

FORMULATION, STABILITY AND PHARMACOKINETIC STUDY OF PACLITAXEL LOADED POLY (L-LACTIDE) NANOPARTICLES

B J MISHRA, P TRIVEDI*

School of Pharmaceutical Sciences, Rajiv Gandhi Proudyogiki Vishwavidyalaya, Bhopal, Madhya Pradesh, India

The surface modification of nanoparticles has been largely explored for improving the release kinetics of drug molecules. In this study we have described the formulation of paclitaxel loaded poly (L-lactide) nanoparticles conjugated with L-cysteine as the surface modifying ligand and established the in vivo release kinetics of the formulation relative to pure drug solution and stability analysis at different storage conditions. The results obtained from the study exhibited particles of size < 200 nm, encapsulation efficiency of 92.65% and sustained release over a period of 24 h. The elimination half life of the drug from the nanoparticles was significantly increased. The nanoparticles were found to be most stable at the refrigerated conditions of storage.

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1. Introduction

The selective localization of drug to the proposed site of action has got clear therapeutic advantages such as reduced toxicity and smaller drug levels. Developing an efficient drug delivery system has been the area of interest amongst the researchers in last two decades. The use of colloidal carrier linked delivery systems is believed to reduce the undesired effect of drugs through controlled biodistribution at the target site [1]. The major drawback associated with these particulate delivery systems is the rapid clearance from circulation by the reticuloendothelial system (RES) [2,3]. Various approaches have been used to reduce the uptake of the carrier by RES. The most promising approach is the surface modification of the carrier by dysopsonic polymer such as poly (ethylene glycol) (PEG) [4,5]. Pegylation refers to the decoration of a particle surface by the covalent grafting, entrapment or adsorption of PEG chains. PEG chains can also be incorporated as copolymers throughout the particle. Modification of the surface of the particulate carrier also allows for targeting the carrier to desired site [6].

The main objectives of designing nanoparticulate delivery systems are controlling the particle size, modulating the surface properties and release kinetics of the encapsulated drug molecule to achieve an optimum dosage regimen. The nanoparticulate delivery systems not only tend to increase the stability of drugs but also are able to control the release of drug molecules over a longer period [7].

Several modifications of nanoparticle surface by targeting ligands have been reported in scientific databases for improvising targeting, stability and release of drugs from delivery systems. Glycosylated nanoparticles have been widely studied [8,9], fluorescein-PLGA conjugated nanoparticles for in vivo detection for targeting efficiency [10], folate decorated nanoparticles for targeting to tumors [11], polysorbate 80 coated nanoparticles for brain targeting [12], poly (L-lysine)-GRGDS surface modifier for PLA [13], fatty acids modified conjugates for improved targeting [14], amongst others, have been prepared successfully.

The purpose of the present work was to evaluate the pharmacokinetic profile of L-cysteine (L-cys) modified paclitaxel (PTX) loaded poly L-lactide (PLA) nanoparticles after i.v.

*Corresponding author: prof_piyushtrivedi@yahoo.com

administration in rats. The stability of the nanoparticles was also evaluated in terms of size and drug content in the nanoparticles after 6 months at different conditions.

2. Experimental

Paclitaxel was obtained as a kind gift from Cipla Pharmaceuticals Limited, India. All other chemicals used in the study were of analytical grade. Deionized water, filtered through 0.22 μ m nylon filter was used throughout the study.

2.1 Preparation of PLA nanoparticles

PLA nanoparticles containing PTX were formulated by simple emulsion solvent evaporation technique. PLA and PTX were dissolved in dichloromethane (DCM). This solution was added to a 0.5% w/v aqueous solution of Pluronic-F 68 over a period of 15 min. The emulsion formed was stirred for 3 h to remove DCM.

2.2 Determination of drug content in the nanoparticles

The nanoparticles were centrifuged at 15000 rpm for 30 min at 4°C. The supernatant was decanted and pellet was washed thrice with water to collect nanoparticles. The pellet obtained was dispersed in 10 mL water followed by lyophilization using mannitol (0.1% w/v).

The drug loading efficiency was determined by HPLC. The mobile phase comprised of acetonitrile/water in the ratio 50/50 on a Luna C-18 column at flow rate of 1.0 mL/min and detection wavelength of 228 nm [15].

Lyophilized nanoparticles were dissolved in acetonitrile and vortexed to get a clear solution. 20 μ L of this solution was injected to the column and analyzed by HPLC. Percent encapsulation efficiency (%EE) was defined as the ratio of measured PTX and the initial amount of PTX encapsulated in the nanoparticles.

$$\%EE = \text{amount of PTX in nanoparticles} / \text{initial amount of PTX} \times 100$$

The amount of drug loading (%DL) was defined as the ratio of drug measured in the nanoparticle to the polymer mass used for formulation of the nanoparticles.

$$\%DL = \text{amount of PTX in nanoparticles} / \text{polymer mass} \times 100$$

2.3 Surface modulation of nanoparticles with L-cysteine

Nanoparticles were activated with EDC in activation buffer (mixed phosphate buffer, pH 5.5) and incubated with 300 μ L solution of L-cysteine in coupling buffer (pH 8.2-8.5).

2.4 Physicochemical characterization of L-cys nanoparticles

The average particle size and poly dispersity index of the surface modified nanoparticles was determined by dynamic light scattering in water using a Malvern zetasizer. The experimental values are average of three different formulations.

2.5 Stability analysis

In order to investigate the physical and chemical stability of the nanoparticles, the samples were stored in amber colored glass vials with rubber stopper and the stability study was conducted at 4°C, 40°C and room temperature for 6 months [16]. The samples were analyzed after 6 month for changes in size, PDI and drug content.

2.6 Pharmacokinetic evaluation

All the experiments were approved by the Institutional Animal Ethical Committee and were performed in accordance to the guidelines of the ethical committee. Healthy male albino rats (200-250 g) were used in the study. Animals were maintained on standard pellet diet with free access to water in a 12-h light and dark cycle. The aqueous nanoparticle dispersion equivalent to 6 mg/Kg of PTX in saline was administered to rats by intravenous route in tail vein. Three rats per group were used for the study. All the animals were fasted overnight with access to water *ad libitum* before dosing.

About 0.5 mL blood samples were collected from retro orbital plexus of rat eye at 0, 1, 2, 4, 6, 8 and 24 h after dosing. The estimation of PTX in blood was performed by HPLC. The chromatographic conditions comprised of C-18 column with PDA detector; wavelength of estimation was 228 nm and mobile consisting of water, acetonitrile and methanol (40:30:30) at a flow rate of 1.0 mL/min [17].

To a 200 μL of serum sample obtained from animals, was added 200 μL of acetonitrile and the mixture was vortexed for 1 min and centrifuged at 13000 rpm for 8min at 37°C. 20 μL of the supernatant was injected onto HPLC.

2.7 Pharmacokinetic parameters of PTX in serum

The pharmacokinetic parameters were determined by analyzing the data by Wagner-Nelson method. Clearance, half-life, Area under curve (AUC_{0-24}), volume of distributions and elimination rate constant were determined in blood samples.

2.8 Statistical analysis

The data obtained are a mean of three readings and was statistically analyzed by *student's t test*. All the results represent a statistical significance with $p < 0.05$.

3. Results and discussions

In this study, nanoparticles of PTX were obtained by simple emulsion solvent evaporation process and the stability analysis and *in vivo* pharmacokinetic analysis of the formulation was performed. The method used for encapsulation of drug substance into nanoparticles usually depends on the solubility of the drug and polymer.

3.1 Characterization of L-cys modified nanoparticles

The amount of conjugation was determined by Ellman's reagent assay for determination of sulfhydryl groups, suggesting a conjugation of 72 % L-cysteine to nanoparticle surface. The encapsulation efficiency of the nanoparticles was found to be 92.65 ± 0.002 % with a drug loading capacity of 1.854 %. The average particle size of the L-cys conjugated nanoparticles was found to be 263 ± 8 nm.

3.2 Stability analysis

The L-cys modified nanoparticles were subjected to stability analysis at room temperature, 4°C and 40°C/75 % RH. The formulation was evaluated for physical appearance, particle size and drug content (% EE and % DL). The results are summarized in table 1. The results revealed that there was not much difference in the % EE and % DL of the nanoparticles stored at 4°C. The size of the nanoparticles increased very marginally at this storage temperature. The nanoparticles tend to aggregate at room temperature and in the accelerated conditions of storage as revealed by the increased average particle size. The amount of degradation of the particles was significantly high at 40°C/75 % RH decreasing the amount of the drug content in the nanoparticles to around 60 %.

Table 1. Stability parameters of Nanoparticles at different storage conditions.

Parameter	Room temperature	4°C	40°C
Particle size	315 nm	270 nm	567 nm
% drug loading	1.208	1.742	1.126

3.3 Pharmacokinetic parameters of PTX in serum

The L-cys conjugated nanoparticles and pure drug solution of PTX were subjected to pharmacokinetic analysis in rats. The serum drug concentration of PTX solution and the L-cys conjugated nanoparticles is presented in figure 1. The pharmacokinetic parameters are summarized in table 2. The values obtained for area under curve and the elimination half life of PTX nanoparticles were found to be much higher (3-4 times) than the pure drug solution. The clearance rate of the nanoparticles was significantly lower than that of pure drug as evidenced from the increased steady state release of drug from the nanoparticles over a period of 8-24 h whereas the concentration of the drug reached to null over the same period.

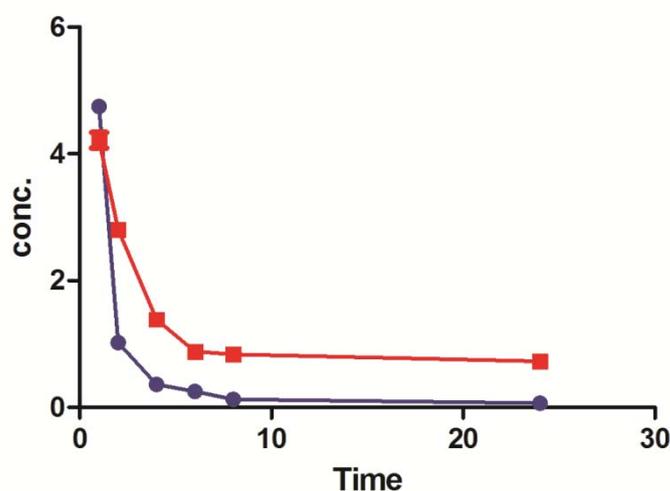


Fig 1. Pharmacokinetic profile of pure drug paclitaxel and nanoparticles

Table 2. Pharmacokinetic parameters of paclitaxel loaded nanoparticles compared to pure drug

PK Parameters	Units	PTX solution	PTX CNP
Elimination rate, K_{el}	l/h	0.0605	0.0217**
Elimination half life, $t_{1/2}$	H	11.45455	31.93548**
Volume of distribution, V_D	mL/kg	1.483313	1.659522**
AUC ₀₋₂₄	µg.h/mL	6.8579	24.2402**
AUC _{0-∞}	µg.h/mL	133.8347	333.2258**

** $p < 0.05$, at 95% CI

4. Conclusion

The objective of the study was accomplished by formulating paclitaxel loaded nanoparticles using PLA and conjugating them with L-cysteine to modify the surface properties. The study led to the conclusion that the nanoparticles conjugated to L- cysteine were stable and was able to produce prolonged release of the drug at therapeutic levels. The storage of the nanoparticles should be at refrigerated conditions. Hence it can be concluded that the formulation developed in this study could be used as a sustained release formulation of delivery of drug molecules.

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