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ORIGINAL RESEARCH

Novel free paclitaxel-loaded poly(L-γ-glutamylglutamine)–paclitaxel nanoparticles

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Abstract: The purpose of this study was to develop a novel formulation of paclitaxel (PTX) that would improve its therapeutic index. Here, we combined a concept of polymer–PTX drug conjugate with a concept of polymeric micelle drug delivery to form novel free PTX-loaded poly(L-γ-glutamylglutamine) (PGG)–PTX conjugate nanoparticles. The significance of this drug formulation emphasizes the simplicity, novelty, and flexibility of the method of forming nanoparticles that contain free PTX and conjugated PTX in the same drug delivery system. The results of effectively inhibiting tumor growth in mouse models demonstrated the feasibility of the nanoparticle formulation. The versatility and potential of this dual PTX drug delivery system can be explored with different drugs for different indications. Novel and simple formulations of PTX-loaded PGG–PTX nanoparticles could have important implications in translational medicines.

Keywords: paclitaxel, polymeric micelle, poly(L-γ-glutamylglutamine)–paclitaxel, nanoconjugate, nanoparticles

Introduction

Paclitaxel (PTX; Bristol-Myers Squibb, New York, NY, USA), which is extracted from the bark of the Pacific Yew tree,[1] is known as Taxol® (Cremophor–ethanol formulation of PTX) and is approved by the US Food and Drug Administration (FDA) for the treatment of ovarian cancer, breast cancer, and lung cancer. However, the formulation of PTX has a low therapeutic index due to its inability to selectively target tumor tissues and to the toxic side effects of the Cremophor–ethanol diluent.[2,3] Recently, other formulations have been explored for improving the therapeutic response of PTX. Polymeric micelle formulation of PTX is one of the recent formulations and clinical trials are now underway. NK105 is one of the polymeric micelle formulations that contains amphiphilic block copolymers of poly(ethylene glycol)-modified poly(aspartate) with 4-phenyl-1-butanol modification and entrapped PTX.[4] Hamaguchi et al reported that NK105 had higher antitumor activity than PTX and reduced neurotoxicity in mouse models.[4] Results of clinical trial studies of NK105 showed that it was well tolerated[5] and efficacious in patients with gastric cancers.[6] Another polymeric micelle formulation of PTX is Genexol-PM® (Samyang Pharmaceuticals, Daejeon City, Korea),[7] amphiphilic micelle-forming diblock copolymers of poly(D,L-lactide) and poly(ethylene glycol), and PTX incorporated within the core. A Phase I clinical trial study showed that Genexol-PM delivered higher PTX drug doses without additional toxicity,[8] and it also had a better response rate in patients with metastatic breast cancer[9] compared with Taxol.[10–12] In addition to polymeric micelles, polymer–PTX conjugate is another formulation of

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PTX. Several polymer conjugates have been actively pursued and extensively reviewed.[13,14] Poly(L-glutamic acid)–PTX conjugate (CT-2103) was considered one of the most successful polymer–PTX conjugates to date.[14] CT-2103 was investigated in multitrial Phase III studies,[15,16] yet it has not been approved by the FDA. CT-2103 is currently undergoing a Phase III clinical trial in combination with carboplatin against chemotherapy-naïve advanced non-small cell lung cancer in women with estradiol >25 pg/mL.[17] Recent formulations of PTX have shown encouraging results.

Previously, we developed a new poly-(L-γ-glutamylglutamine)–paclitaxel nanoconjugate (PGG–PTX).[18] PGG–PTX was demonstrated to be efficacious in antitumor activity in vivo and outperformed Abraxane® (Abraxis Bioscience, Summit, NJ, USA) (albumin-bound PTX nanoparticle) in some mouse models.[19] Pharmacokinetics and tissue distribution of PGG–PTX resulted in prolonged half-life of total taxane, extractable taxanes, and active free PTX in both the plasma and tumor compartments compared with the Cremophor–ethanol formulation of PTX in BABL/c nude mice bearing lung cancer NCI-H460 xenografts.[20] We speculated that hybridizing polymer–PTX conjugate with free PTX would create a novel class of PTX nanoparticle formulation and improve the therapeutic index of PTX.

Materials and methods

Materials

PTX was obtained from NuBlocks (Vista, CA, USA). Ethanol was purchased from Sigma-Aldrich, St Louis, MO, USA. PGG–PTX was synthesized in our laboratory, as described previously.[18] ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectra were recorded at 400 MHz with a JEOL spectrometer (JEOL USA Inc., Peabody, MA, USA) at room temperature. ^{1}H chemical shifts were reported in parts per million (ppm). Purified deionized water was prepared by the Milli-Q plus system from Millipore (Billerica, MA).

BABL/c nude mice were purchased from Sino-British SIPPR/BK Lab Animal Ltd (Shanghai, China). All the mice were acclimated for at least 1 week before experimentation. All the studies were performed in accordance with the approved animal protocols.

Preparation of free PTX-loaded PGG–PTX nanoparticles

The free PTX-loaded PGG–PTX nanoparticles were prepared as follows. A solution of free PTX (75 mg) in ethanol (5 mg/mL) was added dropwise into a solution of PGG–PTX (1 g) in distilled water at a concentration of 25 mg/mL, while gently stirring at room temperature. The solution was then opened to air overnight (~15 hours) to allow slow evaporation of ethanol and formation of the nanoparticles. The residual ethanol was removed by vacuum distillation with a rotary evaporator. The colorless nanoparticle solution was filtered with a 0.45 μm pore-sized microfiltration membrane before dynamic light scattering (DLS) studies and transmission electron microscopy (TEM) studies of particle sizing and before injecting to maximum tolerated dose (MTD) and efficacy studies.

^{1}H-NMR characterization

To show the PTX-loading characteristics of the PTX-loaded nanoparticles, the nanoparticle solution was freeze-dried, and the nanoparticles were reconstituted in deuterated water (D_2O) or deuterated methanol (CD_3OD) at room temperature. ^{1}H-NMR spectra of the free PTX in CD_3OD, PGG–PTX nanoconjugate in D_2O, and PTX-loaded PGG–PTX nanoparticles D_2O and CD_3OD were recorded on a Varian 400 MHz spectrometer (Varian, Palo Alto, CA, USA).

Transmission electron microscopy

The morphologies PGG–PTX nanoconjugate and PTX-loaded PGG–PTX nanoparticles were observed by using a TEM H-7000 (Hitachi, Tokyo, Japan), an electron microscope operating at an accelerating voltage of 75 kV. Negative staining was performed as follows: 1) a drop of sample solution was placed onto a copper grid coated with carbon, 2) the sample drop was taped with a filter paper to remove surface water and air-dried for 5 min followed by the application of 0.01% phosphotungstic acid to deposit the micelles on the grid, and 3) the samples were air-dried before observation.

Dynamic light scattering measurements

The particle size of the free PTX-loaded PGG–PTX nanoparticles obtained directly from the nanoparticle formulation was determined by DLS using a Zetasizer Nano-ZS (Malvern Instruments Inc, Malvern, UK) equipped with an He–Ne laser (4 mW, 633 nm) light source and 90° angle scattered light collection configuration. The solution of the free PTX-loaded PGG–PTX nanoparticles was further diluted to the final concentration of approximately 2 mg/mL and was allowed to equilibrate for 2 min at 25°C before the measurements. The hydrodynamic diameter of the free PTX-loaded PGG–PTX nanoparticles was calculated based on the Stokes–Einstein equation. Correlation function was curve fitted by cumulant method to calculate mean size and polydispersity index. All measurements were repeated three times, and mean particle size was presented as the average diameter with standard deviation.

Maximum tolerated dose studies

MTD was defined as the dose that produced 15% weight loss. MTD for PTX-loaded PGG–PTX nanoparticles administered via tail vein injection was investigated in healthy BABL/c nude mice (20 ± 2 g). The nude mice were divided into four groups (n = 5), which were injected with: 1) saline as a control, 2) 210 mg/kg of total PTX of PTX-loaded PGG–PTX nanoparticles, 3) 230 mg/kg of total PTX of PTX-loaded PGG–PTX nanoparticles, and 4) 250 mg/kg of total PTX of PTX-loaded PGG–PTX nanoparticles. Mice survival and variation in body weight were observed and recorded daily over 14 days.

Therapeutic efficacy experiments

Female athymic nude mice were randomly divided into four groups (n = 6). Each group was injected subcutaneously in their shoulders with 0.1 mL of suspension cells containing 4×10^6 viable NCI-H460 human lung cancer cells. Tumors were allowed to grow until they reached an average volume of about 100 mm^3. Tumor size in mm^3 was estimated from the formula $(w^2 \times l)/2$, where "l" is the longest diameter of the tumor and "w" is the diameter perpendicular to the longest diameter measured in millimeters. A single dose of 1) saline, 2) 50 mg/kg PTX formulated in Cremophor–ethanol, 3) 180 mg/kg total PTX equivalent of PTX-loaded PGG–PTX nanoparticles, or 4) 230 mg/kg total PTX equivalent of PTX-loaded PGG–PTX nanoparticles in saline was administered intravenously(IV) via tail vein injection. Body weight change and tumor volume of the mice were measured daily for the duration of the study until total tumor burden of the saline injection as a control reached 3000 mm^3, at which time all the mice were terminated from the study.

Statistics

The tumor volume data were shown as mean ± standard error of the mean. The statistical significance of differences in the data between two groups was calculated by means of repeated measures. The slope of the regression of log (tumor volume) on time was determined for each individual tumor and the mean of the slopes of all tumors within a group was compared using Student's *t*-test. A value of $P < 0.05$ was considered statistically significant.

Results and discussion

Polymers have been extensively explored for drug delivery of PTX.[4,7,13,14] Polymers can be used either as polymeric PTX micelles or polymer–PTX conjugates. Polymeric micelles employing di-block copolymers are an effective formulation to solubilize PTX. The formulation results in the formation of micellar nanoparticles, which are intended for tumor passive targeting, known as enhanced permeability and retention effects.[21,22] PTX conjugated onto PGG is another formulation. Previously, we reported the synthesis, characterization, and in vitro evaluation of PGG–PTX conjugate.[18] The PGG–PTX conjugate intriguingly self-assembled into nanoparticles whose size remained in the range of 12–15 nm (volume) over the concentration range from 25 to 2000 μg/mL in saline.[18] PGG–PTX conjugate was further demonstrated to possess superior in vivo antitumor efficacy[19] and to exhibit prolonged half-life pharmacokinetics.[20] Polymeric PTX micelles and PGG–PTX conjugate have their own unique properties.

In this study, we presented the feasibility of combining polymeric micelles and polymer conjugates into one nanoparticle delivery system of PTX. The novel nanoparticle delivery system featured entrapped PTX and conjugated PTX. A schematic formulation of PTX-loaded PGG–PTX nanoparticles is shown in Figure 1. The design of the nanoparticle formation was simple. A solution of free PTX in ethanol (5 mg/mL) was added to a solution of polymer–PTX conjugate in water (25/mL) while being stirred, and the nanoparticles were formed after the ethanol was slowly evaporated overnight. The percentage of PTX relative to PGG–PTX conjugate was 7.5% weight by weight. To our knowledge, this is the first polymeric nanoparticulate containing entrapped free PTX drug and conjugated PTX drug united into one delivery system.

Encapsulation of free PTX with PGG–PTX conjugate was demonstrated by comparing ^{1}H-NMR spectra of free PTX in CD_3OD, PGG–PTX conjugate in D_2O, and PTX-loaded PGG–PTX nanoparticles in CD_3OD and D_2O. ^{1}H-NMR spectra of PTX in CD_3OD (A), PGG–PTX conjugate in D_2O (B), PTX-loaded nanoparticles in D_2O (C), and CD_3OD (D), respectively, are shown in Figure 2. When PTX was encapsulated inside the nanoparticles, the characteristic peaks of PTX were hardly seen in D_2O due to their insufficient mobility in D_2O. However, in CD_3OD, resonance peaks corresponding to the PTX were clearly observed. This observation was consistent with ^{1}H-NMR studies of the other polymer micelles in D_2O reported.[23,24] The ^{1}H-NMR results indicated that PTX was successfully entrapped into the hydrophobic inner core of the polymeric nanoparticles.

Further DLS and TEM studies confirmed the formation of PTX-loaded PGG–PTX nanoparticles. The average particle size and the unimodal size distribution of both PGG–PTX conjugate and PTX-loaded PGG–PTX were examined by DLS, and the results are illustrated in Figure 3(A) and (B). The Z-mean diameter of PGG–PTX conjugate in water was approximately 20 nm, with a narrow polydispersity index (PDI) of 0.27. The particle size of PGG–PTX conjugate in water was consistent with the previous study of the conjugate in saline, which was about 12–15 nm.[18] The particle size

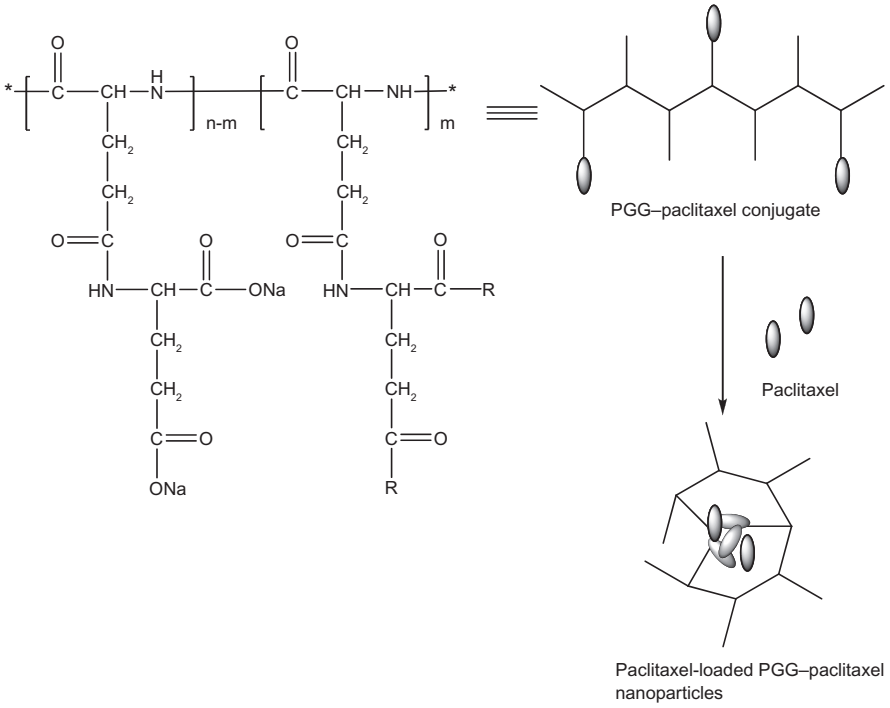

Figure 1 Schematic of formation of free paclitaxel-loaded PGG–PTX nanoparticles.
Abbreviations: PGG, poly(L-γ-glutamylglutamine); PTX, paclitaxel.

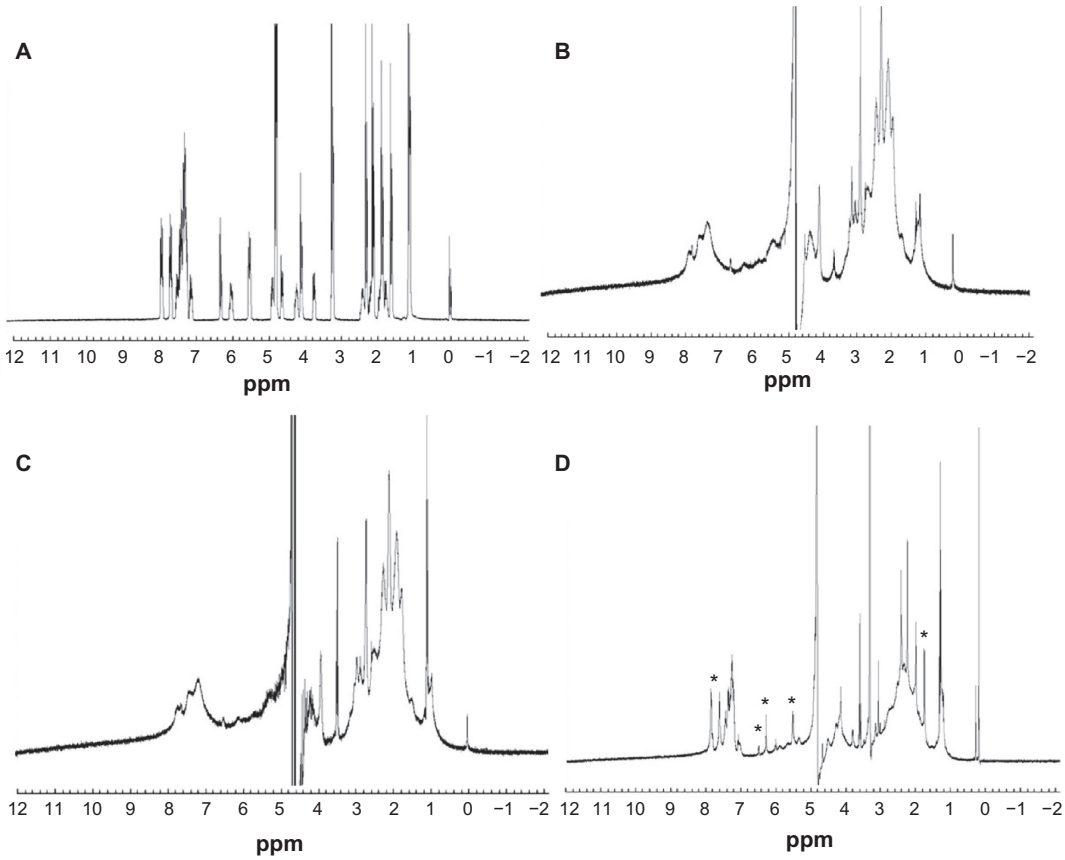

Figure 2 A) ^{1}H-NMR spectra of paclitaxel in CD_3OD; **B**) PGG–PTX nanoconjugate in D_2O; **C**) paclitaxel-loaded PGG–PTX nanoparticles in D_2O; and **D**) paclitaxel-loaded PGG–PTX nanoparticles in CD_3OD.
Note: *Free paclitaxel protons.
Abbreviations: PGG, poly(L-γ-glutamylglutamine); ppm, parts per million; PTX, paclitaxel.

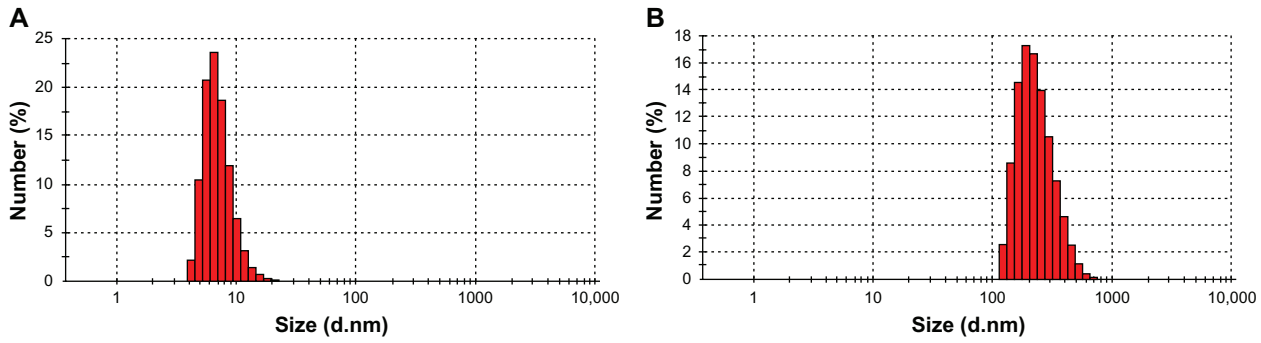

Figure 3 The particle size distribution of PGG–PTX nano-conjugate **A**) and paclitaxel-loaded PGG–PTX nanoparticles **B**) measured by DLS.
Abbreviations: d.nm; diameter in nanometers; DLS, dynamic light scattering; PGG, poly(L-γ-glutamylglutamine); PTX, paclitaxel.

and distribution of free PTX-loaded nanoparticles in water were 270 nm and 0.08 PDI, respectively. Furthermore, TEM images of PGG–PTX conjugate and PTX-loaded nanoparticle exhibited a spherical shape of moderate uniform particle size, as shown in Figure 4(a) and (b). The particle size of PGG–PTX conjugate observed by TEM was about 20 nm, which was consistent with the DLS data. However, particle size of PTX-loaded PGG–PTX nanoparticle observed by TEM was about 50 nm, which was smaller than that determined by DLS. We speculated that the particle size determined by DLS represents their hydrodynamic diameter, whereas that obtained by TEM is related to the collapsed micelles after water evaporation. The TEM diameter of the PTX-loaded nanoparticles was smaller than the DLS diameter.

To confirm the applicability of the free PTX-loaded PGG–PTX nanoparticles, the nanoparticles were investigated for antitumor activity in mouse-bearing human lung NCI-H460 cancer models. Prior to antitumor efficacy study, MTD of the nanoparticles needed to be determined. With respect to PTX-loaded PGG–PTX nanoparticles, MTD was defined as the dose that yielded 15% weight loss. The MTD studies for the PTX-loaded nanoparticles were carried out in healthy BABL/c nude mice. The mice were divided into three experimental groups and one saline control group (n = 5 for each group). The mice of experimental groups were administered with a single IV tail vein injection of 1) 210 mg/kg, 2) 230 mg/kg, and 3) 250 mg/kg

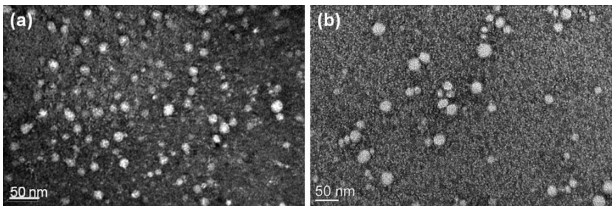

Figure 4 TEM micrograph of the PGG–PTX nanoconjugates **a**) and paclitaxel–loaded PGG–PTX nanoparticles **b**).
Abbreviations: PGG, poly(L-γ-glutamylglutamine); PTX, paclitaxel; TEM, transmission electron microscopy.

of total PTX of PTX-loaded PGG–PTX nanoparticles. Mice survival was observed, and their body weight change was measured and recorded daily for the duration of the study. No mice died during the MTD study period. The details of their body weight change are shown in Figure 5. When the doses of the PTX-loaded nanoparticles were administered, 5%–15% body weight loss was observed. The body weight of the healthy BABL/c nude mice was consistent with the escalation of the doses. On the basis of the results, MTD of the PTX-loaded nanoparticles was decided at 230 mg/kg of total PTX. With the MTD of the PTX-loaded nanoparticles, a study of antitumor efficacy was carried out on BABL/c nude mice bearing NCI-H460 human lung cancer xenografts. The mice were randomly divided into two experimental groups (180 and 230 mg/kg of the total PTX equivalence of PTX-loaded nanoparticles), one positive control group (50 mg/kg of PTX), and one saline negative control group (n = 6 for each group). The mice received a single IV tail vein injection after 12 days of subcutaneous implantation with the NCI-H460 human lung cancer cells when the mean tumor volume reached 100 mm^3. The antitumor activity of the nanoparticles was compared with that of PTX and saline by measuring tumor volume and survival rates of the mice. Results of the efficacy study are shown in Figure 6. The results indicated that the PTX-loaded nanoparticles inhibited tumor growth. At the dose of 230 mg/kg of the total PTX equivalents, PTX-loaded nanoparticles showed stronger tumor regression with statistically significant differences compared with that of 50 mg/kg of PTX ($P = 0.036$), 180 mg/kg of the total PTX of the PTX-loaded nanoparticles ($P = 0.019$), and saline ($P = 0.006$) after administration. However, it seemed that the PTX-loaded PGG–PTX nanoparticles exhibited higher toxicity in mice-bearing human lung NCI-H460 cancer (Figure 6, bottom) compared with that of healthy mice (Figure 5) based on their body weight loss. Surprisingly, the dose of 180 mg/kg, PTX equivalents, of the nanoparticles appeared to lead to more weight loss than that of 230 mg/kg (Figure 6, bottom). No statistical significance of

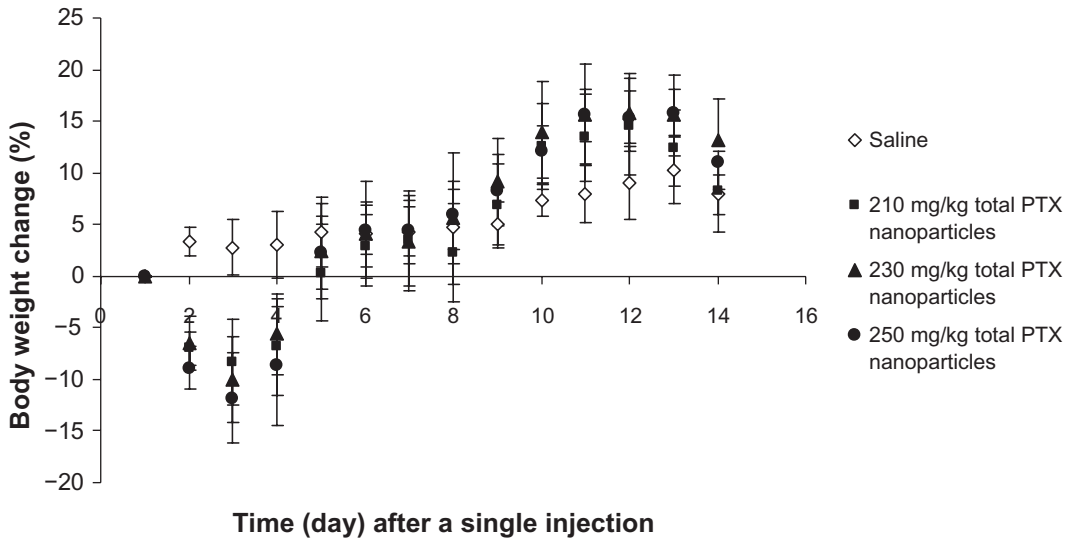

Figure 5 Maximum tolerated dose of paclitaxel-loaded PGG–PTX nanoparticles. Body weight change as a function of time in the normal *nu/nu* mice (n = 5) with a single IV tail vein injection of either saline (◊), 210 mg PTX/kg (■), 230 mg PTX/kg (▲), or 250 mg PTX/kg (●) paclitaxel-loaded PGG–PTX nanoparticles. Vertical bars ±SEM.
Abbreviations: IV, intravenous; PGG, poly(L-γ-glutamylglutamine); PTX, paclitaxel; SEM, standard error of the mean.

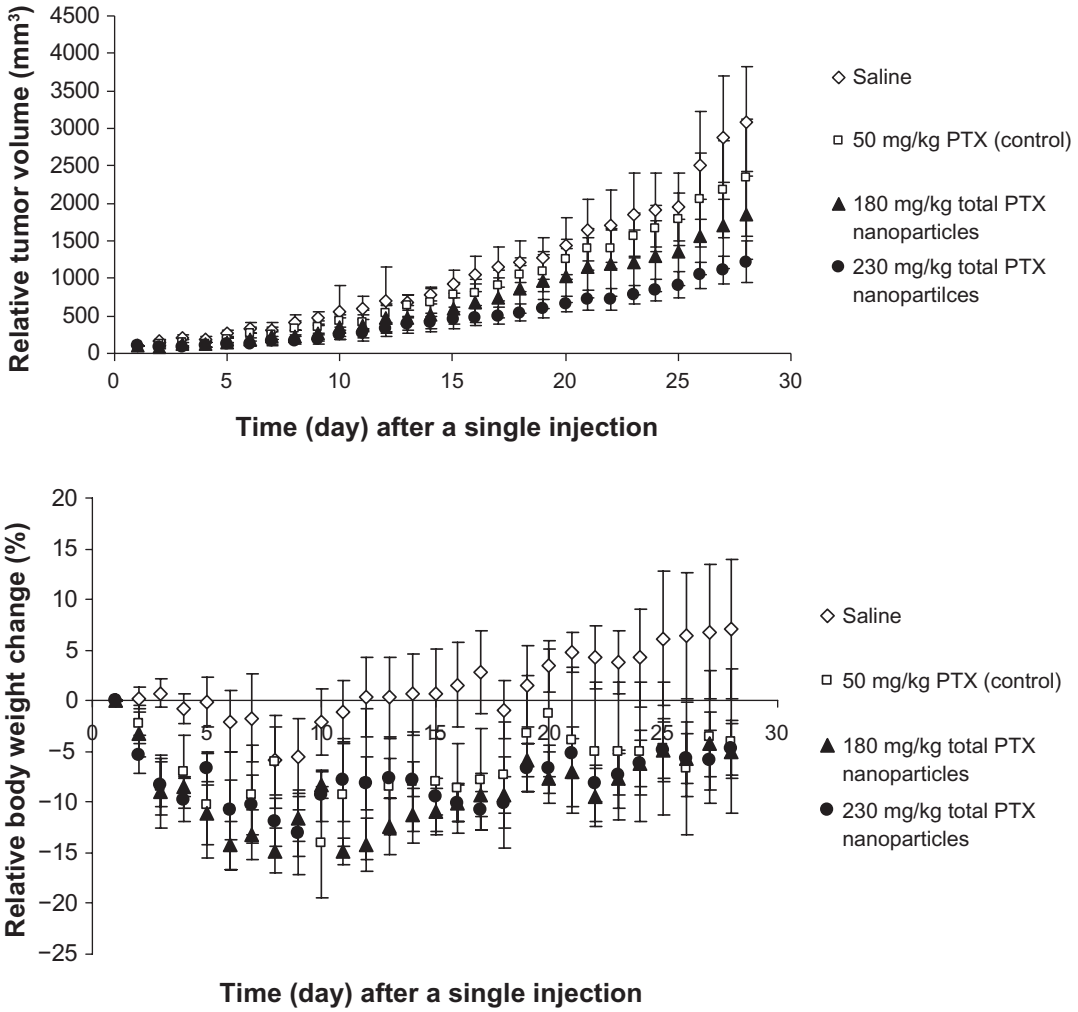

Figure 6 Relative tumor volume (top) and body weight change (bottom) as a function of time in nude mice (n = 6) bearing NCI-H460 tumors treated with a single IV tail vein injection of either saline (◊), 50 mg PTX/kg (□) (paclitaxel Cremophor–ethanol formulation), 180 mg PTX/kg (▲) (paclitaxel-loaded PGG–PTX nanoparticles), or 230 mg PTX/kg (●) (paclitaxel-loaded PGG–PTX nanoparticles). Vertical bars ±SEM.
Abbreviations: IV, intravenous; PGG, poly(L-γ-glutamylglutamine); PTX, paclitaxel; SEM, standard error of the mean.

tumor inhibition was observed between 180 mg/kg of the total PTX of the PTX-loaded nanoparticles and 50 mg/kg of PTX ($P = 0.934$). The antitumor efficacy of 230 mg/kg of the total PTX of the novel PTX-loaded nanoparticles confirmed the feasibility of a dual PTX drug delivery system to be applicably useful for chemotherapy.

Conclusion

The results of this study contribute two important scientific issues. First, we provide the first and most simple method of forming nanoparticles featuring free PTX and conjugated PTX in the same drug delivery system. Second, these nanoparticles can effectively inhibit tumor growth. The versatility and potential of this dual drug system can be explored with different drugs for different indications. Developing novel and simple PTX-loaded PGG–PTX nanoparticles could have important implications in translational medicines.

Acknowledgments

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Disclosure

No conflicts of interest were declared in relation to this paper.

References

1. Wani MC, Taylor HL, Wall ME, Coggon P, McPhail AT. Plant antitumor agents. VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*. *J Am Chem Soc*. 1971;93:2325–2327.
2. Weiss R, Donehower RC, Wiernik PH. Hypersensitivity reactions from Taxol. *J Clin Oncol*. 1990;8:1263–1268.
3. Meerum Terwogt JM, Nuijen B, Huinink Ten Bokkel WW, Beijnen JH. Alternative formulations of paclitaxel. *Cancer Treat Rev*. 1997;23: 87–95.
4. Hamaguchi T, Matsumura Y, Suziki M, et al. NK105, a paclitaxel-incorporating micellar nanoparticle formulation, can extend in vivo antitumour activity and reduce the neurotoxicity of paclitaxel. *Br J Cancer*. 2005;92:1240–1246.
5. Kato K, Hamaguchi T, Yasui H, et al. Phase I study of NK105, a paclitaxel-incorporating micellar nanoparticle, in patients with advanced cancer. *J Clin Oncol*. 2006;24(18S):2018.
6. Chin K, Kato K, Yoshikawa T, et al. Phase II study of NK105, a paclitaxel-incorporating micellar nanoparticle as second-line treatment for advanced or recurrent gastric cancer. *J Clin Oncol*. 2010:28(15S):4041.
7. Kim SC, Kim DW, Shim YH, et al. In vivo evaluation of polymeric micellar paclitaxel formulation: toxicity and efficacy. *J Control Release*. 2001;72:191–202.
8. Kim TY, Kim DW, Chung JY, et al. Phase I and pharmacokinetic study of Genexol-PM, a Cremophor-free, polymeric micelle-formulated paclitaxel, in patients with advanced malignancies. *Clin Cancer Res*. 2004;10:3708–3716.
9. Lee KS, Chung HC, Im SA, et al. Multicenter phase II trial of Genexol-PM, a Cremophor-free, polymeric micelle formulation of paclitaxel, in patients with metastatic breast cancer. *Breast Cancer Res Treat*. 2008;108:241–250.
10. Di Leo A, Gomez HL, Aziz Z, et al. Phase II, double-blind, randomized study comparing lapatinib plus paclitaxel with placebo plus paclitaxel as first-line treatment for metastatic breast cancer. *J Clin Oncol*. 2008;26:5544–5552.
11. Jones SE, Erban J, Overmoyer B, et al. Randomized phase III study of decetaxel compared with paclitaxel in metastatic breast cancer. *J Clin Oncol*. 2005;23:5542–5551.
12. Paridaens R, Biganzoli L, Bruning P, et al. Paclitaxel versus doxorubicin as first-line single-agent chemotherapy for metastatic breast cancer: a European organization for research and treatment of cancer randomized study with cross-over. *J Clin Oncol*. 2000;18:724–733.
13. Duncan R. The dawning era of polymer therapeutics. *Nat Rev Drug Discov*. 2003;2:347–360.
14. Haag R, Kratz F. Polymer therapeutics: concepts and applications. *Angew Chem Int Ed*. 2006;45:1198–1215.
15. O'Brien MER, Socinski MA, Popovich AY, et al. Randomized phase III trial comparing sing-agent paclitaxel poliglumex (CT-2103, PPX) with single-agent gemcitabine or vinorelbine for the treatment of PS 2 patients with chemotherapy-naïve advanced non-small cell lung cancer. *J Thoracic Oncol*. 2008;3:728–734.
16. Paz-Ares L, Ross H, O'Brien M, et al. Phase III trial comparing paclitaxel poliglumex vs docetaxel in the second-line treatment of non-small-cell lung cancer. *Br J Cancer*. 2008;98:1608–1613.
17. US National Institute of Health. ClincalTrails.gov. Available from: http://clinicaltrials.gov/. Accessed 2010 Dec 02.
18. Van S, Das SK, Wang X, et al. Synthesis, characterization, and biological evaluation of poly(L-γ-glutamylglutamine)-paclitaxel nanoconjugate. *Int J Nanomedicine*. 2010;5:825–837.
19. Feng Z, Zhao G, Yu L, Gough D, Howell SB. Preclinical efficacy studies of a novel nano-particle-based formulation of paclitaxel that out-performs Abraxane. *Cancer Chemother Pharmacol*. 2010;65:923–930.
20. Wang X, Zhao G, Van S, et al. Pharmacokinetics and tissue distribution of PGG-paclitaxel, a novel macromolecular formulation of paclitaxel, in nu/nu mice bearing NCI-460 lung cancer xenografts. *Cancer Chemother Pharmacol*. 2010;65:515–526.
21. Matsumura Y, Maeda H. A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumouritropic accumulation of proteins and the antitumor agent smanc. *Cancer Res*. 1986;46:6387–6392.
22. Maeda H, Wu J, Sawa T, Matsumura Y, Hori K. Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. *J Control Release*. 2000;29:17–23.
23. Heald CR, Stolnik S, Kujawinski KS, et al. Poly(lactic acid)–poly(ethylene oxide) (PLA–PEG) nanoparticles: NMR studies of the central solidlike PLA core and the liquid PEG corona. *Langmuir*. 2002;18;3669–3675.
24. Hrkach JS, Peracchia MT, Domb A, Lotan N, Langer R. Nanotechnology for biomaterials engineering: structural characterization of amphiphilic polymeric nanoparticles by 1H NMR spectroscopy. *Biomaterials*. 1997;18:27–30.