P-035 **Novel polymer coupled lipid nanoparticle of docetaxel for *in-situ* application**

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INTRODUCTION AND OBJECTIVES

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] for the treatment of cancer but associated with toxicities as neutropenia, peripheral neuropathy and hypersensitivity reactions. The marketed formulation available is also responsible for hypersensitivity reactions [Capri et al, 1996].

In view of these observations there is a strong rationale for reformulating taxanes using safer and better-tolerated excipients suitable for chronic cases. The literature reveals that the payload especially efficiency achieved with docetaxel in conventional liposomes is less and associated with storage stability problems [Immordino et al, 2003]. These problems were addressed by employing a novel technique for formulation of polymer coupled *in-situ* lipid nanoparticles of docetaxel (LP1) which can be applied for bed side reconstitution, further minimizing cost and stability issues.

MATERIALS 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 LP1

Ethanolic solution containing 50mg HSPC, 15mg EPG and 15mg cholesterol along with 12mg of docetaxel was prepared by dissolving in 1ml of ethanol. PL1 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, LP1 were extensively dialyzed against buffer overnight at 4°C.

Characterization

Size and zeta potential of LP1 were determined by Zetasizer Nano ZS (Malvern,UK). Morphology was examined by transmission electron microscope (TEM, Philips CM-10). 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 assay was linear over the tested concentration range (20–1000 ng). The release profile of LP1 was determined in PBS (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 MTT assay using HepG-2 cells and MCF-7 cells in the exponential growth phase.

Table 1 : Composition of LP1

Contents	EE (%)	Drug Conc. (mg/ml)
HSPC:EPG:Chol (3.5:1:2)	96.4±1.8	0.98±0.08

RESULTS AND DISCUSSION

A novel *in-situ* polymer coupled lipid nanoparticle of docetaxel for bed-side reconstitution was prepared by ethanol injection method. Lipid composition (Table-1) was optimized for 5-15 mol% entrapment of docetaxel. 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 average mean diameter of LP1 was 113.3±4.5 nm (PDI: 0.38). It reveals that the all the vesicles are in the range and distribution is monodisperse.

ζ-potential value of LP1 was found to be (+) 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. *In situ* lipid nanoparticle greatly avoid stability problem as it forms at bed side of patients by injecting drug lipid concentrate in chitosan solution. Stability of drug lipid concentrate was well established up to six-months for degradation and precipitation. Ethanol con-

centration used in *in-situ* formulation is low and under acceptable FDA limit so there is no need for removal of ethanol after in situ LP1 formulation. This novel technique helps in cost effective management for hepatic and breast carcinomas. Morphological evaluation of these particles by TEM shows circular bilayer structure along with outer polymer layers (Figure 1)

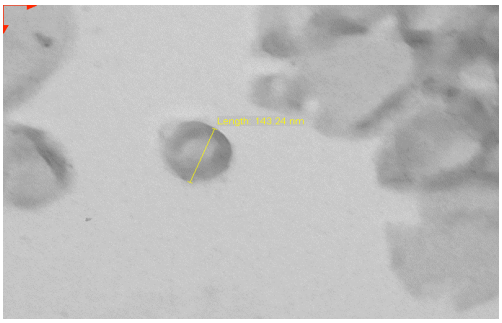

Figure 1: TEM (LP1)

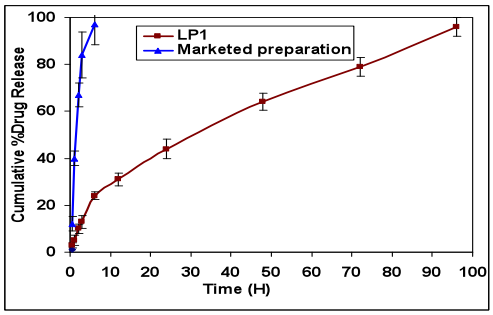

Figure 2: Drug release profile

The release profile indicates complete drug release from marketed docetaxel formulation within 6h while LP1 controlled drug release for more than 96h. Burst release from LP1 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) [Immordino et al, 2003]. The $t_{50\%}$ of LP1 was found to be 36h. The cytotoxicity of LP1 was evaluated and compared with marketed formulation in-vitro by the MTT assay on HePG-2 and MCF-7 cell lines (Liang et al, 2006). LP1 exposed at different conc. showed dose dependent increase in cytotoxicity in both cell line studied.

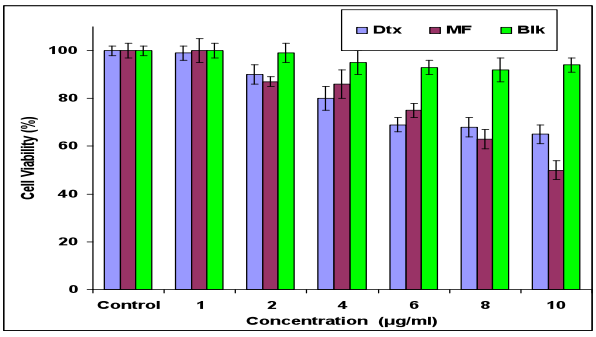

Figure 3: % cell viability in MCF-7 cells

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

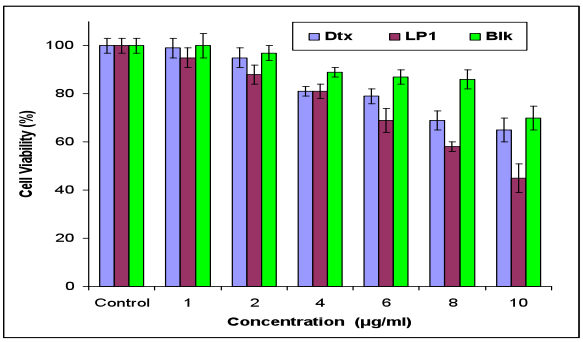

Figure 4: % cell viability in HepG-2 cells

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 as plateform technology for development of various in-situ formulations. Further investigations are still underway to gather toxicity profile of the proposed formulation.

REFERENCES

- Rowinsky E.K. et al. (1997) *The development and clinical utility of the taxane class of antimicrotubule chemotherapy agents.* Annu Rev Med 48 353–374.
- Capri G. et al. (1996) *The role of taxanes in the treatment of breast cancer*. Semin Oncol 23 68–75.
- Immordino M. L. et al. (2003) *Preparation, characterization, cytotoxicity and pharmacokinetics of liposomes containing docetaxel.* J Control Rel 91 417-429.
- Liang H.F. et al. (2006) *Paclitaxel loaded poly (γ - glutamic acid)-poly (lactide) nanoparticles as a targeted drug delivery system against cultured HepG-2 cells.* Bioconj Chem 17 291-299.