Lipopolymeric Nanoparticles: Platform Technology for Aerosolized Combination Chemotherapy

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ABSTRACT

The present study describes conceptualization and formulation of a novel nanoscale delivery vehicle as a platform for combination chemotherapy, to be given in aerosolized form for lung cancer treatment. The formulation (PSCs) is prepared by conjugation of phosphatidyl serine based lipid nanovesicles encapsulating paclitaxel (anti-cancer agent) with dopamine (anti-angiogenic agent) loaded chitosan nanoparticles. The size of the nanoparticles was found to be 347.2 ± 43 nm. Drug release kinetics was studied for both the drugs and a faster release of dopamine was observed, 50% release in 10 hours as compared to 11% release of paclitaxel. The formulation maintained the airway patency and formed aerosols, around 60% of which were deposited in the stage II corresponding to terminal airways, indicating its feasibility to be given via inhalation route. Further, enhanced cellular uptake and low IC50 of 31 nM in A549 cells show the significant therapeutic potential of the formulated nanoparticles.

Keywords: nanoparticles, aerosol, combination therapy, anti-angiogenic agent, lung cancer

1 INTRODUCTION

Lung cancer causes maximum percentage of deaths among those caused by different cancers in the world [1]. As surgical resection is beneficial only for cases of primary lung cancer, chemotherapy is widely used for majority of lung carcinoma patients. Conventional chemotherapy has certain disadvantages associated with it like systemic toxicity, low therapeutic index due to poor bioavailability and non-specific deposition. These can be overcome by using nanoparticles as delivery vehicles for these chemotherapeutic agents. The aim of the present study is to devise a nanoscale delivery vehicle for effective codelivery of two anti-cancer agents with different solubilities.

Combination therapy offers advantage for cancer treatment as two different pathways involved in the cancer development can be targeted simultaneously. So, the present formulation is used for encapsulation of a chemotherapeutic agent as well as an anti-angiogenic agent. Paclitaxel is a potent anti-cancer agent and has been used in the first line treatment of lung cancer. It is encapsulated in a lipid nanovesicle and it shows high encapsulation efficiency due to its hydrophobic nature. Thus it eliminates the requirement and side effects associated with Cremophor which is used in Taxol, the widely used marketed formulation of paclitaxel. The anti-angiogenic agent used in the study is dopamine, which is found to inhibit VEGF induced angiogenesis [2, 3]. To obtain high encapsulation of hydrophillic dopamine, chitosan nanoparticles are used. The application of anti-angiogenic agents with cytotoxic agents have been studied to improve the efficacy of therapy in cancers [4, 5].

The present study aims at formulating a nanocarrier, that can be given in aerosolized form for lung cancer treatment. Local delivery of chemotherapy via inhalation for primary or metastatic lung cancer would increase drug exposure of the lung tumour while minimizing systemic side effects. The non-invasive administration via inhalation also has advantages like avoidance of first pass metabolism, direct delivery to site of action for treatment of pulmonary diseases, and availability of large surface area for local drug action [6]. The formulation is optimized to have maximum deposition in the terminal airways and due to the incorporation of surface active lipids, a high airway patency is maintained. The formulation was evaluated against non small cell lung carcinoma to determine its therapeutic potential.

Therefore, this study aims to devise and optimize a nanoscale platform for efficient delivery of two drugs targeting different pathways involved in cancer development, and to evaluate the feasibility of this formulation for aerosol delivery.

2 METHODS

2.1 Preparation of PSCs nanoparticles

Lipid nanovesicles are prepared by thin film hydration method desribed previously [1], using using 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1,2-dioleoyl-sn-glycero-3-phospho-L-serine (sodium salt) (DOPS) (Lipoid, Germany) in 7:3 molar ratio. Paclitaxel (Fresenius Kabi India Pvt.Ltd.) is added in 1:2 molar ratio with lipids before thin film preparation.

Chitosan (Marine Chemicals, Mumbai, India) nanoparticles are prepared by ionic gelation method [7]. Dopamine hydrochloride (Sigma Aldrich Chemicals) was added to chitosan solution in 1:2 (w/w) ratio.

PSCs nanoparticles are prepared by adding an aliquot of the chitosan nanoparticles (1 mg/ml) solution to the liposomal suspension (2 mg/ml). 100mg N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) and 50mg N-hydroxy succinimide (NHS) were then added to this mixture of nanoparticles. The carbodiimide was solublized using a vortex mixer. The mixture was reacted for 2 hours at room temperature and was centrifuged. The supernatant of the centrifuged solution was discarded and pellet was washed once before resuspending it in TES buffer. Blank nanoparticles are prepared in similar way without addition of drugs in the respective nanoparticles.

2.2 Size and charge measurements

PSCs was characterized for size distribution by dynamic light scattering (DLS) using laser particle analyzer (BI 200SM, Brookhaven Instruments Corporation). The nanovesicles were also characterized for surface charge by determining their zeta potential using zeta potential analyzer (ZetaPALS, Brookhaven Instruments Corporation).

Transmission electron microscopy (TEM) of PSCs was done as per the negative staining protocol and imageswere analyzed by a transmission electron microscope, model: CM200 (Philips) operating at 120 kV.

2.3 Drug release studies

Encapsulation efficiency of paclitaxel in the nanovesicles was determined by break opening them using methanol and quantifying the drug using reverse phase HPLC (Agilent 1100 Binary LC pump liquid chromatograph). Encapsulation efficiency of dopamine in chitosan nanoparticles is determined spectrophotometrically by quantifying the amount of drug in supernatant after centrifugation.

In vitro release study of both the drugs from PSCs was performed by dialysis bag method at pH 7.4 and 37 °C temperature conditions with proper sink [1].

2.4 Airway patency and *in vitro* lung deposition

Capillary surfactometer (CS) from Calmia Biomedicals (Toronto, Ontario) was used to study the airway patency and surfactant ability of PSCs. The airway patency of our formulation was studied as % opening time of capillary over an observation period of 120 seconds and was compared against to that of Taxol and dopamine solution.

In vitro lung deposition studies for PSCs were performed using glass twin impinger apparatus (Copley Scientific, Nottingham, UK), adapted from apparatus A of European and British Pharmacopoeia [1].

2.5 *In vitro* cytotoxicity

PSCs was evaluated on A549 non small cell lung aenocarcinoma and NCI-H460 human lung cancer cell line for cytotoxicity. Both the cell lines were purchased from National Centre for Cell Sciences (NCCS) Pune, India. The cytotoxicity of paclitaxel containing PSCs is carried out in both the cell lines and IC50 values were calculated using GraphPad Prism 4 software.

2.6 Cellular uptake studies

Cellular uptake and internalization studies were carried out for PSCs in A549 cell line. Hydrophobic dye nile red was incorporated in lipid nanovesicles and hydrophillic calcein was loaded in chitosan nanoparticles. Dye loaded PSCs was incubated with cells for 3 hours, and then analysed using confocal laser scanning microscope (CLSM) (Olympus Fluoview, FV500, Tokyo, Japan). Images were acquired with 60X oil immersion objective using the Fluoview software (Olympus, Tokyo, Japan). Cells incubated with equivalent amount of free dye were used as control.

3 RESULTS AND DISCUSSION

3.1 Preparation and physiochemical charaterization of PSCs

Size of individual nanoparticles and PSCs are summarized in Table 1. The size is further confirmed by TEM image as shown in Fig. 1.

Nanoparticle	Hydrodynamic diameter (nm)	Polydispersity index
Liposomes	238.2 ± 7.4	0.11 ± 0.02
Chitosan nanoparticles	101.3 ± 15.3	0.21 ± 0.02
PSCs	347.2 ± 43	0.25 ± 0.03

Table 1: Particle sizes as obtained by DLS

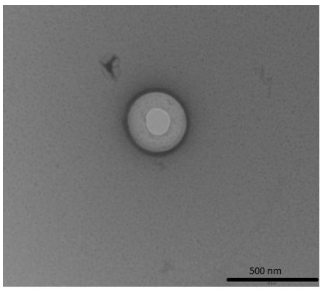

Figure 1: TEM image of PSCs (scale bar =500 nm)

From TEM images as well as zeta potential comparision (Fig. 2), it is clear that chitosan nanoparticles form a coating on the surface of liposomes as a result of EDC linkage between the carboxy groups of serine based liposomes with amine groups on chitosan nanoparticles.

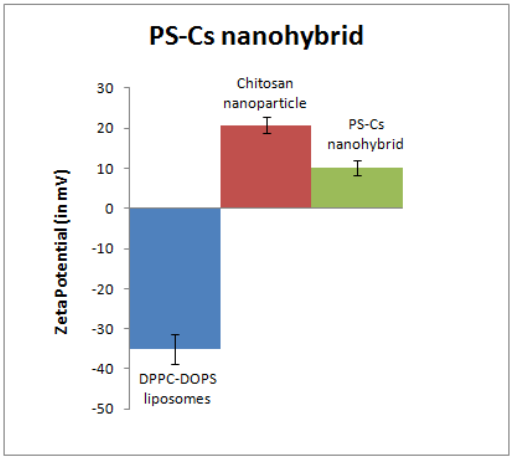

Figure 2: Zeta potential of PSCs and associated nanoparticles

3.2 Drug release studies

Paclitaxel was loaded in lipid nanovesicles at a high encapsulation efficiency of 85.4 ± 4 %, due to its hydrophobic nature and thus affinity for lipid bilayer. Dopamine was encapsulated with 20.5 ± 5.3 % loading efficiency in chitosan nanoparticles.

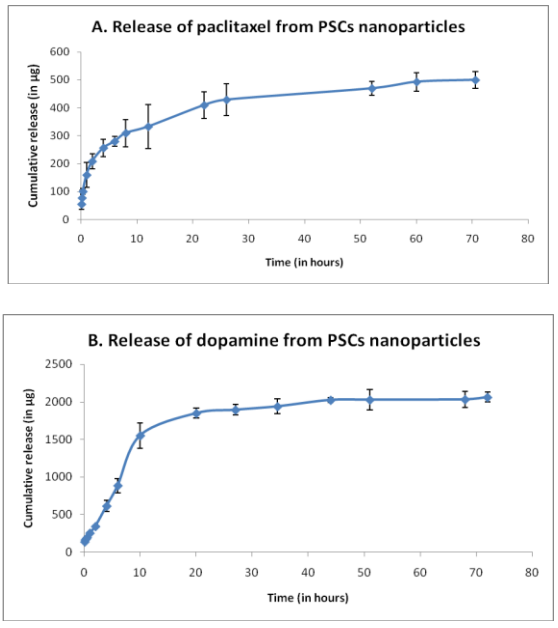

Figure 3: *In vitro* release of drugs from PSCs at 37°C

Drug release kinetics were studied for both the drugs from PSCs, as shown in Fig. 3. A faster release of dopamine was observed, 50% release in 10 hours as compared to 11% release of paclitaxel. Initial burst release of dopamine will cause the nanoparticles to get entrapped in tumor tissue due to vascular shutdown, followed by sustained release of paclitaxel causing tumor cell death.

3.3 Airway patency

Airway patency of the PSCs formulation is compared with individual paclitaxel and dopamine drug solutions after being evaluated in terms of % opening time of capillary in capillary surfactometer. PSCs had shown 97.8 ± 1.6 % capillary opening time as compared to 1.2 ± 0.4 % for Taxol and 18 ± 3.8 % for dopamine solution for the entire observation period of 120 seconds. This clearly indicates the superior surfactant properties of the formulation which makes it suitable for aerosol administration without the risk of airway blocking.

3.4 *In vitro* lung deposition

In vitro lung deposition of paclitaxel loaded formulation was studied using a glass twin impinger. Fig. 4 shows the deposition in two stages of the impinger as a result of 2 minute nebulization. The deposition pattern depends on factors like mass median aerodynamic diameter (MMAD), density and speed of impaction of the aerosol droplets.

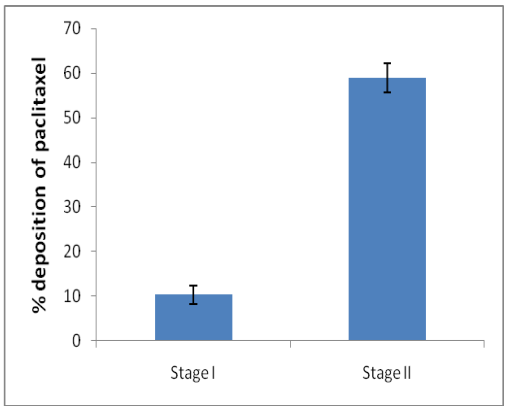

Figure 4: % deposition of paclitaxel in two stages of twin impinger after 2 minutes of nebulization of PSCs

Higher deposition in stage II is correlated to a higher deposition in alveolar region. At an airflow of 60 l/min, the cutoff aerodynamic diameter for deposition in lower impingement chamber is 6.4 µm. Thus, a statistically significant ($p<0.05$) deposition of PSCs in lower chamber of the impinger shows its capability to reach terminal airways and suitability for aerosol administration.

3.5 *In vitro* cytotoxicity

Cytotoxicity was evaluated by SRB (Sulforhodamine B) assay in A549 and NCI-H460 cells by incubating the formulation for a period of 48 h. As shown in Fig. 5, the IC50 of PSCs containing paclitaxel is significantly lower ($p<0.05$) than paclitaxel alone in both the cell lines. It shows clear advantage of the formulation in increasing the therapeutic potential of the drug.

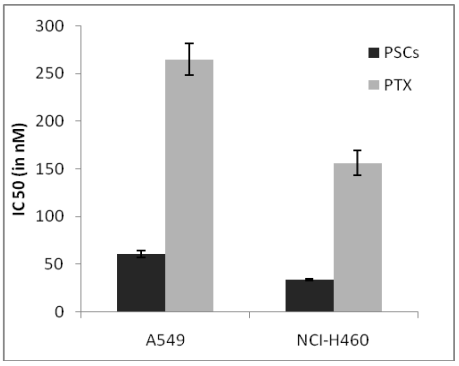

Figure 5: *In vitro* cytotoxicity profile of the formulation PSCs and paclitaxel (PTX) in A549 and NCI-H460 cells

3.6 Cellular uptake of PSCs

Cellular internalization of PSCs in A549 cells is determined by using dye loaded formulation. Nile red was entrapped in lipid bilayer of nanovesicles and calcein is encapsulated in chitosan nanoparticles. As seen in Fig. 6, both red and green dyes are colocalized in the cells with formulation, indicating the uptake of PSCs as compared to free dye which is not internalized by the cells.

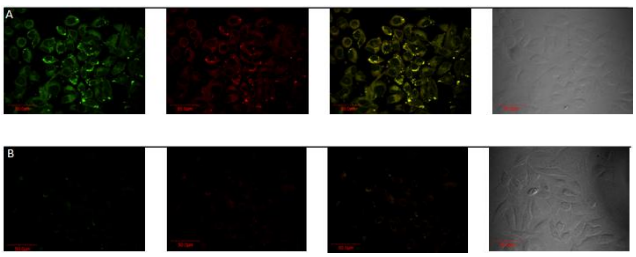

Figure 6: CLSM images of A549 cells after 3h of incubation with dye loaded PSCs particles (A) and free dye (B)

The z scan or depth scan of these cells is also taken to prove that the particles are internalized by the cells and not just localized on the cell surface. The z-scan is carried out from -10 μM to 10 μM with maximum fluorescence intensity at 0 position, indicating the complete internalization of the dye loaded PSCs nanoparticles.

4 CONCLUSION

Lipopolymeric nanoparticles have been prepared and characterized for dual drug delivery. As the formulation shows high airway patency and increased alveolar deposition in suitable *in vitro* models, it is feasible to administer it as an aerosol. *In vitro* studies show that the formulation is internalized in A549 cells and significantly reduces the IC50 as compared to the free drug. The study suggests that the lipopolymeric nanoparticles developed are promising for therapy in lung cancer.

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