Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v30i16.2570

L-THREONINE DECORATED PACLITAXEL POLY (L-LACTIDE) NANOPARTICLES: FORMULATION, PHARMACOKINETIC AND STABILITY STUDY

Brijeshkunvar Mishra[1*], Jagdish Chandra Pati[2], Richa Mishra[3], Manmeet Singh Saluja[2]

[1*]Research Scholar, Sunrise University, Alwar, Rajasthan.
[2]Research Supervisor, Sunrise University, Alwar, Rajasthan.
[3]Director, RB Science (Research Lab), Bhopal, Madhya Pradesh.

***Corresponding Author:** Brijeshkunvar Mishra,
*Research Scholar, Sunrise University, Alwar, Rajasthan. Email- bjmishra08@gmail.com

Abstract
A lot of research has been done on surface modification of nanoparticles to enhance the kinetics of drug release. The formulation of paclitaxel-loaded poly (L-lactide) nanoparticles conjugated with L-threonine as the surface-modifying ligand has been described in this study, along with the formulation's in vivo release kinetics in comparison to pure drug solution and stability analysis under various storage conditions. According to the study's findings, the particles had an average size of 200 nm or less, had an encapsulation efficacy of 92.6 percent, and released continuously for 24 hours. The drug's nanoparticle elimination half-life was dramatically lengthened. The most stable storage settings for the nanoparticles were determined to be in a refrigerator.

Keywords: Surface-modification, stability, nanoparticle, L-threonine, stability

1. Introduction

With less toxicity and lower drug levels, the selective localization of a medication to the desired site of action has obvious therapeutic benefits. Over the past two decades, researchers have been interested in creating an effective medication delivery system. Through carefully managed biodistribution at the target site, colloidal carrier linked delivery systems are thought to lessen the adverse effects of medications (Putney 1998). The reticuloendothelial system's (RES) fast clearance from circulation is the main disadvantage of these particle delivery systems (Proffitt et al. 1983; Gref et al. 1994). The absorption of the carrier by RES has been decreased using a variety of techniques. The most promising method (Storm et al. 1995; Kaul and Amiji 2002) involves coating the carrier with a dysopsonic polymer, such as poly (ethylene glycol) (PEG). The process of decorating a particle surface with PEG chains through covalent grafting, trapping, or adsorption is known as pegylation. Additionally, PEG chains can be inserted into the particle as copolymers. Targeting the particulate carrier to the targeted spot is also possible with surface modification of the particulate carrier (Gulyaev et al. 1999).

To create an ideal dosing regimen, the key goals of creating nanoparticulate delivery systems include managing the particle size, varying the surface characteristics, and modifying the release kinetics of the drug molecule. In addition to improving drug stability, nanoparticulate delivery methods have the potential to regulate drug molecule release over a longer time periods (Kaul and Amiji 2004).

The surface of nanoparticles can be altered in a number of ways by targeting ligands to improve drug targeting, stability, and release from delivery devices. Folate decorated nanoparticles for targeting to tumours (Zhiping et al. 2007), polysorbate 80 coated nanoparticles for brain targeting (Sun et al. 2004), poly (L-lysine)-GRGDS surface modifier for PLA (Quirk et al. 2001), fatty acids modified conjugates for improved targeting efficiency (Fahmy et al. 2005) are just a few examples of the glycosylated nanoparticles that have been extensively studied (Benjamin and Mark 2002; Egleton et al. 2001)

In continuation to our previous study on paclitaxel loaded nanoparticles (Mishra and Trivedi, 2013), the current study was undertaken with an aim to assess the pharmacokinetic characteristics of poly L-lactide (PLA) nanoparticles loaded with L-threonine (L-thr) modified paclitaxel (PTX) following intravenous delivery in rats. After six months under various circumstances, the stability of the nanoparticles was assessed in terms of their size and drug content.

1. Experimental

Paclitaxel was received as a generous gift from India's Cipla Pharmaceuticals Limited.
Analytical-grade compounds were employed for all other substances in the investigation.
Throughout the investigation, deionized water was filtered using a 0.22 m nylon filter.

3.1 Preparation of PLA nanoparticles

By using the simple method of emulsion solvent evaporation, PLA nanoparticles containing PTX were created. Dissolved in dichloromethane (DCM) were PLA and PTX. Over the course of 15 minutes, this solution was added to a 0.5% w/v aqueous solution of Pluronic-F 68. To get rid of DCM, the emulsion was agitated for three hours (Mishra and Trivedi, 2013).

3.2 Determination of drug content in the nanoparticles

At 4°C, the nanoparticles were centrifuged for 30 minutes at a speed of 15000 rpm. To gather nanoparticles, the supernatant was decanted and the pellet washed three times with water. The resulting pellet was mixed with 10 mL of water before being lyophilized with mannitol (0.1% w/v).
Using HPLC, the drug loading effectiveness was assessed. On a Luna C-18 column, the mobile phase was composed of acetonitrile and water in a 50/50 ratio, with a flow rate of 1.0 mL/min and a detection wavelength of 228 nm (Lirong 2005).
A transparent solution was obtained by dissolving lyophillized nanoparticles in acetonitrile and vortexing the mixture. This solution was injected into the column at a volume of 20 L for HPLC analysis. The ratio between the observed and original amounts of PTX that were encapsulated in the nanoparticles was called percent encapsulation efficiency (%EE).
%EE = amount of PTX in nanoparticles/ initial amount of PTX x 100
The ratio of the drug mass measured in the nanoparticle to the polymer mass utilised to make the nanoparticles was called the amount of drug loading (%DL).
% DL = amount of PTX in nanoparticles/ polymer mass x 100

3.3 Surface modulation of nanoparticles with L-threonine

300 µg of a freshly made L-Threonine (L-thr) solution in coupling buffer were added to the EDC activated nanoparticle suspension with constant stirring for five hours at room temperature. The resultant suspension containing L-thr attached nanoparticles was lyophilized, kept in a desiccator, and rinsed with storage buffer (PBS, pH 7.4).

3.4 Physiochemical characterization of L-thr nanoparticles

Using a Malvern zetasizer and dynamic light scattering in water, the amount of conjugation, average particle size, and poly dispersity index of the surface modified nanoparticles were assessed. Three distinct formulations were used to calculate the average experimental values.

3.5 Stability analysis

The samples were kept in amber-colored glass vials with rubber stoppers while the stability research was carried out at 4°C, 40°C, and room temperature for 6 months in order to examine the physical and chemical stability of the nanoparticles (Aterman 2007). After six months, the samples were examined for changes in size, PDI, and drug content.

3.6 Pharmacokinetic evaluation

The Institutional Animal Ethical Committee gave its approval to all of the trials, which were carried out in accordance with its rules. The study employed male albino rats (weighing 200–250 g) in good health. Animals were kept in 12-hour light/dark cycles and fed a conventional pellet diet with free access to water. Rats were given an intravenous infusion of an aqueous nanoparticle suspension containing 6 mg/Kg of PTX in saline in the tail vein. For the investigation, three rats per group were utilised. Before dosing, all of the animals were allowed unlimited access to water after an overnight fast.

At 0, 1, 2, 4, 6, 8 and 24 hours following dosage, approximately 0.5 mL of blood samples were taken from the retro orbital plexus of the rat eye. PTX levels in blood were calculated using HPLC. According to Rajendar and Narayanan (2009), the chromatographic settings included a C-18 column with a PDA detector, an estimation wavelength of 228 nm, and a mobile phase made up of water, acetonitrile, and methanol at a flow rate of 1.0 mL/min.

A 200 µL sample of animal serum was mixed with 200 µL of acetonitrile, vortexed for one minute, and then centrifuged at 13000 rpm for eight minutes at 37 °C. The supernatant was fed into HPLC in a volume of 20 µL.

3.7 Pharmacokinetic parameters of PTX in serum

The Wagner-Nelson method was used to analyse the data and determine the pharmacokinetic parameters. Blood samples were used to determine clearance, half-life, Area Under Curve (AUC_{0-24}), volume of distributions, and elimination rate constant.

3.8 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, PTX nanoparticles were created using a simple emulsion solvent evaporation procedure, and the formulation's stability and *in vivo* pharmacokinetic investigation were carried out. The process used to incorporate a drug ingredient into nanoparticles often depends on how soluble the drug and polymer are.

3.1 Characterization of L-thr modified nanoparticles

The average particle size obtained was 131.7 nm with a PDI of 0.497 for model nanoparticles, 167.1 nm with a PDI of 0.428 for PTX loaded nanoparticles. The nanoparticles displayed strong negative Zeta potential of -13.0 mV for model nanoparticles and -17.0 mV for PTX loaded particles. The negative surface charge of PLA nanoparticles at physiologic pH contributes to the formation of stable spherical particles. The amount of L-thr coupled to the nanoparticles was determined by amine-group titration. The threonine-conjugated nanoparticles were treated with picric acid and TEA to form a salt of polymer bound NH_2 groups. The concentration of amino groups in this solution is determined colorimetrically, suggesting a conjugation of 64.8 % Lthreonine 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 %.

3.2 Stability analysis

The stability of the L-thr modified nanoparticles was examined at room temperature, 4°C, and 40°C/75% RH. The formulation's physical characteristics, particle size, and drug content (% EE and % DL) were assessed. Table 1 provides a summary of the findings. The findings showed that the EE and DL of the nanoparticles kept at 4°C did not differ significantly. At this storage temperature, there was a very slight increase in nanoparticle size. The increasing average particle size indicates that the nanoparticles have a tendency to agglomerate at room temperature and under accelerated storage conditions. At 40°C/75% RH, a considerable amount of the particles degraded, bringing the drug content of the nanoparticles down to about 60%.

Table 1. Stability parameters of threonine decorated nanoparticles

Parameter	Room temperature	4°C	40°C
Particle size	413 nm	309 nm	617 nm
%drug loading Shape	1.289 Spherical	1.671 Spherical	1.108 Irregular

3.3 Pharmacokinetic parameters of PTX in serum

The L-thr 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-thr conjugated nanoparticles is presented (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 (2-3 times) than the pure drug solution. The clearance rate of the conjugated 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.

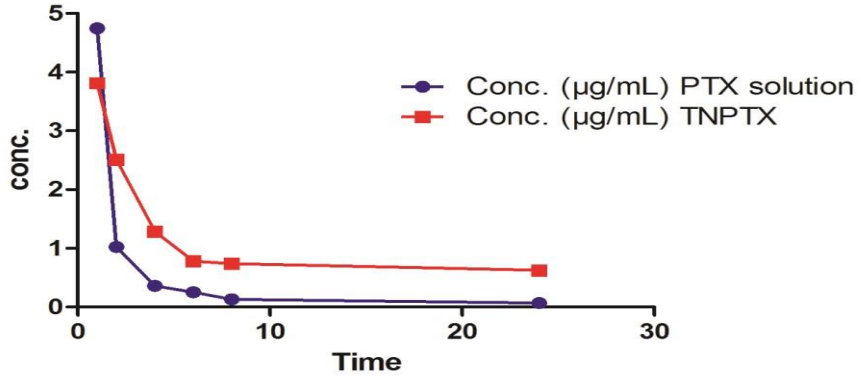

Figure 1 *In vivo* release of PTX from pure PTX solution and TNPTX

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

PK Parameters	Units	PTX solution	CNPTX
Elimination rate, K_{el}	l/h	0.0605	0.0217**
Elimination half life, $t_{1/2}$	H	11.45455	25.0312**
Volume of distribution, V_D	mL/kg	1.483313	1.5916**
AUC0-24	µg.h/mL	6.8579	17.8902**
AUC0-∞	µg.h/mL	133.8347	271.4126**

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

4. Conclusion

By creating paclitaxel-loaded nanoparticles using PLA and conjugating them with L-threonine to change the surface characteristics, the study's goal was achieved. According to the study's findings, L-threonine-conjugated nanoparticles were stable and capable of producing extended drug release at

therapeutic levels. The nanoparticles should be kept in a refrigerator during storage. So it follows that the formulation used for this study could be used to deliver therapeutic molecules in a sustained release formulation.

References

1. Putney SD (1998) Encapsulation of proteins for improved delivery. Curr Opin Chem Biol 2: 548-552.
2. Proffitt RT, Williams LE, Presant CA, Tin GW, Ulina JA, Gamble RC, Baldeschwieler JD (1983) Liposomal blockade of the reticuloendothelial system: improved tumor imaging with small unilamellar vesicles. Science 220: 502-505.
3. Gref R, Minamitake Y, Peracchia MT, Trubetskov V, Torchilin V, Langer R (1994) Biodegradable long-circulating polymeric nanospheres. Science 263: 1600-1603.
4. Storm G, Belliot SO, Daemen T, Lasic DD (1995) Surface modification of nanoparticles to oppose uptake by the mononuclear phagocytic system. Adv Drug Del Rev 17: 31-48.
5. Kaul G, Amiji M (2002) Long-circulating poly(ethylene glycol)-modified gelatin nanoparticles for intracellular delivery. Pharm Res 19: 1061-1067.
6. Gulyaev AE, Gelperina SE, Skidan IN, Antropov AS, Kivman GY, Kreuter J (1999) Significant transport of doxorubicin into the brain with polysorbate 80- coated nanoparticles. Pharm Res 16: 1564-1569.
7. Kaul G, Amiji M (2004) Biodistribution and targeting potential of poly(ethylene glycol)modified gelatin nanoparticles in subcutaneous murine tumor model. J Drug Target 12: 585-591.
8. Benjamin GD, Mark AR (2002) Drug delivery systems based on sugar-macromolecular conjugates. Curr Opin Drug Del 5 (2): 279-288.
9. Egleton RD, Mitchell SA, Huber JD, Palian MM, Polt R, Davis TP (2001) Improved blood-brain barrier penetration and enhanced analgesia of an opioid peptided by glycosylation. J Pharm Exper Ther 299 (3): 967-972.
10. Tosi G, Rivasi F, Gandolfi F, Costantino L, Vandelli MA, Forni F (2005) Conjugated poly (D,L-lactide-co-glycolide) for the preparation of in vivo detectable nanoparticles. Biomater 26:4189-4195.
11. Zhiping Z, Sie Huey Lee, Si-Shen Feng (2007) Folate-decorated poly (lactide-coglycolide)-vitamin E TPGS nanoparticles for targeted drug delivery. Biomater 28: 18891899.
12. Sun W, Xie C, Wang H, Hu Y (2004) Specific role of polysorbate 80 coating on the targeting of nanoparticles to the brain. Biomater 25: 3065-3071.
13. Quirk RA, Chan WC, Davies MC, Tendler SJB, Shakeshe KM (2001) Poly (L-lysine) GRGDS as a biomimetic surface modifier for poly (lactic acid). Biomater 22: 865-872.
14. Fahmy TM, Samstein RM, Harness CC, Mark Saltzman W (2005) Surface modification of biodegradable polyesters with fatty acid conjugates for improved drug targeting. Biomater 26: 5727-5736.
15. Lirong C (2005) Nanoparticles of biodegradable polymers for delivery of therapeutic agents and diagnostic sensitizers to cross the blood brain barrier for chemotherapy and mri of the brain. Masters thesis: National university of Singapore
16. Aterman KC (2007) A critical review of gastro retentive controlled drug delivery Pharm Dev Technol 12: 1-10.
17. Rajender G, Narayanan NGB (2009) Sensitive and validated HPLC method for determination of paclitaxel in human serum. Indian J Sci Technol 2 (5): 52-54.
18. Mishra BJ, Trivedi P (2013) Formulation, stability and pharmacokinetic study of paclitaxel loaded poly(L-lactide) nanoparticles. Digest J Nanomater Biostruct 8: 1829-1833
19. Mishra BJ, Kaul A, Trivedi P (2015) L-Cysteine conjugated poly-L-lactide nanoparticles containing 5-fluorouracil: Formulation, characterization, release and uptake by tissues *in vivo*. Drug Delivery 22 (2): 214-222.