetylphosphate. Proniosomes with maltodextrin as the carrier were prepared by a slurry method using surfactants like span 20 (F1), span 60 (F2), tween 20 (F3) and tween 80(F4). 10 g of maltodextrin powder was added to a 250-mL round-bottom flask and the entire volume of surfactant mixture (14.5 mL) was added directly to the flask. The flask was attached to the rotary evaporator with the rotation speed set at 60 RPM and temperature to 37^0c. Vacuum was applied until the powder appeared to be dry and free flowing. The flask was removed from the evaporator and the proniosomes were sealed in screw cap vials, until further use.

Scanning electron microscopy (SEM)

Particle shape analysis was done by SEM using JEOL JSM-T330A scanning microscope. Cleaned brass specimen studs were used for mounting the samples. Wet solvent paint was applied on these studs and while the paint was wet, the proniosome powder was placed on each stud and allowed to dry. Thereafter, photomicrographs were taken [12].

Measurement of angle of repose

The angle of repose of the dry proniosome powder was measured by the funnel method [13]. Briefly, the maltodextrin powder or proniosome powder was poured into a funnel which was fixed at a position so that the orifice of the funnel was 10 cm from the base. When the powder was poured into the funnel, it flowed down to form a cone on the flat surface. The height of the cone (h) and the radius (r) of its base were measured, and the angle of repose computed using Eq 1.

$$\varnothing = \tan^{-1} h/r \ \dots\dots\dots\dots\dots (1)$$

Drug loading and rehydration of proniosomes

Irinotecan hydrochloride trihydrate (10 mg) was dissolved in 10 ml of PBS (pH 7.4). The drug solution was added to a vial containing proniosome powder, the vial was capped, attached to a vortex mixer (Remi Equipment Pvt Ltd, Mumbai, India) and agitated at maximum speed for 2 min to yield niosomes.

Determination of drug entrapment efficiency:

The degree of incorporation of irinotecan hydrochloride trihydrate in the proniosomes (entrapment efficiency) was evaluated. Following centrifugation of the aqueous noisome suspension [14], amount of the free drug in the supernatant as well as the amount of the incorporated drug were determined spectrophotometrically model UV-1700, Shimadzu Corporation, Japan) at 369 nm. Entrapment efficiency (EE, %) was computed using Eq 2.

$$EE \ (\%) = 100 \ (Wo - Wf)/Wo \ \dots\dots\dots (2)$$

where Wo is the total amount of drug used in preparing the proniosomes and Wf is the amount of free drug in the supernatant.

In vitro drug release studies

Niosomes equivalent to 10 mg of irinotecan hydrochloride trihydrate were taken into a tube with one end of the tube closed with a dialysis membrane. The tube was palced vertically in a beaker containing 50 ml of PBS (pH 7.4) in such a way that it just touched the surface of the buffer solution. The whole set-up was placed on a magnetic stirrer rotating at 50 rpm with the temperature of the buffer maintained at $37 \pm 1 \ ^0$C. One ml aliquot of the release medium (buffer) was withdrawn at time intervals of 1, 2, 4, 8, 16, 24 h and replaced with the same volume of PBS on each occasion. The withdrawn samples were filtered through 0.45 μm membrane filter (Elix, Mill-Q), diluted suitably and determined spectrophotometrically (model UV-1700, Shimadzu Corporation, Japan) at 369 nm [15].

In vivo drug distribution studies

This study was performed to compare the targeting efficiency of the drug-loaded proniosomes with that of the plain drug in terms of degree of targeting to various organs of the reticuloendothelial system (RES), namely, liver, lung, spleen, kidney, heart and brain [16]. Nine healthy adult rats weighing 200-250 g were selected and fasted for 12 h prior to the the test. The animals were divided into 3 groups of 3 rats each. Group I received niosomes (batch F_2) equivalent to 810 mcg of irinotecan hydrochloride trihydrate intravenously in the tail vein after redispersing the niosomes in sterile PBS. Group-II rats received 810 mcg of pure (free) irinotecan hydrochloride trihydrate intravenously while Group-III rats were treated as vehicle control and injected intravenously with sterile PBS. All the animals of Group I and II were anaesthetized using Ketamine HCl (22mg/kg i.m) prior to administration of pure drug and formulation (F_2) respectively to reduce painful symptoms.

The rats were sacrificed 3 h later by cervical dislocation and their liver, lungs, spleen and kidneys isolated. The organs of each rat were homogenized separately in 5 mL ethanol using a tissue homogenizer (Remi Equipment Pvt Ltd, Mumbai, India) and the homogenate centrifuged at 15,000 rpm for 30 min. The supernatant was collected, filtered through a 0.45 μ filter, and analyzed spectrophoto-metrically at 369 nm after appropriate dilution with PBS [17].

Stability studies

The purpose of the stability testing was to determine the stability of the proniosomes over time under a variety of conditions, namely, temperature, humidity and light. The best batch of irinotecan-loaded proniosomes (F2) was used for test. All the preparations were divided into three sets and were stored at 4 ± 2 ^{o}C in a refrigerator, ambient temperature and humidity (25 ± 2 °C/60 **±** 5 %RH), and 37 ± 2 ºC/65 ± 5 %RH in a humidity control oven (Lab Care, Mumbai, India). Three months later, drug content and *in vitro* drug release of the formulations were determined by the methods discussed previously [18].

Statistical analysis

The data (mean ± standard deviation) were analyzed by Student's t-test using Statistica for Windows (version 5.0, Statsoft, Inc, USA). Significant difference was set at a probability level of $p < 0.05$.

RESULTS

Morphology of dry proniosome powder

Scanning electron microscopy of uncoated maltodextrin (Fig 1A) and dry proniosome powder (Fig 1B) reveals that there is a slight difference in the appearance of their surfaces. The powder in (Fig 1B) appears to be smoother indicating a thin and uniform coating over the maltodextrin powder. Also, based on the scale of the micrograph, no significant change in size of particles was seen. This shows that there is no aggregation of the particles due to surfactant coating. Furthermore, the scanning electron micrograph of the dried proniosome-derived niosome dispersions (Fig 1C) suggests that the niosomes generated from proniosomes were discrete and uniform.

Angle of repose

The angle of repose of the various formulations were 31.20 ± 0.75, 32.40 ± 1.62, 31.70 ± 1.07 and 32.60 ± 2.04 for F1, F2, F3 and F4, respectively. Angle of repose of pure maltodextrin was 32.8±1.87. The angle of repose data for the proniosome formulations and pure maltodextrin indicate that there no significant difference ($p > 0.05$) in their flow properties. This is, however, not consistent with the scanning electron microscopic findings for proniosomes, where it was observed that the proniosome surface was smoother.
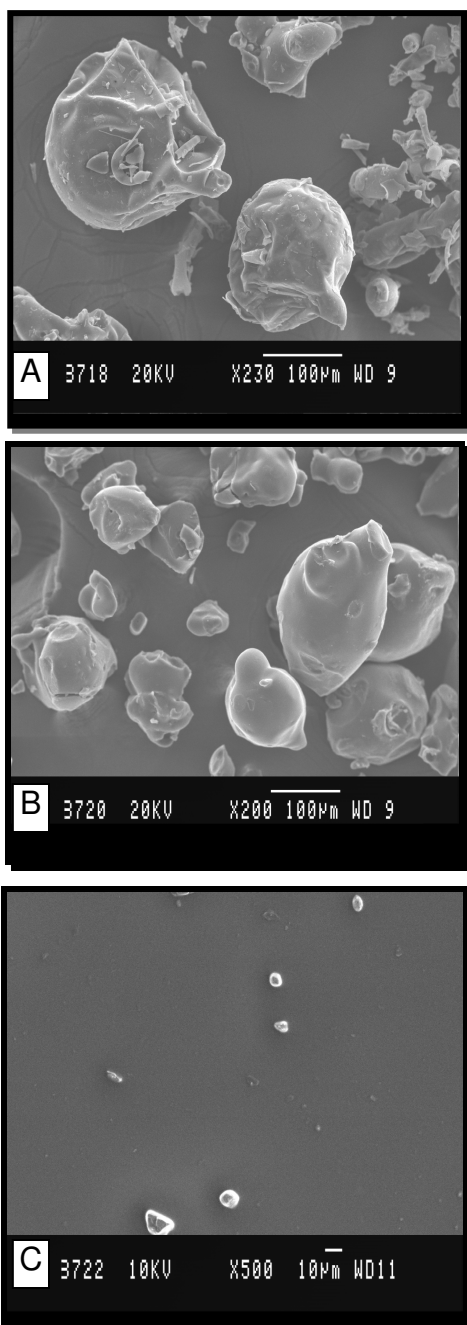
Fig 1: Scanning electron micrograph of (A) maltodextrin powder (uncoated), (B) proniosomes (F2), and (C) niosomes derived from proniosomes

Drug entrapment efficiency

Table 1 shows the entrapment data. The entrapment efficiency of the proniosomal formulations ranged from 69.84±3.5% to 74.94±2.7%for formulations F1 to F4. On the other hand, the entrapment efficiency of conventional niosomes was 61.17±3.5 which is statistically different due to the larger vesicle size of the latter. As the size of vesicle increased, surface area decreased leading also to decrease in drug entrapment.

Table 1: Drug entrapment efficiency of irinotecan-loaded proniosomes (mean ± SD, n = 3)

Formulation code	Entrapment Efficiency (%)
F1	73.58±2.4
F2	74.94±2.7
F3	69.84±3.5
F4	70.46±3.1
Conventional niosomes	61.17±3.5

In vitro drug release

As Fig 2 indicates, cumulative drug release for F-1 to F-4 after 24 h was 96.33%, 95.88% , 98.16% and 97.38% , respectively. On the other hand, cumulative release of pure irinotecan in 5 h was 98.76 %.

In vivo drug distribution

Formulation F-2 with optimum particle size, and with good entrapment efficiency and *in vitro* release, was selected for the *in vivo* drug targeting studies. The distribution of the drug at the various organs following intravenous injection are shown in Fig 3. The mean distribution data, which are a measure of targeting efficiency of drug-loaded proniosomes, was 25.38% for liver, 9.64% in lungs, 9.26% in spleen, 5.7 % in kidney,7.69% in heart, and 21.62% in brain.whereas accumulation of pure drug was 22.46% in liver, 8.86% in lungs, 9.68% in spleen, 11.14% in kidneys, 18.26% in heart and 12.3% in brain of the injected dose.
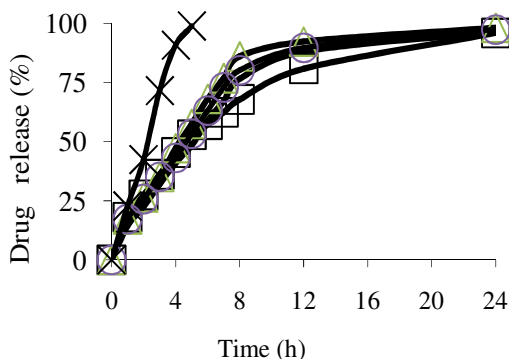
Fig 2: Cumulative drug release of proniosomal formulations and pure drug (◊ = F1, □ = F2, Δ = F3, ○= F4, × = pure drug)
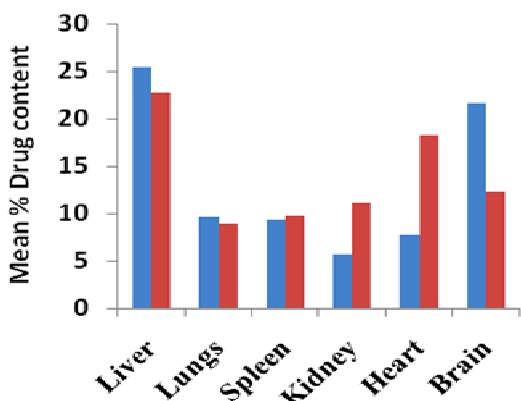

Fig 3: Drug distribution (targeting efficiency) of formulation F2 (blue) and free Drug (red) in selected organs

Stability results

The results of stability studies, shown in Fig 4, indicate that angle of repose, entrapment efficience and drug release rate of the formulations did not change significantly ($P>0.05$) after storage under various conditions for 3 months, thus suggesting that the formulations were stable.

DISCUSSION

Irinotecan is an effective anticancer agent and widely used in colon cancer therapy. However, its clinical use has been limited by dose-related side effects such as severe diarrhea and extreme suppression of the immune systems. Therefore, it is necessary to provide an alternative vesicular drug delivery system for irinotecan in the form of proniosomes which will have advantages of controlled drug release, site specificity, increased drug stability, high drug payload and absence of carrier bio-toxicity.
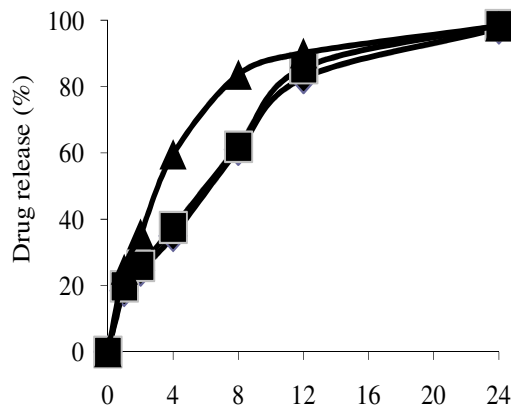

Fig 4: Cumulative drug release of F2 formulation after 3 months following storage at 4 °C (♦), at room temperature (■), and (▲) at 37 °C/65% RH

Preparation of the proniosomes on a maltodextrin carrier was relatively straightforward but it was necessary that the surfactant solution used be incorporated in very small amounts and that complete drying be ensured before further additions are made. As proniosomes are a dry powder, further processing is possible. Angle of repose data indicate that the fluidity of proniosome dry powder is equal to or better than that of maltodextrin powder; therefore, further processing of proniosome powder should not pose any problems. Hydration of the proniosome powder is much easier than the long shaking process required to hydrate surfactants in the conventional dry film method and this can be implemented in a 'point-of-use' application.

Drug entrapment efficiency studies revealed that the change in nonionic surfactant had a significant effect on entrapment of hydrophilic drug, Irinotecan hydrochloride trihydrate.

Entrapment efficiency of proniosomes composed of tweens was relatively low as compared to spans. Higher entrapment efficiency of vesicles of span 60 was predictable because of its higher alkyl chain length. A larger alkyl chain lowers the HLB value of a surfactant and this tends to increase entrapment efficiency of the drug.

The *in-vitro* release of all the four batches of proniosomes showed bi-phasic release with an initial burst effect over the first hour. Thereafter, drug release followed a steady pattern approximating Higuchi matrix release. The burst release in the first hour can be attributed to the drug loaded on the surface of the vesicles as well as to the unentrapped drug in the niosomal suspension. The drug is adsorbed in the lipophilic region (between the bilayers) of the proniosomes where it may undergo rapid ionization and is released until equilibrium is reached [19]. Drug release was regulated by diffusion throughout the swollen niosomal membrane, after the initial burst release, with the remaining drug released over a period of 24 h. Cumulative drug release from F2 (containing Span 60) was lower than from other formulations due to the higher alkyl chain length of the surfactant; the higher the chain length the slower the release. Drug release from the proniosome-derived niosomes was far more controlled than from the conventional niosomes, and may therefore offer improved bioavailability of some poorly soluble drugs with poor solubility.

The *in vivo* drug distribution results reveal that the drug loaded proniosomes showed preferential drug targeting to liver followed by brain, lungs, spleen, heart and kidneys. Compared to pure drug, higher concentrations of drug was targeted to the organs like liver after administering the dose in the form of proniosomes.Higher drug targeting to liver and brain is attributed to the increased lipophilicity of irinotecan loaded proniosomes.

Stability studies of formulation F2 reveal that there was an overall increase in the drug release. These results may be attributed to phase transition of surfactant and lipid causing vesicles leakage to some extent during storage. From the stability data it can be concluded that 4º C is the most suitable condition for storage of irinotecan loaded proniosomes.

Thus, by developing a dry proniosomal formulation, problems related to the hydrolysis of active ingredient or surfactants are eliminated. Since a suspension is formed, precipitation and aggregation are avoided. Maltodextrin-based proniosomes satisfied all these requirements thus indicating that proniosomes are a promising drug carrier.

CONCLUSION

The slurry method used is simple and suitable for laboratory-scale preparation of irinotecan proniosomes. Proniosomes offers an alternative colloidal carrier approach in achieving drug targeting as irinotecan proniosomes were retained at targeted sites and are capable of releasing there drug for the extended period of time.

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