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Lipid based nanocarrier system for the potential oral delivery of decitabine: Formulation design, characterization, *ex vivo*, and *in vivo* assessment

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ABSTRACT

The aim of this study was to design and fabricate nanostructured lipid carrier (NLC) for the potential oral delivery of decitabine (DCB). NLC was prepared by cold homogenization technique and optimized by the Box–Behnken experimental design. It was further characterized by particle size, zeta potential, transmission electron microscopy (TEM), atomic force microscopy (AFM), differential scanning calorimetry (DSC), X-ray diffraction (XRD), *in vitro* release study, and stability study. Moreover, *ex vivo* and *in vivo* efficacy of the NLC was assessed by gut permeation study, γ scintigraphy imaging, and MTT assay. NLC was found to have particle size (116.64 ± 6.67 nm), zeta potential (−31.8 ± 0.96 mV) and sustained drug release (80.23 ± 4.67%) up to 24 h. TEM and AFM proved that the particles were spherical in shape and smooth surface. DSC and XRD studies had demonstrated the reduced crystallinity and stability enhancing effect of the NLC. Stability studies revealed the changes in the observed parameters up to 45 days were not significantly differences ($p > 0.05$). *Ex vivo* gut permeation study showed 4-folds increment in the permeation of drug compared with the plain drug solution. γ Scintigraphy imaging and MTT assay results inferred that DCB loaded NLC possesses excellent cytotoxic activity against cancer cells. Thus, NLC holds high potential for the oral delivery of DCB to treat cancer cells and future prospects for the industrial purpose.

1. Introduction

DCB, a novel drug that inhibits deoxyribonucleic acid (DNA) methylation and approved by the U.S. Food and Drug Administration (USFDA) to Myelodysplastic Syndromes (MDS) in May 2006 (Gore et al., 2006). DCB is an analogue of the natural nucleoside 2′-deoxycytidine reported to have therapeutic activity against various solid tumors and MDS by inhibiting the enzyme DNA methyl transferases, which results in hypomethylation of DNA (Jones and Taylor, 1980; Issa et al., 2005). MDS is a heterogeneous group of bone marrow disorders characterized by ineffective hematopoiesis resulting in anemia, neutropenia, and thrombocytopenia with survival time range from weeks to years (Heaney and Golde, 1999; Silverman and Mufti, 2005).

The reported oral bioavailability of the drug is low (3.9–14%) (Liu, 2012) because it was sparingly soluble in water (22.67 mg/mL), less permeable and unstable in the acidic condition (Garcia et al., 2010). It is also found that DCB gets metabolized by the enzyme cytidine deaminase which is present in the liver (Stresemann and Lyko, 2008). Reduction of the particle size to the nano scale is one of the major approach to enhance the oral bioavailability of the drug (Liversidge and Cundy, 1995). There is a need to design such nanoformulation that could protect DCB from acidic condition and release in the intestinal medium.

Although some colloidal drug carriers like polymeric nanoparticles, nanosuspensions, nanoemulsions had tried to overcome the problems like solubility and bioavailability of the many drugs but they possess the disadvantages of the mammalian tissue toxicity due to use of organic solvents, limited physical stability and leakage of drug during storage (Blasi et al., 2011; Radtke et al., 2005; Muller et al., 1996). Hence, the current focus of the research based on the search of bio-compatible lipids as a carrier for low bioavailable drugs to minimize the above mentioned problems.

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NLC has emerged as a potential drug carrier with lower incidence of tissue toxicity due to use of bio-compatible and biodegradable lipids (Muller et al., 1996; Narvekar et al., 2012). It has reported that lipid protects drug from acidic degradation, increases intestinal permeability, promotes oral absorption through lymphatic transport by reducing first pass metabolism and higher amount of drug loading which enhances the availabile of drug to the systemic circulation (Xie et al., 2011; Pouton, 2006; Porter et al., 2007; Charman, 2000). In addition, NLC showed sustained release of the drug from the lipid matrix which results in the prolongation of the drug concentration within therapeutic window (Xie et al., 2011). Hence, NLC is becoming one of the best selected drug delivery system among the researchers.

Basically, lipid nanocarriers are composed of solid lipids combine with liquid lipids to enhance the drug loading and stabilized by using aqueous surfactant solution. These are produced using high pressure homogenization, ultrasonication, solvent injection, high shear homogenization and solvent evaporation techniques (Neupane et al., 2013; Mehnert and Mader, 2001; Uner and Yener, 2007).

This study thus, aims to design and fabricate thermodynamically stable NLC of DCB by cold homogenization technique using high pressure cell homogenizer. The prepared NLC was characterized for various parameters and its efficacy against cancer cells was evaluated.

2. Materials and methods

2.1. Materials

DCB was taken as the gift sample from the Dabur Research Foundation, India. Transcutol® HP (diethylene glycol monoethyl ether), Compritol® 888 ATO (glyceryl behenate) and Precirol® ATO 5 (glyceryl palmitostearate) were kindly donated by Gattefosse (Mumbai, India). Tween® 80 (polyoxyethylene (20) sorbitan monooleate) (SD Fine Chem), Poloxamer 188 (BASF) and Solutol® HS 15 (polyoxyl 15 hydroxystearate) (BASF) were used as surfactant. Deionized water was obtained from interchanged columns Milli-Q (Millipore, U.S.A). Ammonium acetate (Merck, Bombay), sodium bisulfite (Merck), Methanol HPLC Grade (SRL) were used.

2.2. Selection of excipients

Solid lipid and liquid lipid were selected based on saturation solubility study and miscibility study among them. For saturation solubility study, 500 mg of the solid lipid was kept in the 5 mL glass vials and heated to 5–10 °C above their melting point in water bath. To this, DCB was added in small increments of 1 mg and kept for shaking up to 24 h in the water bath. Solid lipid was selected by observing the loss of transparency on addition of the drug to the melted lipid. Liquid lipid was selected from the miscibility study between solid lipid and liquid lipid. On the other hand, selection of surfactant was carried out by preparation of placebo formulations with different surface active agents. The prepared formulations were evaluated for stability upon storage, particle size, particle sedimentation (data not shown).

2.3. Preparation of DCB loaded NLC

2.3.1. Particle size reduction of DCB in Transcutol® HP before formulating to NLC

DCB was subjected to reduce the particle size prior to formulate the NLC to achieve high entrapment efficiency and drug loading capacity using high pressure cell homogenizer (FPG 12800, Stansted, UK) at room temperature (Kasongo et al., 2012). DCB (15% w/w) was dispersed in the binary mixture of Transcutol® HP and Tween® 80 (2% w/w), and then the mixture was vortexed to produce coarse dispersion. The coarse dispersion was homogenized using the high pressure cell homogenizer for five cycles at pressure 500 and 1000 bar (*e.g.*, 5 × 500 and 5 × 1000 bar). Samples after each five cycles were taken for particle size monitoring using a light microscope to monitor the progress of particle size reduction during each five cycles. The product obtained after homogenization was used for the further production of NLC.

2.3.2. Production of NLC

DCB loaded NLC was prepared by using DCB dispersed in Transcutol® HP as liquid lipid and Precirol® ATO5 as solid lipid, and cold high pressure homogenization technique (Kasongo et al., 2012) with slight modification using high pressure cell homogenizer (FPG 12800, Stansted, UK). Transcutol® HP (4% w/v) containing DCB was added to melted Precirol® ATO 5 (8–16% w/v) heated at 70 °C with continuous stirring. The molten lipid phase was poured immediately into dry ice to solidify it. After solidification, the dried mixture was ground by using mortar and pestle to get fine powder. The powdered mixture was then dispersed in cold aqueous solution of Tween® 80, Polaxomer 188 and Solutol® HS 15 (1:2:3) ratio as the surfactant (4–8% w/v) by using Diax 9000 (Heidolph, Germany) at 5400 rpm for 8 min to produce coarse pre-dispersion. The resulted pre-dispersion was then homogenized using the high pressure cell homogenizer at cold condition at the pressure of 500 and 1000 bar for 8–12 cycles to produce NLC dispersion.

Finally, NLC dispersion was subjected to the lyophilization to produce NLC powder using mannitol as cryoprotectant in the ratio of 2% w/v of the total formulation. Liquid sample was frozen at −20 °C for 24 h and subjected to lyophilization using freeze dryer (Heto Dry Winner, Denmark) at −10 °C for 24 h. The obtained NLC powder sample was subjected to further evaluation.

2.4. Design of the experiment by using response surface methodology (RSM)

The experimental design was performed by using RSM in which 3-factors 3-levels study and 17 experimental runs were obtained with the help of Design Export® Software (Version 8.0.7.1, Stat-Ease Inc., MN, USA). This experimental design was used to investigate the effect of independent variables on various dependent variables. The independent variables were % w/v of lipid concentration (X_1), % w/v of surfactant concentration (X_2) and number of homogenization cycles (X_3) represented by −1, 0, +1, analogous to the low, middle and high levels respectively, while the dependent variables were particle size (Y_1), poly dispersity index (Y_2) and %entrapment efficiency (Y_3) as described in Table 1.

Table 1
Variables and their coded levels in the Box–Behnken design.

Factors	Coded levels		
Independent variables	Low level (−1)	Medium level (0)	High level (+1)
X_1 = lipid concentration (%w/v)	8	12	16
X_2 = surfactant concentration (%w/v)	4	6	8
X_3 = no. of homogenization cycle	8	10	12

Dependent variables	Constraints
Y_1 = particle size (nm)	Optimum (100–200 nm)
Y_2 = poly dispersity index (PDI)	Minimum
Y_3 = entrapment efficiency (%w/w)	Maximum

nm = nanometer, mV = millivolt, %w/v = percentage weight/volume.