Novel polymer coupled lipid nanoparticle based *in-situ* docetaxel formulation

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INTRODUCTION

Docetaxel, belonging to the taxane class of anticancer agents, is perhaps the most important chemotherapeutic agent which has emerged over the past several decades (Rowinsky et al, 1997; Gelmon et al, 1994) for the treatment of ovarian carcinoma, advanced breast cancer, lung cancer and head/neck cancer. The toxicities associated are neutropenia, peripheral neuropathy and hypersensitivity reactions. The marketed formulation available is also responsible for hypersensitivity reactions (Vanhoefer et al, 1997; Capri et al, 1996).

In view of these observations there is a strong rationale for reformulating taxanes using safer and better-tolerated excipients suitable especially for chronic cases. The literature reveals that the payload efficiency achieved with docetaxel in conventional liposomes is not higher and additionally the conventional liposomes are associated with storage stability problems (Immordino et al, 2003). The problem of low payload efficiency and stability was addressed by employing a novel technique of polymer coupled lipid nanoparticle based *in-situ* docetaxel formulation which can further be applied for bed side reconstitution, greatly avoiding stability problem.

MATERIAL AND METHODS

Hydrogeneted soya phosphatidyl cholines (HSPC), egg phosphatidylglycerol (EPG) were purchased from Avanti. Cholesterol, chitosan, triton X-100, dialysis bag (12KD) was purchased from Sigma. Docetaxel was donated by Dabur Research Foundation, India. The water used throughout the experiment was purified with a Milli Q system from Millipore Co., USA.

Preparation of polymer coupled lipid nanoparticle based *in-situ* docetaxel formulation: Ethanolic solution containing 50mg HSPC, 15mg EPG and 15mg cholesterol along with 12mg of docetaxel was prepared by dissolving in 1ml of ethanol. Chitosan coupled lipid nanoparticle were prepared by rapidly injecting ethanolic solution in 10 ml of 0.02% chitosan solution with the help of 1ml insulin syringe (Needle- 29G).To separate un-incorporated drug, docetaxel-liposomes were extensively dialyzed against buffer.

S. No.	Phospholipid Composition	Encapsulation Efficiency(%)	Docetaxel Conc.(mg/ml)
LP 1	HSPC/EPG/CHOL. (3.5:1:2) [In 0.02% Chitosan Solution]	96.4±1.8	0.98±0.12

Table. 1. Characteristics of polymer coupled lipid nanoparticle based *in-situ* docetaxel formulation

Characterization: Size and zeta potential of docetaxel coupled lipid nanoparticle were determined by Zetasizer Nano ZS (Malvern,UK). The size & ζ-potential value was the average of three successive measurements. Morphology of coupled lipid nanoparticle was examined by transmission electron microscope (TEM, Philips CM-10). To separate unincorporated drug, polymer coupled lipid nanoparticle based *in-situ* docetaxel formulation were extensively dialyzed against buffer overnight at 4°C. Vesicles were lysed with triton X-100. The amount of docetaxel incorporated in nanoparticulate vesicles was determined by HPLC (Merck C-18 column, 5µm). The column was eluted with acetonitrile–water (60:40). Detection was done by UV adsorption measurement at 229 nm (flow rate: 1 ml /min). The drug concentration was calculated from standard curves. The assay was linear over the tested concentration range (20–1000 ng). The release profile of polymer coupled lipid nanoparticle (LP1) was determined in phosphate buffer (pH=7.4) using dialysis membrane. The in-vitro cytotoxicity of formulations compared with docetaxel drug solution (containing tween 80 & absolute ethanol, similar to marketed formulation) was measured by proliferation assay utilizing tetrazolium dye, MTT (Mosmann et al, 1983). The experiments were carried out using HepG-2 cells and MCF-7 cells in the exponential growth phase.

RESULTS AND DISCUSSION

A novel *in-situ* polymer coupled lipid nanoparticle based docetaxel formulation for bed-side reconstitution was prepared by ethanol injection method. Lipid composition (Table-1) was optimized for 5-15 mol% entrapment of docetaxel. Drug was determined in supernatant as well as in lysed formulation to confirm mass balance. It was found that ~12 mol% docetaxel was completely encapsulated. The maximum drug loading in vesicles was 96.4±1.8% with 12 mol% docetaxel in overall formulation. The reason behind high encapsulation is associated with good solubility of both drug & lipids in ethanol. When rapidly injected in 0.02% chitosan solution, small vesicles of lipid form in nanometer size range along with docetaxel incorporated in lipid layers. Chitosan coating formed on outer lipid layer due to electrostatic attraction with highly anionic lipid (e.g. EPG) present in lipid layers. The mean particle size & size distribution data has been represented in Fig.1. The average mean diameter of polymer coupled lipid nanoparticle is 113.3±4.5 nm (PDI – 0.38). It reveals that the all the vesicles are in the range and distribution is monodisperse.

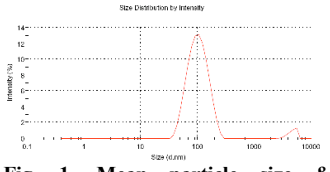

Fig. 1. Mean particle size & size distribution of plain & chitosan coated docetaxel liposomes (LP1)

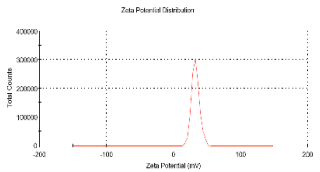

Fig. 2. Mean zeta potential of plain & chitosan coated docetaxel liposomes (LP1)

ζ-potential value (Fig. 2) of LP1 is (+) 35.7±2.9 mV (SD±4.1). Higher cationic charge proves chitosan covering on lipid nanoparticles. This shows the suitability of ethanol injection method for preparation of nano-sized vesicles with narrow size distribution (PDI) and enhanced stability as surface charge is sufficient to stabilize the vesicles. As lipid solution is supplied separately and chitosan coupled lipid nanoparticles forms at bed side only by injecting in 0.02% chitosan solution,

so it avoids enormous stability problem associated with particulate type of drug delivery systems. Stability of lipid solution was well established up to three months where degradation as well as any type of precipitation was not observed. Ethanol concentration used in *in-situ* formulation is low and under acceptable FDA limit so there is no need for removal of ethanol and it can directly be used in patients as ready to use as lipid nanoparticulate formulation ruling out long term shelf life stability problem associated with liposomes. This novel in-situ technique helps in cost effective management for hepatic and breast carcinomas. Morphological evaluation of these particles by transmission electron microscopy (TEM) shows circular bilayer structure along with outer polymer layers (Fig 3) with size similar to mean diameter determined by zetasizer.

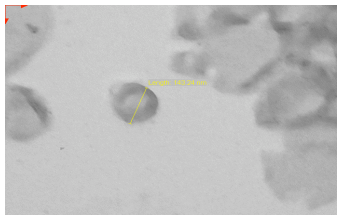

Fig. 3. TEM image of polymer coupled lipid nanoparticle (LP1).

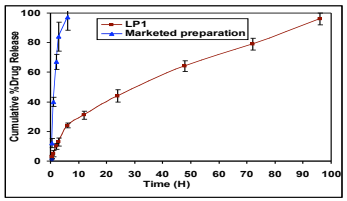

Fig. 4.Drug release profile of LP1 and marketed formulation in PBS (pH=7.4). (n=3).

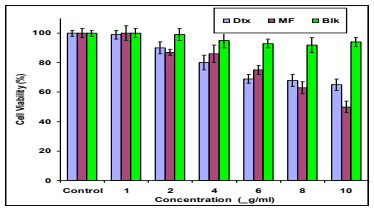

Fig. 5. % cell viability in MCF-7 cells after the exposure of various concentrations of drugs for 96h

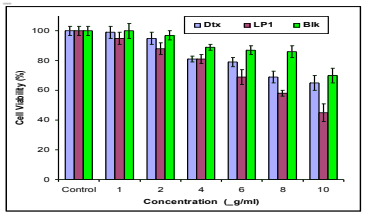

Fig. 6. % cell viability in HepG-2 cells after the exposure of various concentrations of drugs for 96h

The release profile indicates complete drug release from marketed docetaxel formulation within 6h while polymer coupled lipid nanoparticle controlled drug release for more than 96h. Burst release from polymer coupled lipid nanoparticle is 4±0.9% as compared to 15% in marketed formulation. This also shows that most of the drug is encapsulated inside the system only and very less drug present on the surface. Chitosan coupled lipid vesicles proved to be excellent release controlling coating membrane and it demonstrated superiority over docetaxel liposomes (48h) also reported in literature (Immordino et al, 2003). The $t_{50\%}$ of LP1 was found to be 36h.

The cytotoxicity of LP1 was evaluated in-vitro by the MTT assay and compared with marketed formulation. The assay is based on mitochondrial dehydrogenase cell viability (Liang et al, 2006). The study was carried out on HePG-2 and MCF-7. When docetaxel loaded liposomes were exposed at different concentration there was dose dependent increase in cytotoxicity in both cell line studied.

The inhibition of the growth of cells by the LP1 was higher when compared to that of a marketed docetaxel formulation ($p< 0.05$). At the concentration of 10µg/ml, LP1 showed 47% cell viability compared to 65% in case of marketed docetaxel formulation at similar concentration in MCF-7 cell lines. Similarly results were seen in HePG-2 cells also. Highly cationic vesicles due to chitosan coupling act synergistically with docetaxel and thus showed significant higher cytotoxicity compared to marketed docetaxel formulation.

CONCLUSIONS

In conclusion, the most significant finding from our study is that it was possible to in-situ formulate polymer coupled lipid docetaxel in-situ formulation with almost complete encapsulation and good size distribution without using costly equipment and cumbersome processing. Moreover chitosan coating further extended the release and proved to be good release controlling barrier.

Novel method of in-situ liposomes preparation almost solves the most troublesome problem of liposome stability. Polymer coupled lipid docetaxel in-situ formulation showed dose dependent significant increase in cytotoxicity in MCF-7 & HePG-2 cancer cell lines. Moreover, the proposed system provides ample of opportunity to in-situ formulate various drugs easily. Further investigations are still underway to gather toxicity profile of the proposed formulation.

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