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ORIGINAL ARTICLE

Poly(ethylene oxide)-modified poly(beta-amino ester) nanoparticles as a pH-sensitive system for tumor-targeted delivery of hydrophobic drugs: part 3. Therapeutic efficacy and safety studies in ovarian cancer xenograft model

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Abstract
Purpose The objective of this study was to evaluate the anti-tumor efficacy and lack of systemic toxicity of paclitaxel when administered in pH-sensitive poly(ethylene oxide) (PEO)-modified poly(beta-amino ester) (PbAE) nanoparticles in mice bearing human ovarian adenocarcinoma (SKOV-3) xenograft.
Methods Paclitaxel-encapsulated PEO-modified PbAE (PEO–PbAE) nanoparticles were prepared by the solvent displacement method. PEO-modified poly(epsilon-caprolactone) (PCL) (PEO–PCL) nanoparticles were used as a non pH-responsive control formulation. Efficacy studies were conducted in SKOV-3 tumor-bearing athymic *(Nu/Nu)* mice at an equivalent paclitaxel dose of 20 mg/kg with the control and nanoparticle formulations. Safety of the drug when administered in the control and nanoparticle formulation was determined from blood cell counts and changes in body weight of the animals.
Results The formulated paclitaxel-containing PEO–PbAE and PEO–PCL nanoparticles had a particle size in the range of 100–200 nm and a surface charge of + 39.0 and − 30.8 mV, respectively. After intravenous administration of paclitaxel in these formulations, the tumor growth was inhibited significantly. Both of the formulated nanoparticles tested have shown improved therapeutic efficacy as compared to the paclitaxel aqueous solution. Additionally, significantly lower toxicity profile of paclitaxel was observed with PEO-modified nanoparticles as compared to the aqueous solution formulation
Conclusion PEO-modified PbAE nanoparticles are a unique pH-sensitive drug delivery system that elicits enhanced efficacy and safety profile in solid tumor therapy.

Keywords Biodegradable · pH sensitive · Nanoparticles · Poly(beta-amino ester) · Poly(epsilon-caprolactone) · Tumor targeting · Efficacy · Safety

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Introduction

The major limitation with systemic cancer chemotherapy is the lack of tumor selectivity, resulting in severe dose-limiting adverse effects. Furthermore, another serious limitation is the generation of multidrug-resistant tumor cells under the influence of long-term treatment, which results in suboptimal drug concentrations at the tumor site. It is, therefore, desirable to maintain a steady infusion of the drug into the tumor interstitium to accomplish continuous extermination of the

rapidly dividing cells resulting in tumor regression [1]. The pathophysiology of tumor tissue, characterized by angiogenesis, hypervasculature, a defective vascular architecture, an impaired lymphatic drainage, and acidic tumor microenvironment seems to be a universal feature of solid tumors that can be exploited for tumor targeted delivery using long-circulating polymeric conjugates and other colloidal drug carriers [2]. Due to the hyper-permeability of the tumor vasculature and the lack of lymphatic drainage, blood-borne macromolecules and colloidal particles are preferentially distributed in the tumor due to the enhanced permeability and retention (EPR) effect. Maeda's group first described the EPR effect of tumor vasculature, which has subsequently been examined and confirmed by other investigators [3, 4]. Concentrations of polymer–drug conjugates in tumor tissues can reach levels as high as 10–100 times greater than would be seen after administration of the free drug [5].

Advances in nanotechnology have resulted in the development of novel drug delivery systems such as polymeric nanoparticles, liposomes, polymeric micelles, and nanoemulsions that promise to enhance the efficacy of anticancer therapy with less toxicity [6–8]. Using poly(ethylene glycol)-modified liposomes, Jain and colleagues [9, 10] have found that the effective pore size of most peripheral human tumors range from 200 to 600 nm in diameter, with a mean of about 400 nm. Tumors that grow in the brain, on the other hand, tend to have smaller pore size of less than 400 nm in diameter. The circulating liposomes and nanoparticles with a size of less than 400 nm will be trapped and retained in the tumor due to the EPR effect. Colloidal delivery systems, such as liposomes and polymeric nanoparticles, with a positive surface charge are taken preferentially in the tumor and retained for longer duration as compared to negatively charged or neutral particles [11, 12]. The properties of the tumor microvasculature, therefore, are amenable to the application of nanoparticulate systems for targeted delivery [13].

Gref et al. [14] were the first to propose the concept of long-circulating solid Poly(D, L-lactide-co-glycolide) nanoparticles prepared by surface modification with poly(ethylene oxide) (PEO) for systemic drug delivery. PEO surface modification is very popular for colloidal drug delivery systems since this polymer has had a long history of safe use in biological and pharmaceutical products. Surface-bound PEO chains extend into the aqueous physiological environment, repelling proteins (e.g., C3b) and decreasing antibody formation by the steric repulsion mechanism. This effect results in an increase in plasma circulation time of the nanoparticle formulation [15]. Polymeric nanoparticles modified in this manner offer a number of advantages for drug delivery to tumors over other carrier systems. First, by appropriate selection of the base polymer, one could encapsulate drugs with different physical–chemical properties. Second, the loading capacity of drugs in polymeric nanoparticles tends to be significantly higher than in liposomes or micelles. Third, the drug is dispersed throughout the matrix, which will prevent "burst release" in the plasma. Fourth, based on the choice of polymer type, such as one with pH-sensitive solubility profile, the encapsulated drug can be readily available at the tumor site where the pH tends to be lower than in the systemic circulation. Lastly, polymeric nanoparticle surfaces can be easily modified with targeting ligands for site-specific delivery.

Poly(beta-amino ester) (PbAE) is synthetic, hydrolytically degradable, biocompatible cationic polymers that are synthesized by an addition reaction between primary or secondary diamines and diol-diacrylates [16, 17]. Since there are a number of different types of diamines and diol-diacrylates, the properties of the PbAE such as water-solubility, cationic charge density, crystallinity, and degradation kinetics can be easily controlled to meet the specific requirements of a particular application. PbAE used in the present study is a representative biodegradable and biocompatible hydrophobic polymer that has pH-responsive solubility profile. Among United States Food and Drug Administration (FDA)-approved biocompatible and biodegradable polyesters, poly(epsilon-caprolactone) (PCL) possesses unique properties such as higher hydrophobicity and neutral biodegradation end products [18, 19]. Low molecular weight PCL (e.g., 10–20 kDa) degrades quickly in the biological environment, especially in the presence of lipases [20]. Over the years, an array of drug delivery systems has been developed using PCL as polymeric material [21–23].

The hydrophobic nature of PbAE and PCL plays a key role in efficient surface modification strategy using ABA-type PEO/poly(propylene oxide) (PPO)/PEO triblock copolymers (Pluronic®). The surface modification is purely dependent on the hydrophobic interactions between the center-block (PPO) of the stabilizing surfactant and the polymeric core (PbAE or PCL nanocarrier) [24, 25]. In the present study, we have used Pluronic® F-108 having 56 residues of propylene oxide (PO) and 122 residues of ethylene oxide (EO) for surface modification of the nanoparticles. By blending PbAE and PCL with Pluronic® copolymers in the right proportion, the hydrophilic PEO side-arms remain in the mobile state as they extend outwards from the particle surface and provide stability to the

particle suspension by a repulsion effect through a steric mechanism of stabilization involving both enthalpic and entropic contributions [26]. The end result of such an assembly is a stable, pH-responsive faster eroding (PEO–PbAE) or slowly eroding (PEO–PCL) nanoparticulate system that is less prone to be phagocytosed by scavenger cells, such as macrophages of the reticuloendothelial system.

Because of the low pH of the tumor interstitial microenvironment resulting from lactic acid production due to hypoxia, or acidic intracellular organelles, there is a need for tumor-targeted drug delivery systems that undergo rapid, pH-sensitive dissolution [27, 28]. In previous studies, we have formulated and characterized the PEO–PbAE nanoparticulate formulations for releasing the hydrophobic cytotoxic drug in the acidic tumor microenvironment and/or after internalization in tumor cells via non-specific endocytosis and consequently releasing the drug under the influence of the intracellular pH-trigger [29–31]. The previous studies included evaluation of cellular uptake and cytotoxicity comparisons as well as biodistribution, pharmacokinetic, and paclitaxel accumulation in the tumor mass in SKOV-3 ovarian adenocarcinoma xenograft model. The results of these studies showed that PEO-modified PbAE nanoparticles provide pH-responsive delivery of paclitaxel in the cells and led to higher drug concentrations in the tumor mass than when the drug was administered either in non pH-responsive (i.e., PEO–PCL) nanoparticles or in aqueous solution formulations.

As a follow-up, the present study examines the anticancer efficacy and toxicity of paclitaxel administered intravenously either as an aqueous solution or in PEO–PbAE (pH-sensitive) and PEO–PCL (non pH-sensitive) nanoparticulate system to female athymic mice bearing subcutaneous SKOV-3 human ovarian adenocarcinoma xenograft.

Materials and methods

Materials

A representative hydrophobic PbAE (MW ~ 10 kDa) was synthesized by the addition reaction of 4,4(-trimethyldipiperidine with 1,4-butanediol diacrylate in dimethylformamide for 48 h at 50(C. PCL, with a number average molecular weight of 14.8 kDa (as verified by gel-permeation chromatography), was purchased from Polysciences Inc. (Warrington, PA, USA). Pluronic® F-108 NF was kindly provided by the Performance Chemical Division of BASF Corporation (Parsipanny, NJ, USA). Paclitaxel powder was purchased from LC Laboratories (Woburn, MA, USA). Paclitaxel solution, available as a commercial injection in Cremophore EL-ethanol (50:50 mixture) (Onxol®) was purchased from Ivax Pharmaceuticals (Miami, FL, USA). SKOV-3, human ovarian adenocarcinoma cells were kindly supplied from Dr. Michael Seiden's laboratory at the Massachusetts General Hospital (Cambridge, MA, USA). The cell culture media was purchased from Fischer Scientific (Pittsburgh, PA, USA). WBC diluting fluid was purchased from ENG Scientific, Inc. (Clifton, NJ, USA). Platelet diluting fluid was purchased from Ricca Chemical Company (Arlington, TX, USA). All the other chemicals and reagents were of analytical grade and were used as supplied. Deionized distilled water (NanoPure II, Dubuque, IA, USA) was used for preparation of all aqueous solutions.

Preparation and characterization of paclitaxel-containing PEO-modified nanoparticles

Paclitaxel-loaded (at 10% w/w) PEO-modified PbAE and PCL nanoparticles were prepared by the solvent displacement method as described earlier [30]. Briefly, a solution of PbAE or PCL (85 mg) and Pluronic® F-108 (15 mg) was prepared in absolute ethanol or in acetone for PCL (5 ml) and was introduced into a pre-cooled (< 15°C) purified water maintained under vigorous magnetic stirring. For preparation of drug-loaded nanoparticles, paclitaxel was dissolved along with the polymers blends of PbAE–Pluronic® F-108 or PCL–Pluronic® F108 in the organic phase before introduction into the aqueous medium. The lyophilized nanoparticles were re-suspended and diluted suitably in deionized distilled water (pH 7.0), and the particle size and mean zeta potential was measured [28, 29].

In vivo studies

Cell culture The hypodiploid human ovarian adenocarcinoma cell line, SKOV-3, was maintained in RPMI®-1640 medium supplemented with 10% (v/v) FCS and 1% (v/v) penicillin/streptomycin combination. Cells were cultured in a humidified atmosphere of 95% of air and 5% CO_2 at 37°C in an incubator. Media were routinely changed every 3 days. For subculture, cells growing as monolayer cultures were released from the tissue flasks by treatment with 0.05% trypsin/EDTA. The viability and cell count was monitored using Trypan blue dye exclusion method. Then the cells were harvested during the logarithmic growth

phase and re-suspended in serum-free medium (SFM) prior to inoculation in animals.

Experimental model The experimental protocol involving use of animals was approved by the Institutional Animal Care and Use Committee at Northeastern University. Female athymic mice (*Nu/Nu* strain), 4–6 weeks old, weighing about 25 gm were purchased from Charles River Laboratories (Cambridge, MA) and were housed under controlled laboratory conditions (temperature 25 ± 1°C, relative humidity 50 ± 10% and 12 h/12 h light/dark cycle) in polycarbonate cages having free access to sterilized rodent pellet diet and acidified drinking water. The animals were allowed to acclimatize for at least 48 h prior to any experiments.

Tumor model development Approximately, 4 million SKOV-3 cells suspended in 100 µl of SFM were injected subcutaneously into the dorsal side of mice under light isoflurane anaesthesia. Solid tumors developed within 7–10 days post-tumor cell inoculation and as soon as tumor volume reached 45 ± 5 mm^3 after day 9, the animals were selected for experimental treatment. The tumor-bearing mice were randomly allotted to four different control and treatment groups (i.e., untreated control, paclitaxel in solution treatment, paclitaxel in PEO–PbAE nanoparticle treatment, and paclitaxel in PEO–PCL nanoparticle treatment) such that the means of each group were similar.

In vivo administration of the formulations Six lightly anaesthetized animals per group were used for the experiments. Therapy was initiated by intravenous administration of a single dose of paclitaxel aqueous solution, drug-loaded PEO–PbAE and PEO–PCL nanoparticles. The formulations were diluted and suspended in sterile phosphate buffered saline. Each lightly anesthetized tumor-bearing animal received a paclitaxel dose of 20 mg/kg either in aqueous solution, PEO–PbAE, or PEO–PCL nanoparticles by intravenous administration of 0.1 ml over 20 s periods through the tail vein. Aqueous paclitaxel solution was prepared from the commercial Onxol® formulation.

Evaluation of therapeutic efficacy The tumor size was measured twice weekly with a vernier calipers in two dimensions. Individual tumor volumes (V) were calculated using the formula $V = [\text{length} \times (\text{width})^2]/2$ where length (L) is the longest diameter and width (W) is the shortest diameter perpendicular to length [32]. Growth curves for groups of tumors are presented as the mean volume relative to the values on the first day of the treatment (RTV). $\text{RTV} = (V_x - V_0)/V_0$, where V_x is the volume at day x and V_0 is the volume on the day of treatment (day 0) [33]. The time taken for a tumor to triple its initial volume (43 ± 1 to 129 ± 3 mm^3) was determined. The difference between mean values of this parameter for individual tumors in treatment and control groups was defined as the tumor growth delay (GD) achieved as a result of therapy [34]. Tumor volume doubling time (DT) calculations were determined by the equation $V_F = V_i\, e^{Kt}$, where V_F is final tumor volume, V_i is the initial tumor volume and t is the number of days between V_F and V_i measurements. According to the formula, DT (in days) equals $0.693/K$, where $K = \text{In}\,(V_F/V_i)$ [35]. At each time point, treated versus control tumor volume ratio values were calculated as percentage for each experimental group. The lowest values obtained within 4 weeks after treatment were used for evaluating the efficacy of the compounds. At the end of the experiment the animals were sacrificed by cervical dislocation and the tumor mass was harvested and weighed.

Evaluation of safety For safety evaluation of the control and nanoparticulate formulations, the body weight of each mouse was determined twice weekly and related to the first day as percent change in body weight. In addition, blood samples were withdrawn from the retro-orbital venous plexus at day 25 after treatment and blood parameters (white blood cells and platelet counts) were determined [36].

Statistical analysis Data sets were analyzed using a commercially available software package (InStat 2.03, Graphpad Software, Inc.). A student's t test was used to determine the validity of the differences between control and treatment data sets. A P-value of less than 0.05 was considered significant.

Results and discussion

In recent years, a number of pH-responsive polymeric drug carriers have been developed to release the polymer bound drug at the tumor site either extracellularly, resulting from the slightly acidic pH in tumor tissue, or intracellularly, in acidic endosomes or lysosomes after cellular uptake of the drug-polymer conjugate [37]. In order to exploit the pH differences between systemic circulation and the tumor mass, we have focused our work on development of nanoparticlulate drug delivery formulations that exploits the low pH environment in the tumor and upon internalization in the cells to enhance increased local concentrations of the cytotoxic drug at the target site.

Paclitaxel in PEO–PbAE nanoparticle formulations

Spherical nanoparticles having a smooth surface and distinct boundary were obtained by solvent displacement method. The particles obtained were in the range of 100–150 nm with a mean diameter of 113 nm and the surface charge is 39.4 mV. The PCL nanoparticles had a mean diameter of about 200 nm, and the surface charge was −30.8 mV (Table 1). Inclusion of paclitaxel did not alter the particle size or surface charge, which may be due to the uniform distribution of the drug in the polymer matrix rather than predominant surface localization [29].

In a previous study [31], we have shown that PEO–PbAE nanoparticles were internalized very efficiently in SKOV-3 cells and release the encapsulated payload rapidly in response to low pH of the endosomal/lysosomal vesicles. Intracellular concentrations of paclitaxel were significantly higher when administered in the nanoparticle formulations as compared to the control aqueous solution. The enhanced bioavailability of paclitaxel from PEO–PbAE nanoparticles in the cells and increased residence time of the drug led to higher cell-kill efficiency. In vivo studies in SKOV-3 tumor-bearing mice showed that the PEO–PbAE and PEO–PCL nanoparticles had long circulation times upon intravenous administration [30]. The concentration of paclitaxel delivered to the tumor mass was significantly greater with the PEO–PbAE nanoparticles than the other formulations tested. At 1 h post-administration, for instance, there was a 5.2-fold higher concentration of paclitaxel in the tumor when delivered in PEO–PbAE nanocarriers and 2.2-fold higher concentrations with PEO–PCL nanocarriers as compared to the aqueous solution [31].

Table 1 Particle size and surface charge of the control and paclitaxel-containing poly(ethylene oxide)-modified nanoparticle formulations

Nanoparticle formulation	Particle size (nm)	Surface charge (mV)
Blank PEO–PCL	198.0 ± 20[a]	−34.4 ± 1.3
Paclitaxel-loaded PEO–PCL	191.5 ± 12	−30.2 ± 2.1
Blank PEO–PbAE	169.4 ± 17	50.9 ± 2.5
Paclitaxel-loaded PEO–PbAE	147.9 ± 8.0	44.3 ± 1.3

Control and paclitaxel-loaded poly(ethylene oxide)-modified poly(epsilon-caprolactone) (PEO–PCL) and poly(beta-amino ester) (PEO–PbAE) were formulated using the solvent displacement method. Paclitaxel-loaded nanoparticles were prepared with 10% (w/w) drug loading. The efficiency of loading was determined to be > 99%

[a] Mean ± SE ($n = 5$)

Evaluation of therapeutic efficacy

The change in tumor volume as a function of time upon administration of paclitaxel in the control and nanoparticle formulations is shown in Fig. 1. The results clearly show the enhanced efficacy of paclitaxel when administered in PEO–PbAE nanoparticle formulations. On 16 and 28 days post-inoculation, the average tumor volumes in untreated control were 83.5 and 175.4 mm^3, respectively, while the tumor volumes after treatment with paclitaxel in aqueous solution were 39.9 and 143.4 mm^3, respectively. The tumor volumes of mice treated with paclitaxel in PEO–PCL nanoparticles after 16 and 28 days were 33.6 and 134.3 mm^3, respectively. In contrast, upon treatment with paclitaxel in PEO–PbAE nanoparticles, the average tumor volume was only 26.1 and 75.8 mm^3 after 16 and 28 days, respectively. The dynamics of tumor volume response to therapy was determined by measuring the doubling time and growth delay (Table 2). The volume doubling time of control group was 3.92 ± 0.4 days, whereas treatment with PEO–PbAE increased the tumor volume doubling time to 9.31 ± 1.1 days. The control and paclitaxel solution treated tumors grew three-folds in 12 and 16 days respectively, whereas treatment with PEO–PbAE formulation was effective in suppressing the growth of tumors and in 18 days it reaches 1.8-folds. The doubling time and growth delay was increased 2 and 2.5 times with PEO–PbAE nanoparticles compared to untreated animals. The doubling time and growth delay values of PEO–PCL were slightly higher than paclitaxel in solution. Above find-

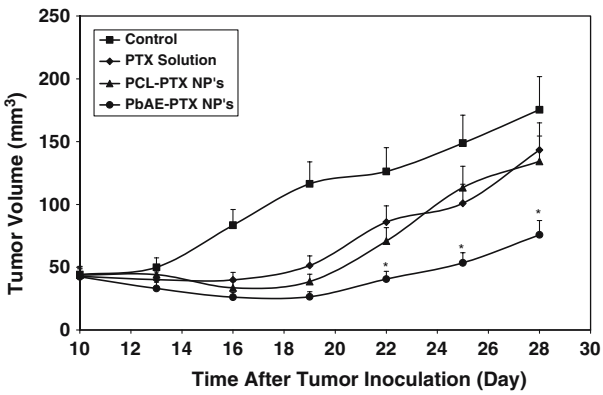

Fig. 1 Change in tumor volume as a function of time in subcutaneous SKOV-3 ovarian adenocarcinoma xenograft-bearing female *Nu/Nu* mice following single dose paclitaxel therapy in aqueous solution (PTX solution), poly(ethylene oxide)-modified poly(epsilon-caprolactone) nanoparticle formulation (PCL-PTX Np's), and poly ethylene oxide)-modified poly beta amino ester) (PbAE-PTX Np's). The intravenous paclitaxel dose of 20 mg/kg was administered in all of the formulations (*Statistically significant at $P < 0.05$ relative to paclitaxel aqueous solution)

Table 2 Tumor volume doubling time and growth delay times of SKOV-3 ovarian cancer xenografts in controls and poly(ethylene oxide)-modified nanoparticle formulations-treated mice

Substance	Growth delay time (days)	Tumor volume doubling time (days)
Cremophor EL: ethanol (1:1)	12.0 ± 1.0[a]	3.9 ± 0.4
Paclitaxel solution	16.1 ± 0.9	4.4 ± 0.5
Paclitaxel-loaded PEO–PCL	17.6 ± 1.4	4.8 ± 0.6
Paclitaxel-loaded PEO–PbAE	> 18 ± 1.3[b]	9.3 ± 1.1[b]

The average tumor doubling time and growth delay time of each group was calculated according to the formula described in "Materials and methods"

[a] Mean ± SD ($n = 4$)

[b] $P < 0.05$ relative to paclitaxel aqueous solution

ings demonstrates that paclitaxel reaches its intended destination in sufficient amounts when it is delivered through pH sensitive PEO–PbAE nanoparticles.

The efficacy results were further supported by measurements of optimum tumor volume ratios, defined as the lowest volumes obtained within 4 weeks after treatment, which are reported in Fig 2. On day 19 post-inoculation of the tumor, the volume ratio was 0.44 for the group receiving paclitaxel in aqueous solution, 0.33 for the PEO–PCL nanoparticle formulation group, and 0.22 for the PEO–PbAE nanoparticle formulation group.

At the time of sacrifice (28 days post-inoculation), the tumor mass was excised and weighed. As shown in Fig. 3, the weight of control (untreated) tumor was 0.54 g. Upon treatment with paclitaxel in solution, the

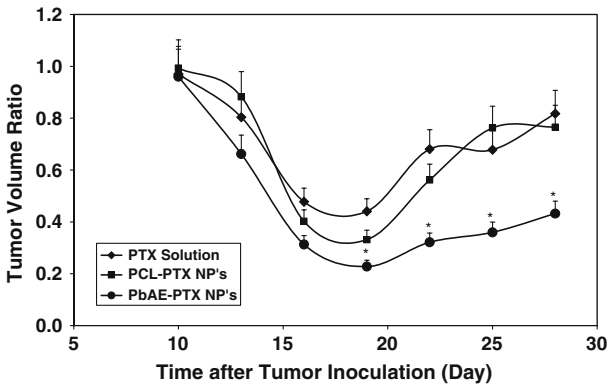

Fig. 2 Change in tumor-to-control volume ratios as a function of time in subcutaneous SKOV-3 ovarian adenocarcinoma xenograft-bearing female *Nu/Nu* mice following single dose paclitaxel therapy in aqueous solution (PTX solution), poly(ethylene oxide)-modified poly(epsilon-caprolactone) nanoparticle formulation (PCL-PTX Np's), and poly(ethylene oxide)-modified poly(beta amino ester) (PbAE-PTX Np's). The intravenous paclitaxel dose of 20 mg/kg was administered in all of the formulations. (*Statistically significant at $P < 0.05$ relative to paclitaxel aqueous solution)

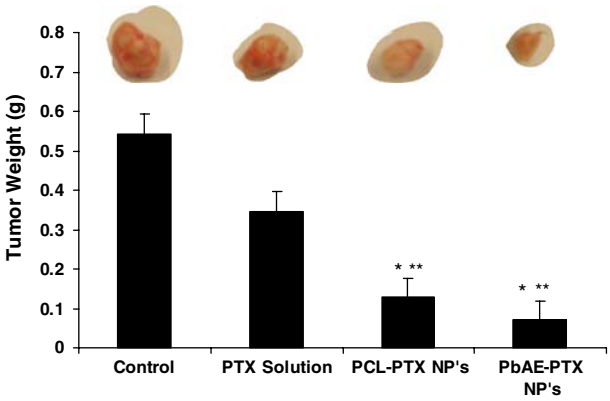

Fig. 3 The weights and gross images of the excised tumor mass from the subcutaneous SKOV-3 ovarian adenocarcinoma xenograft-bearing female *Nu/Nu* mice at day 25 post-administration of single dose paclitaxel therapy in aqueous solution (PTX solution), poly(ethylene oxide)-modified poly(epsilon-caprolactone) nanoparticle formulation (PCL-PTX Np's), and poly(ethylene oxide)-modified poly(beta amino ester) (PbAE-PTX Np's). The intravenous paclitaxel dose of 20 mg/kg was administered in all of the formulations. (*Statistically significant at $P < 0.05$ relative to untreated control, **statistically signifcant at $P < 0.05$ relative to paclitaxel aqueous solution)

tumor weight decreased to 0.35 g. Administration of paclitaxel in PEO–PCL and PEO–PbAE nanoparticle formulations resulted in significant decrease in tumor weight of 0.13 and 0.07 g, respectively. The significantly lesser tumor volumes, volume ratios, and tumor weight upon administration of paclitaxel in PEO–PbAE nanoparticle formulations is attributed to improvement in the bioavailability of the drug at the tumor site as well as in the cells upon administration in pH-sensitive polymeric nanoparticulate formulations. PEO–PCL nanoparticles were not as effective in suppressing tumor growth probably due to slow drug release from the nanoparticles after localization in the tumor mass

Evaluation of safety

Safety of paclitaxel administered in the aqueous solution and in nanoparticulate formulations was evaluated by measuring the body weight changes as a function of time and the changes in blood cell counts. These parameters are generally used as safety indicators in cancer chemotherapy. The weight of untreated tumor-bearing animals measured on day 10 post-inoculation (first day of therapy) was 16.5 g and decreased to 14.8 g on day 16 post-inoculation (Fig 4). The average body weight of the control group started out lower than all of the test groups. For the paclitaxel-treated animals, the body weight remained within 22–24 g per animal over the course of therapy. In addition, there was no

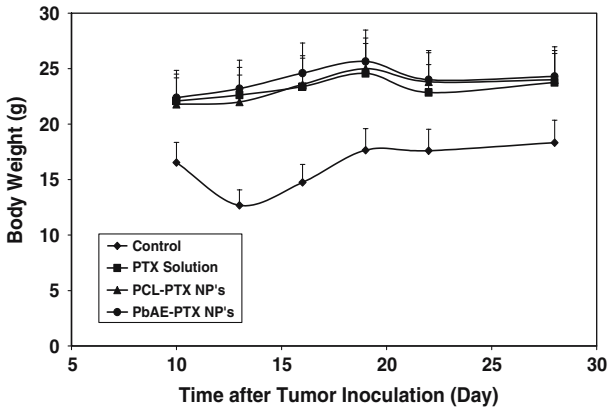

Fig. 4 Change in body weight of as a function of time in subcutaneous SKOV-3 ovarian carcinoma xenograft-bearing female *Nu/Nu* mice following single dose paclitaxel therapy in aqueous solution (PTX solution), poly(ethylene oxide)-modified poly(epsilon-caprolactone) nanoparticle formulation (PCL-PTX Np's), and poly(ethylene oxide)-modified poly beta amino ester) (PbAE-PTX Np's). The intravenous paclitaxel dose of 20 mg/kg was administered in all of the formulations

Table 3 White blood cells and platelet counts on day 28 post-inoculation of tumor mass in the control and poly(ethylene oxide)-modified nanoparticle formulations to SKOV-3 tumor-bearing female *Nu/Nu* mice

Treatment	White blood cell counts (10^9per l)	Platelet counts (10^{12}per l)
Normal mice	4.78 ± 0.37	1.48 ± 0.03
Cremophor EL: ethanol (1:1)	9.75 ± 0.60[a]	1.01 ± 0.08
Paclitaxel solution	6.28 ± 0.40	1.15 ± 0.06
Paclitaxel-loaded PEO–PCL	7.38 ± 0.30	1.07 ± 0.02
Paclitaxel-loaded PEO–PbAE	5.48 ± 0.40	1.25 ± 0.02

White blood cells and platelets were collected from blood samples of control and treatment animals at 28 days post-inoculation of the tumor mass

[a] Mean ± SD (n = 4)

difference between the weights of animals receiving paclitaxel in aqueous solutions or in the nanoparticle formulations. The results show that paclitaxel administered as a single dose in the aqueous solution or in nanoparticle formulation did not induce any toxicity that would affect the body weight of the tumor-bearing animals. Hematological toxicity was determined by measuring the white blood cells and platelet counts at day 28 post-inoculation of tumor mass. The results presented in Table 3 show that there were no significant differences in the white blood cell and platelet counts of animals treated with single dose of paclitaxel either in aqueous solution, PEO–PCL nanoparticles, or PEO–PbAE nanoparticles.

Conclusions

PEO-modified PbAE and PCL nanoparticles can be prepared successfully by blending the respective polymers with Pluronic® F-108. The PPO central block gets anchored onto nanoparticle surface more strongly than mere physical adsorption leaving the hydrophilic PEO chains mobile on surface leading to steric stabilization of the nanocarrier. When used for in vivo delivery of paclitaxel to ovarian cancer xenograft in athymic mice, PEO-modified PbAE nanoparticles have shown significant tumor growth inhibition compared to the free paclitaxel at the same dose level without any toxicity on blood cell parameters. The above data suggests that pH-responsive PEO–PbAE nanoparticles were an effective delivery system for hydrophobic anticancer drugs, such as paclitaxel, to solid tumor.

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