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Antitumor efficacy of solid dispersion of paclitaxel prepared by supercritical antisolvent process in human mammary tumor xenografts

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ABSTRACT

The efficacy of intravenous chemotherapy for breast cancer has been improving with newer agents. However, the fractional improvements in breast cancer progression-free survival were quite modest and these small gains are obtained at the cost of significant toxicity. To address this problem, paclitaxel solid dispersion (PSD), a Cremophor EL-free formulation prepared by supercritical antisolvent process using hydrophilic polymers as carrier, was developed to avoid Cremophor EL-associated toxicities in Taxol®. In this study, we investigated the antitumor activity of PSD as a function of dose from 12 to 24 mg/kg (dose–effect) and compared antitumor activity of 18 mg/kg dose of PSD to that of Taxol® (relative efficacy) in female athymic mice bearing mammary tumor xenografts. In dose–effect study, PSD showed excellent activity and good tolerance at all doses tested with a significant increase in tumor growth inhibition, recurrence time, survival percent, and number of tumor free survivors compared to control ($P < 0.01$). In all of the four doses tested in this study, the magnitude of the increase in effectiveness of PSD was quite substantial and statistically significant with similar degrees of weight loss. In relative efficacy study of PSD and Taxol®, PSD demonstrated a greater degree of tumor growth inhibition with 10 complete tumor regressions (100%) and eight tumor-free survivors (80% cure). Besides, mice treated with PSD regained their initial body weight by day 27 following initial acute weight reductions, whereas mice treated with Taxol® required more than 40 days to regain their initial weight. In conclusion, PSD prepared by supercritical process was very effective and safe, without Cremophor EL-associated toxicities of Taxol®, in human mammary tumor xenografts with possibilities of dose escalation.

1. Introduction

Paclitaxel (PTX) is one of the most potent neoplastic agents available today and is widely used in the treatment of a variety of human neoplastic disorders, including platinum-resistant ovarian cancer, breast and non-small cell lung cancer and leukemia, AIDS-related Kaposi's sarcoma and other cancers (Rowinsky et al., 1990; Holmes et al., 1991; Huizing et al., 1993; Rowinsky and Donehow, 1995; Wall and Wani, 1995). One of the major limitations associated with PTX is its low aqueous solubility due to its extremely hydrophobic nature. Taxol® (Bristol-Myers Squibb), commercially available intravenous formulation of PTX, is 6 mg/mL of PTX in a 50:50% (v/v) mixture of Cremophor EL (polyethoxylated castor oil derivative) and ethanol.

The amount of Cremophor EL necessary to deliver the required doses of PTX in Taxol® is significantly higher than that given with any other marketed formulations containing Cremophor EL. Therefore, Taxol® has encountered many problems including pharmacological, pharmacokinetic, and pharmaceutical problems during standard clinical practice (Weiss et al., 1990; Holmes et al., 1991; Rowinsky and Donehow, 1995; Rowinsky and Donehower, 1995). Besides, since chemotherapy is generally given at the highest tolerated dose, toxic side effects of Cremophor EL in Taxol® deny the possibilities to administer higher doses of PTX which is considered to be very important in the clinical practice (Desai et al., 2006; Adams et al., 1993; Riondel et al., 1986; Arbuck et al., 1993). Therefore, various PTX formulations without Cremophor EL have been investigated to administer PTX using liposomes, microspheres, micelles, nanoparticles, and prodrugs (Tarr et al., 1987; Alkan-

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Onyuksel et al., 1994; Dordunoo et al., 1995; Shieh et al., 1997; Bilensoy et al., 2008). However, all these formulations demonstrated problems of complicated preparative procedure and/or invariably low stability.

Recently, protein-bound PTX nanoparticles for injection (Abraxane) have been introduced for the treatment of breast cancer (Feng et al., 2010). However, it was reported that the fractional improvement in breast cancer progression-free survival for this formulation was quite modest (Gradishar et al., 2005). Hence, current approaches are mainly focused on developing PTX formulation that is effective and safe with possibilities of elevated dose, preparation on a large scale, and stability for longer periods of time (Panchagnula, 1998).

Recently, we reported preparation and characterization of paclitaxel solid dispersion (PSD) using supercritical antisolvent (SAS) process and evaluated its in vivo toxicity in ICR mice (Park et al., 2008, 2009). SAS technique, analogous to spray drying, allows drug–polymer interaction in molecular level and aids in generation of small, even, and easily wettable particles that are difficult or even impossible to obtain by traditional techniques such as milling, crystallization, and spray drying (Kompella and Koushik, 2001; Jung and Perrut, 2001). PSD prepared using hydrophilic polymers by this method showed enhanced solubility and stability for a longer period of time (Park et al., 2008).

In this study, we investigated the antitumor efficacy of PSD as a function of dose and compared its relative efficacy to that of Taxol® in athymic mice bearing MDA-MB-231 human mammary tumor xenografts. Antitumor activity and tolerance were evaluated in terms of tumor inhibition and body weight loss, respectively. The response was assessed by number of nonspecific deaths, number of partial and complete tumor regressions, number of tumor-free survivors, double doubling time, and tumor recurrence time (Desai et al., 2006).

2. Materials and methods

2.1. Materials

The following materials were purchased from various companies and then used as received. Paclitaxel (Natural pharmaceuticals, Inc., USA), hydroxypropyl β-cyclodextrin (HP-β-CD, ISP, Japan), Polyoxyl 40 hydrogenated castor oil (HCO-40, BASF Co., Ltd., Germany), polyvinyl pyrrolidone C-30 (PVP C-30, ISP, Japan), dichloromethane (Daejung Co., Korea), carbon dioxide (CO_2, high purity of 99.99%, Gyeonggi Gas Co., Ltd., Korea), acetonitrile (HPLC grade, Burdick & Jackson, USA), and ethanol (HPLC grade, Burdick & Jackson, USA). All other chemicals were of reagent grade and used without any further purification.

2.2. Preparation and characterization of PSD

The SAS process for preparing PSD was performed by our previously reported method (Park et al., 2008). The SAS process parameters and equipment used for SAS process were described in detail in our earlier article (Park et al., 2008). Briefly, CO_2 from the storage tank was delivered into top of the particle formation chamber using homemade plunger pump until equilibrium pressure (1200 psi) and temperature (40 °C) achieved. Then, the drug solution (flow rate 0.3 mL/min), prepared by dissolving appropriate amounts of PTX, hydrophilic polymers HP-β-CD/PVP C-30, and surfactant HCO-40 in a mixture of dichloromethane and ethanol (3/2, v/v), and supercritical CO_2 (flow rate 10 mL/min) were co-injected through the two-flow spray nozzle in the particle formation chamber filled with supercritical CO_2. After the injection of drug solution, fresh CO_2 was introduced into the chamber to remove residual solvent. During the SAS process, the pressure of the chamber was controlled constantly using a back pressure regulator. The PSD formed on the walls and the bottom of the chamber was collected after reducing the chamber pressure to atmospheric pressure.

2.3. Drug preparation and treatment schedule

PSD formulation contained 1 mg of PTX per 104.6 mg of powder (5 mg PTX per 523 mg). Homogeneous clear solution of PSD for intravenous injection was prepared by mixing appropriate amount of PSD powder into saline using vortex at a very slow speed for 10 min. On each day of injection, aliquots were diluted with saline according to exact body weight and injected within 20 min of preparation. Taxol® (Bristol-Myers Squibb, 6 mg/mL) was stored refrigerated (4 ± 2 °C) between injections. Control group animals received same amount of saline as treatment group.

PSD was administered i.v. at a dose of 12, 15, 18, or 24 mg/kg for five consecutive days (QD × 5) for the dose–effect study. Taxol® was administered i.v. at dosages of 18 mg/kg daily for five consecutive days (QD × 5). The injection volume was fixed at 0.1 mL/10 g of body weight. The control group was treated i.v. with saline for the same period of time. Drug treatment schedule was represented in Table 3.

2.4. Antitumor efficacy study

2.4.1. Animals

Seven-week-old female athymic nude mice were purchased from Charles River Laboratories (Wilmington, DE) and acclimated in the laboratory one week prior to experimentation. The animals were housed in a pathogen-free barrier facility in micro-isolator cages, with five animals per cage in a 12-h light/dark cycle. Mice were fed sterilizable rodent diet (Harlan-Teklad TD8656) *ad libitum*. Cages were changed twice weekly. The animals were observed daily and clinical signs were noted. Animal care and procedures were in accordance with N.I.H. guidelines and were approved by our Institutional Animal Care and Use Committee (IACUC).

2.4.2. Mammary tumor xenograft implantation

To establish tumor model, about 30–40 mg fragments of MDA-MB-231 human mammary tumor were implanted subcutaneously in mice near the right axillary area using a 12-gauge trocar needle and allowed to grow. Tumors were allowed to reach 100–198 mg in weight (100–198 mm^3 in size) before the start of treatment. A sufficient number of mice were implanted so that tumors in a weight range as narrow as possible were selected for the study on day 14 post-implantation. Those animals selected with tumors in the proper size range were assigned to the various treatment groups so that the median tumor weights on the first day of treatment were as close to each other as possible (171–176 mg).

2.4.3. Tumor growth measurement

The subcutaneous tumors were measured and the animals were weighed twice weekly starting with the first day of treatment. Tumor volume was determined by caliper measurements (mm) and using the formula for an ellipsoid sphere: $(L \times W^2)/2 = mm^3$, where L and W refer to the larger and smaller perpendicular dimensions collected at each measurement. Study was carried for a period of 90 days after tumor implantation. Any animal found moribund or any animal whose tumor reached 4000 mg, ulcerated was euthanized for humane reasons prior to study termination.

2.4.4. Antitumor efficacy evaluation

To evaluate the efficacy of PSD (response) in athymic mice bearing MDA-MB-231 human mammary tumor xenografts, response parameters such as number of nonspecific deaths (NSD), number of partial and complete tumor regressions, number of tumor-free survivors (TFS), and median number of days for the tumors in each

group to reach evaluation size, i.e. two tumor mass doublings (double doubling time, DDT), and time to tumor recurrence or overall delay in the growth of the median tumor (tumor growth delay, TGD) were evaluated as mentioned by Desai et al. (2006).

A treated, tumor-bearing animal was presumed to be a nonspecific death if its day of death was significantly less ($P < 0.05$) than the corresponding day of death in the control group and its tumor was less than 400 mg, or if it died with a tumor of 400 mg or less prior to 45 days after the last day of treatment, or with a regressing tumor prior to 15 days after the last day of treatment, or if the treated animal was uniquely specified as a nonspecific death on data input. Tumor regression was scored excluding nonspecific deaths according to the smallest tumor size attained after the beginning of treatment relative to the size at first treatment. Animal with tumor 50% of its size at first treatment, but not complete was considered partial regression, while any unpalpable tumor was scored complete regression. The interval during which a tumor was classified as partial or complete regressor was below 50% of its size at first treatment is measured as duration of regression. The time required for a tumor to double in mass was calculated based on the initial tumor weight at the beginning of the treatment period. When the initial tumor weight has been selected, tumor weights were then examined, beginning with the last recorded value, until a doubling was calculated. Examination from the last recorded value is to ensure that the doubling time was calculated during the final phase of tumor growth and not prior to a tumor regression. Values between measurements were calculated by exponential extrapolation, and a value may be estimated after the final measured weight provided the extrapolated value occurs prior to the animal's death. TGD was the difference in the median of times postimplant for tumors of the treated groups to attain evaluation size compared to the median of the control group. The TGD value was measured excluding nonspecific deaths and any other animal that dies whose tumor failed to attain the evaluation size. All treatments were initiated on day 14 post-implant in animals with median tumor weights of 171–176 mg and the experiment was terminated on day 90 after tumor implantation.

2.5. Statistical analyses

Statistical analyses were carried out using SPSS statistical software (SPSS Statistics, Ver. 17.0). DDT and TGD were analyzed by Kaplan–Meier's techniques. Tumor volume and body weight were compared for statistical differences using ANOVA followed by least significant difference (LSD) post hoc test.

3. Results and discussion

Solid dispersion by hydrophilic polymers has been emerging as an alternative vehicle for hydrophobic drugs as this system tends to stabilize the amorphous hydrophobic drug in hydrophilic polymers through drug–polymer interactions that results in enhanced solubility and stability of the incorporated drug (Aceves et al., 2000; Matsumoto and Zografi, 1999; Oth and Moes, 1989; Pignatello et al., 2001; Taylor and Zografi, 1997). It was reported that solid dispersions formulated with hydrophilic polymers deliver high efficacy, lower toxicity, and favorable pharmacokinetic features of PTX (Lee et al., 2008).

Our earlier study showed possible method of preparation of solid dispersion of PTX by a novel supercritical antisolvent process utilizing hydrophilic polymers that produced nano-sized particles with enhanced water solubility (>20 mg/mL for PSD vs 0.7 μg/mL for pure PTX) and remarkable stability (Park et al., 2008). Additionally, we reported that toxicity studies performed in ICR mice with PSD exhibited lower toxicity and higher safety profile compared to Taxol® in terms of LD_{50} (160 mg/kg PSD vs 31.3 mg/kg Taxol®), nephrotoxicity (no significant change in creatinine clearance up to 50 mg/kg of PSD vs death of all animals at 15 mg/kg dose of Taxol®), and hemolytic activity (10% with PSD vs 40% with Taxol®).

Table 1
Composition of PSD produced by SAS process to study antitumor activity in female athymic mice bearing MDA-MB-231 human mammary tumor xenografts.

Ingredient	Amount (mg)	Use
Paclitaxel	5	Active ingredient
HP-β-CD	100	Hydrophilic polymer
PVP C-30	165	Hydrophilic polymer
HCO-40	250	Surfactant
α-Tocopherol	3	Antioxidant
Total	523	

3.1. PSD preparation and characterization

PSD was prepared by precipitation of PTX from a mixture of dichloromethane and ethanol (3/2, v/v) using our previously reported SAS process (Park et al., 2008). Formulation composition of PSD prepared was shown in Table 1. A thorough characterization of PSD was described in our earlier report (Park et al., 2008) and the properties of prepared PSD and Taxol® were presented in Table 2.

3.2. Efficacy of PSD as a function of dose

The dose–effect relationship of PSD was investigated in athymic mice bearing MDA-MB-231 human mammary tumor xenografts and tumor growth inhibition as a function of PSD dose from 12 to 24 mg/kg was presented in Fig. 1. We selected MDA-MB-231 human mammary tumor model for our study as this is a well accepted and representative model of estrogen-independent breast cancer in research community (Miyazaki et al., 1998; Yano et al., 1992). Treatment of mice with PSD at doses of 12, 15, 18, and 24 mg/kg was well tolerated with no non-specific death in any of groups studied. Tumors in the control group grew well in all 10 mice and the rate of tumor growth in this group was significantly higher than mice treated with PSD. Fig. 1 shows that there was a well defined progressive increase in tumor growth inhibition from 12 to 24 mg/kg. Statistical analyses demonstrated that all dose range tested were significantly efficacious in terms of inhibiting tumor growth compared to control. A significant delay in tumor growth was first detected on day 20 for all doses ($P < 0.05$) except 12 mg/kg dose. Beginning day 23, all doses including 12 mg/kg were found to be statistically significant ($P < 0.001$) compared to control (Table 3).

Median tumor reached two tumor doublings in 5.1 days for saline-treated control group, while it was more than 75 days for

Table 2
Physical properties of Taxol® and PSD produced by SAS process to study antitumor activity in female athymic mice bearing MDA-MB-231 human mammary tumor xenografts.

Properties	PSD	Taxol®
Bulk density	0.32	NA
Solubility[a]	>20 mg/mL	6 mg/mL
Precipitation time[b]	>70 h	<27 h
Turbidity[c]	Clear	Slightly hazy
pH[d]	4.4 ± 0.1	5.8 ± 0.1
Osmolarity[e]	308 mOsm/kg	748 mOsm/kg
Mean particle size	0.37 μm	NT

[a] Solubility of pure paclitaxel powder in water was 0.7 μg/mL.
[b] Precipitation time was the time taken for PTX to precipitate after formulation equivalent to 1000 μg/mL PTX was mixed in water.
[c] Turbidity measured visually upon dilution in dextrose saline.
[d] USP specification of pH for intravenous PTX injection is between 3.0 and 7.0.
[e] Osmolarity measured at formulation concentration of 300 μg/mL and osmolarity of 0.9% (w/w) NaCl is 300 mOsm/kg; NA, not applicable; NT, not tested.

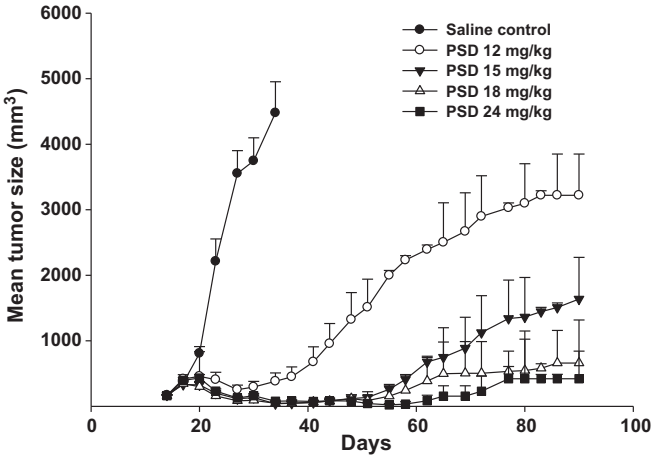

Fig. 1. Tumor growth inhibition in female athymic nude mice bearing MDA-MB-231 human mammary tumor xenografts treated with either saline or PSD at a dose of 12, 15, 18, or 24 mg/kg intravenously for five consecutive days beginning day 14 post-implantation. Each point represents mean ± SEM.

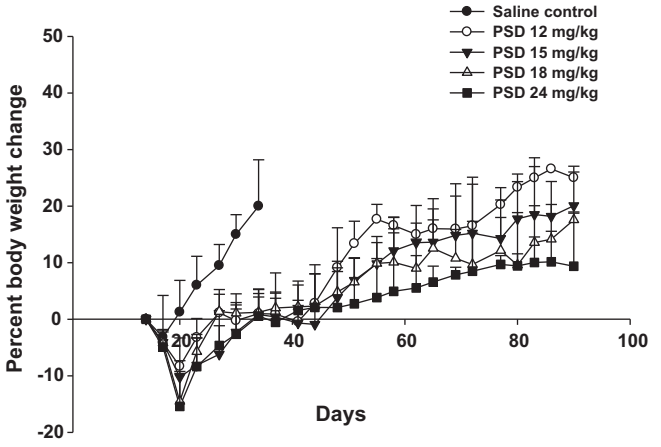

Fig. 2. Mean body weight change in female athymic nude mice bearing MDA-MB-231 human mammary tumor xenografts treated with either saline or PSD at a dose of 12, 15, 18, or 24 mg/kg intravenously for five consecutive days beginning day 14 post-transplantation. Each point represents mean ± SEM.

Table 3
Drug treatment schedule of Taxol® and PSD produced using SAS process to study antitumor activity in female athymic mice bearing MDA-MB-231 human mammary tumor xenografts.

Compound	Animals	Dose	Treatment schedule
Control[a]	10	0	QD × 5
Taxol® (BMS)[b]	10	18	QD × 5
PSD[c]	10	24	QD × 5
	10	18	QD × 5
	10	15	QD × 5
	10	12	QD × 5

[a] Control was saline with an injection volume of 0.2 mL/10 g body weight.
[b] Taxol® with an injection volume of 0.1 mL/10 g body weight.
[c] PSD with designated doses (mg/kg) of PTX with an injection volume of 0.2 mL/10 g body weight; QD × 5 once a day dose for five consecutive days beginning day 14 post-implantation.

15–24 mg/kg dose with mean overall tumor growth delay of more than 70 days (Table 4). It could also be seen from Fig. 1 that 12 mg/kg of PSD was effective enough to delay the tumors up to a period of about 20 days, however tumors appeared to regrew rapidly in most of the animals resulting in no tumor free survivors (0% cure) with two complete regressions and one partial regressions out of 10 animals. Recurrence of tumor was seen in few mice treated with 15–24 mg/kg of PSD as well. However, it was relatively less compared to the mice treated with 12 mg/kg of PSD, and the rate of tumor regrowth or recurrence was found to be inversely proportional to PSD dose. The lowest regrowth was observed with the highest dose of 24 mg/kg. The final estimated mean tumor volume on day 90 in mice treated with PSD doses of 24, 18, and 15 was 10.33%, 15.49%, and 50.79% of that in mice treated with PSD dose of 12 mg/kg.

Mean body weight change of all animals was shown in Fig. 2 and average maximum body weight loss of 8.3%, 10.1%, 14.8%, and 15.4% was observed in mice treated with 12, 15, 18, and 24 mg/kg dose of PSD, respectively. Although, all tested doses produced acute reductions in body weight, weight recovery was more rapid following drug administration. All animals regained their body weight by 5–11 days after the last dose of five-day dose of PSD. There was a slight increase in body weight of all animals during the course of study as a result of natural animal growth. Significant increase in body weight of mice treated with 12 mg/kg of PSD might be due to normal growth coupled with relatively rapid regrowth of tumors (Koziara et al., 2006). The results of the current study provided an additional reason for interest in PSD. In all of the four doses tested in this study, PSD produced similar degrees of weight loss (statistically insignificant) for all doses, but the magnitude of the increase in effectiveness was quite substantial and statistically significant.

In this study, it was worth noting that there was a modest increase in survival percent of animals in all treatment groups compared to control during the study period (Fig. 3). Besides, tumor free survivors (cure) increased with increasing dose of PSD and there were nine, ten, and eight complete tumor regressions out of ten animals treated with PSD dose of 24, 18, and 15 mg/kg, which resulted in nine, eight, and five tumor-free survivors, respectively (Table 4). Thus, it was evident that PSD exhibited significant antitumor activity in athymic mice bearing MDA-MB-231 human mammary tumor xenografts with minimal weight loss, i.e. high tolerability even at high dose.

Table 4
Antitumor efficacy parameters (response) evaluated in female athymic mice bearing MDA-MB-231 human mammary tumor xenografts treated with Taxol® or PSD produced using SAS process.

Compound	Dose (mg/kg)	NSD	Number of tumor regression		TFS	DDT (day)	TGD (day)	LOW (%)
			Partial	Complete				
Control	0	0/10	0/10	0/10	0/10	5.1	–	
Taxol® (BMS)	18	0/10	0/10	9/10	7/10	>76.0	>70.9	13.5
	24	0/10	1/10	9/10	9/10	>76.0	>70.9	15.4
PSD	18	0/10	0/10	10/10	8/10	>76.0	>70.9	14.8
	15	0/10	0/10	8/10	5/10	>75.1	>70.0	10.1
	12	0/10	1/10	2/10	0/10	24.9	19.8	8.3

NSD, total non-specific death; TFS, total tumor free survivor; DDT; double doubling time; TGD; time for tumor growth delay or recurrence; LOW, loss of weight (%) = (initial weight − minimum weight)/initial weight × 100.

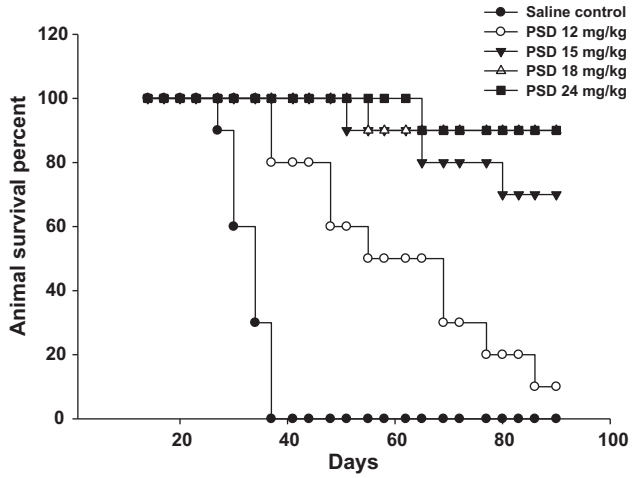

Fig. 3. Survival percent of female athymic nude mice bearing MDA-MB-231 human mammary tumor xenografts treated with either saline or PSD at a dose of 12, 15, 18, or 24 mg/kg intravenously for five consecutive days beginning day 14 post-transplantation.

3.3. Relative efficacy of PSD and Taxol®

To compare the relative efficacy of PSD to that of Taxol®, athymic mice bearing MDA-MB-231 human mammary tumor xenografts were i.v. treated with equal doses of 18 mg/kg of each formulation for a period five consecutive days. Dose selected in this experiment was based on our earlier acute toxicity study performed with PSD and Taxol® (Park et al., 2009). In acute toxicity study, Taxol® was found to be toxic with a dose more than 20 mg/kg, while PSD showed good tolerance and safety with dose as high as 160 mg/kg. Therefore, in this study Taxol® dose was fixed at its maximum tolerant dose of 18 mg/kg for safety concerns with longer study period (Park et al., 2009).

As shown in Fig. 4, Taxol® was effective against MDA-MB-231 mammary tumor and produced statistically significant degrees of tumor growth inhibition relative to growth of tumor in saline-treated control mice ($P < 0.001$). Administration of Taxol® at 18 mg/kg resulted in average maximum body weight loss of 13.5% (Fig. 5). Interestingly, PSD produced a greater degree of inhibition of tumor growth than Taxol® beginning day 19 and continued to be effective until termination of experiment. Time for double tumor doubling was more than 75 days for both formulations. However,

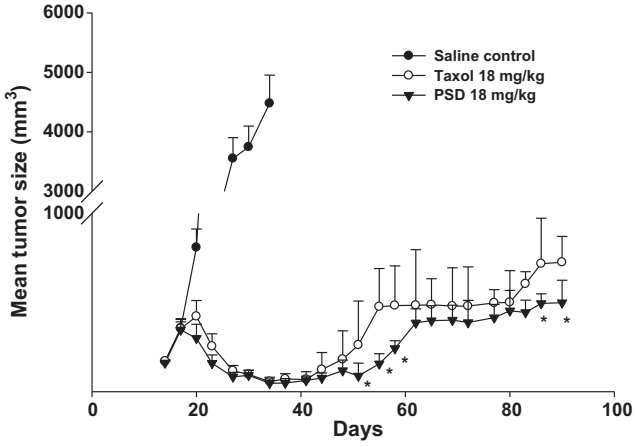

Fig. 4. Tumor growth inhibition in female athymic nude mice bearing MDA-MB-231 human mammary tumor xenografts treated with either saline or 18 mg/kg dose of either PSD or Taxol® intravenously for five consecutive days beginning day 14 post-transplantation. Each point represents mean ± SEM. *$P < 0.05$.

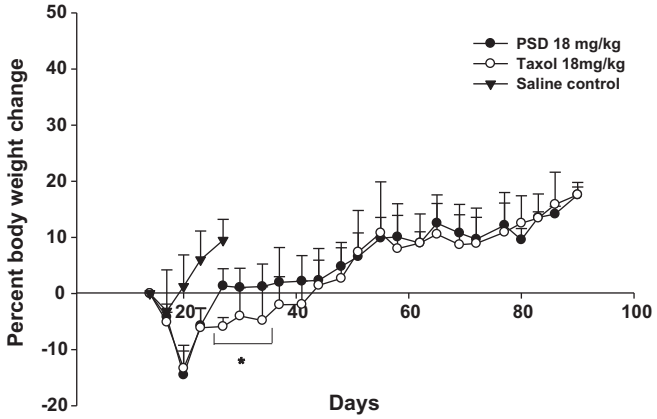

Fig. 5. Mean body weight change in female athymic nude mice bearing MDA-MB-231 human mammary tumor xenografts treated with either saline or 18 mg/kg dose of PSD or Taxol® intravenously for five consecutive days beginning day 14 post-transplantation. Each point represents mean ± SEM. *$P < 0.05$.

treatment of mice with 18 mg/kg of PSD resulted in 10 complete tumor regressions (100%) with eight tumor-free survivors (80% cure). At the same dose, Taxol® produced nine complete tumor regressions with seven tumor free survivors (Table 4).

While mice treated with both formulations showed tumor regrowth in some animals, tumor regrowth or recurrence was higher in mice treated with Taxol®. The recurrence pattern of Taxol® was consistent with the earlier reports (Koziara et al., 2006; Kim et al., 2001; Yano et al., 1992). It was reported that though Taxol® administered at its maximum tolerated dose delayed tumor growth in MX-1 mammary tumor xenografts, tumors regrew as rapidly as those in control mice after 48 days (Kim et al., 2001). Statistical analysis demonstrated that PSD produced significant inhibition of tumor regrowth relative to that of Taxol® during day 51–58 ($P < 0.01$). Besides, comparison of tumor regrowth in mice on day 90 showed that tumor volume in the mice treated with PSD was only 59.9% of that in the mice treated with Taxol®, suggesting slower degree of tumor regrowth in mice treated with PSD. It could also be seen from Fig. 6 that the survival rate of animals treated with 18 mg/kg of PSD was higher than the mice treated with Taxol® at same dose (90% vs 80%) up to 60 days of post-implantation.

As indication of toxicity, the change of body weight during the course of treatment was recorded (Feng et al., 2010). Fig. 5 showed

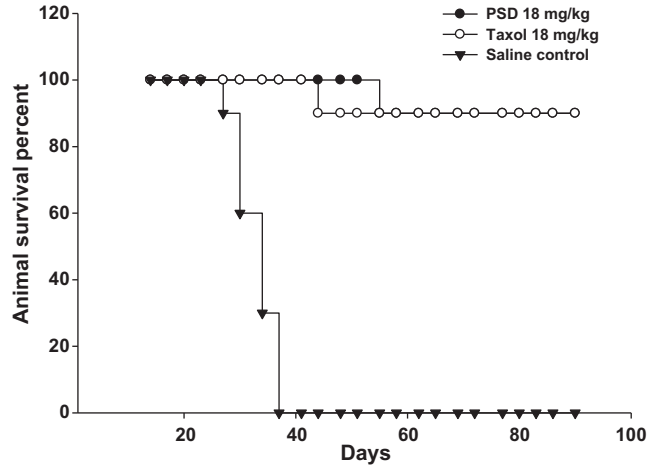

Fig. 6. Survival percent of female athymic nude mice bearing MDA-MB-231 human mammary tumor xenografts treated with either saline or 18 mg/kg dose of PSD or Taxol® intravenously for five consecutive days beginning day 14 post-transplantation. *$P < 0.05$.

that 18 mg/kg dose of Taxol® produced 13.5% of average maximum weight loss with a nadir on day 20. PSD at the same dose produced same degree of weight loss (14.8%) with a nadir on day 20. While both formulations produced equivalent acute reductions in body weight, weight recovery was more rapid following administration of PSD. Mice treated with PSD regained their initial body weight by day 27, whereas mice treated with Taxol® required more than 40 days. These results suggested excellent tolerance of mice to PSD dose as high as 24 mg/kg.

Although PTX administered as Taxol® is one of the most effective anticancer drugs in use today, it bestows considerable toxicity to patients due to the presence of higher amount of Cremophor EL (Alkan-Onyuksel et al., 1994; Bilensoy et al., 2008). Therefore, it is important that novel technologies are developed to reduce toxicities with possibilities of dose escalation. Based on tumor inhibition, survival rate, and evolution of mice weight during the treatment period, mice treated with PSD appeared to be better tolerated and showed higher efficacy and reduced tumor recurrence. Chemotherapy is generally given at the maximum tolerated dose in standard clinical practice and the demonstrated tolerability of PSD at higher dose extends the possibility of administration to its maximum tolerable dose. Given this fact, it is important that we further optimize the dose (dose escalation for maximum tolerated dose) and injection regimen in order to maximize the antitumor efficacy of PSD. Nonetheless, our initial results indicated that PSD prepared by SAS process is effective and safe formulation of PTX and justify further development process.

4. Conclusion

In summary, PSD showed excellent antitumor activity and high tolerance levels in athymic mice bearing MDA-MB-231 human mammary tumor xenografts. Besides, PSD showed possibilities of dose escalation to higher level without Cremophor EL-associated toxicities of Taxol®. The results of this study bolster the validity of a novel Cremophor EL-free PSD formulation prepared by SAS process using hydrophilic polymers as carrier for cancer therapy, combining efficacy, high safety/tolerance, improved stability and possibility of large scale production.

Acknowledgement

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