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Paclitaxel-loaded polymeric microparticles: Quantitative relationships between *in vitro* drug release rate and *in vivo* pharmacodynamics

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ABSTRACT

Intraperitoneal therapy (IP) has demonstrated survival advantages in patients with peritoneal cancers, but has not become a widely practiced standard-of-care in part due to local toxicity and sub-optimal drug delivery. Paclitaxel-loaded, polymeric microparticles were developed to overcome these limitations. The present study evaluated the effects of microparticle properties on paclitaxel release (extent and rate) and *in vivo* pharmacodynamics. *In vitro* paclitaxel release from microparticles with varying physical characteristics (*i.e.*, particle size, copolymer viscosity and composition) was evaluated. A method was developed to simulate the dosing rate and cumulative dose released in the peritoneal cavity based on the *in vitro* release data. The relationship between the simulated drug delivery and treatment outcomes of seven microparticle compositions was studied in mice bearing IP human pancreatic tumors, and compared to that of the intravenous Cremophor micellar paclitaxel solution used off-label in previous IP studies. Paclitaxel release from polymeric microparticles *in vitro* was multiphasic; release was greater and more rapid from microparticles with lower polymer viscosities and smaller diameters (*e.g.*, viscosity of 0.17 *vs.* 0.67 dl/g and diameter of 5–6 *vs.* 50–60 μm). The simulated drug release in the peritoneal cavity linearly correlated with treatment efficacy in mice ($r^2 > 0.8$, $p < 0.001$). The smaller microparticles, which distribute more evenly in the peritoneal cavity compared to the large microparticles, showed greater dose efficiency. For single treatment, the microparticles demonstrated up to 2-times longer survival extension and 4-times higher dose efficiency, relative to the paclitaxel/Cremophor micellar solution. Upon repeated dosing, the paclitaxel/Cremophor micellar solution showed cumulative toxicity whereas the microparticle that yielded 2-times longer survival did not display cumulative toxicity. The efficacy of IP therapy depended on both temporal and spatial factors that were determined by the characteristics of the drug delivery system. A combination of fast- and slow-releasing microparticles with 5–6 μm diameter provided favorable spatial distribution and optimal drug release for IP therapy.

1. Introduction

Cancers originating from organs in the peritoneal cavity (ovarian, pancreatic, colorectal, gastric, liver, peritoneal mesothelioma) account for about 250,000 new cases annually in the US [1]. Peritoneal metastases are common due to locoregional spread (*e.g.*, incidence of 90%, 50% and 32% in ovarian, pancreatic and colon cancer, respectively). Intraperitoneal (IP) therapy delivers high drug concentrations to tumors located in the peritoneal cavity [2–8]. The survival advantage of IP therapy was first demonstrated 17 years ago and has since been confirmed in multiple trials [9–13]. In spite of the demonstrated benefits, several difficulties have prevented IP therapy from becoming a widely practiced standard-of-care [14–16].

Due to the lack of approved IP products by the Food and Drug Administration, the previous IP trials involve off-label use of drugs that are approved for intravenous administration. We have proposed that intravenous formulations do not have the optimal pharmacokinetic properties for IP therapy [16]. For example, in rodents, >99% of an IP dose of paclitaxel dissolved in Cremophor micelles was cleared from the peritoneal cavity in less than 12 h, due to drainage through the lymphatic openings and absorption through the thin peritoneum membrane [17]. Further, the bolus presentation of the entire dose within the peritoneal cavity introduces high local drug concentrations and local toxicity. An alternate approach is to develop specialized delivery systems to optimize IP therapy.

Biodegradable polymeric microparticles, due to their stability and long track record of safety in human patients, represent a suitable system to provide controlled drug release [18–20]. A popular copolymer

Abbreviations: AIC, Akaike Information Criterion; GA, glycolic acid; HPLC, high performance liquid chromatograph; ILS, increase in life span; IP, intraperitoneal; LA, lactic acid; MST, median survival time; PLGA, poly(D,L-lactide-co-glycolide) acid; Tg, glass transition temperature; VIS, inherent viscosity; MP, microparticles; SF, small microparticles with fast release rate; SS, small microparticles with slow release rate; LM, large microparticles with medium release rate.

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is poly(lactide-co-glycolide) (PLGA) that has been used in resorbable sutures, orthopedic fixation devices and drug carriers [21,22]. Drug release from PLGA microparticles is determined by polymer properties (*e.g.*, inherent viscosity or VIS, glass transition temperature or Tg, ratio of copolymers) and by microparticle properties (*e.g.*, size, extent of drug loading). The versatility of PLGA microparticles permits tailoring the design to achieve the desired drug release and residence [23–25]. For example, we have shown that the delivery of paclitaxel in PLGA microparticles resulted in 40% slower clearance from the peritoneal cavity and 3-times higher drug exposure, compared to the intravenous Cremophor micellar paclitaxel solution used off-label in previous IP studies [17].

The present study evaluated the effect of the properties of PLGA microparticles on *in vitro* paclitaxel release, developed a method to use the *in vitro* release data to simulate the *in vivo* drug dosing rate and cumulative delivery, and determined the quantitative relationship between drug delivery/release and *in vivo* pharmacodynamics (*i.e.*, treatment outcome). The *in vivo* studies were performed in mice implanted with IP human pancreatic xenograft tumors.

2. Materials and methods

2.1. Chemicals and reagents

Paclitaxel was obtained from Hande Tech Co. (Houston, TX), polyvinyl alcohol (PVA) from Sigma Chemical Company (St. Louis, MO), and solvents from Fisher Scientific Company (Fair Lawn, NJ). PLGA with VIS ranging from 0.17 to 0.7 dl/g and a copolymer ratio of 50:50 or 75:25 (lactic acid:glycolic acid or LA:GA) were purchased from Birmingham Polymer Inc. (Birmingham, AL). High performance liquid chromatography (HPLC) analysis showed that paclitaxel was >99% pure. Cremophor El was purchased from Sigma Chemical Company (St. Louis, MO). All chemicals and reagents were used as received.

2.2. Preparation and characterization of paclitaxel-loaded PLGA microparticles

PLGA microparticles were prepared as previously described [17]. Briefly, paclitaxel and PLGA were dissolved in dichloromethane, followed by emulsification in an aqueous polyvinyl alcohol solution. The mixing speed of the emulsion dictated the resulting size of the microparticles, producing smaller particles at increased speed. Evaporation of dichloromethane yielded drug-loaded microparticles. The microparticles were collected by centrifugation and washed in distilled water, followed by freeze-drying in a Freezone 4.5 lyophilizer (Labconco Corp., Kansas City, MO).

We prepared seven drug-loaded PLGA microparticles comprising different LA:GA ratios and polymers with different VIS. Particle surface morphology of microparticles was examined using a Phillips XL 30 scanning electron microscope; the SEM images were used to determine the average particle size (at least 300 particles per batch were measured). Tg was determined from thermograms using a differential scanning calorimeter (Perkin-Elmer DSC, Model 7). Paclitaxel-loaded microparticles were dissolved in dichloromethane and analyzed by HPLC, and entrapment efficiency was calculated as (mass of entrapped drug) divided by (mass of drug used in preparation). Yield was calculated as (mass of microparticles produced) divided by (mass of drug plus polymer used in preparation). Drug loading was defined as drug mass divided by mass of drug plus polymer.

2.3. In vitro drug release

Paclitaxel-loaded microparticles containing about 50 μg/ml of paclitaxel-equivalents were suspended in release medium (0.1% w/v Tween 80 in phosphate-buffered saline), at 37 °C in an orbital shaker. At specified time points, samples were centrifuged and the resulting supernatant was analyzed for the concentration of free drug (*i.e.*, not bound to microparticles). The remaining microparticle pellet was resuspended in fresh release medium and the procedures were repeated until the end of the release study, at which time the remaining microparticles were dissolved in dichloromethane and the mass balance was calculated as (cumulative amount of drug released plus amount of drug recovered at the end of study) divided by (total amount of drug loaded into the microparticles before the release study). Initial burst was defined as the dose fraction released during the first 24 h. The duration of the release study was 28 days.

2.4. Calculation of dosing rate and total dose of paclitaxel released/delivered in peritoneal cavity

For the paclitaxel/Cremophor micelles, based on previous studies, we assumed complete drug release on the day of administration as it reflects the simple partition between the aqueous and organic phases [17]. For PLGA microparticles, the *in vivo* release was calculated based on the *in vitro* release, as follows. The data of the *in vitro* drug release over 28 days from individual microparticle preparations were fitted using three equations. Previous studies developed Eq. (1) to describe drug release from an erodible matrix over time where *Constant* consists of erosion rate constant, initial drug concentration and microparticle radius [26,27]; we fitted this equation to the release *vs.* time data and determined the goodness-of-fit.

$$Cumulative\ \%\ released = 100 * \left(1-(1-Constant * time)^3\right) \quad (1)$$

Considering a biphasic first-order drug release, we used Eq. (2) where α and β are the release rate constants for the rapid and slower release processes, respectively.

$$Cumulative\ \%\ released = R1 * \left(1-e^{-\alpha * time}\right) + (100-R1) * \left(1-e^{-\beta * time}\right) \quad (2)$$

We further evaluated the suitability of using a combination of a first-order release and release from an erodible matrix in data fitting. This was accomplished by substituting the second term in Eq. (2) with Eq. (1) (*i.e.*, assuming the slower release was due to polymer erosion), which yielded Eq. (3).

$$Cumulative\ \%\ released = R1 * \left(1-e^{-\alpha * time}\right) + (100-R1) * \left(1-(1-Constant * time)^3\right) \quad (3)$$

The goodness-of-fit by the three models was compared using the Akaike Information Criterion (AIC), which was calculated using the formula $AIC = N * Ln(SSE) + 2 * P$, where N is the number of observations, P is the number of model parameters, and SSE is the residual sums of squares [28].

For combinations of different microparticles, we assumed that each microparticle dose release is independent of each other and calculated the total amount of drug released as the sum of the individual components in the combinations. Nonlinear regression was performed using the NLIN procedure of SAS (Cary, NC).

2.5. Extraction and HPLC analysis of paclitaxel

Paclitaxel was extracted using ethyl acetate and analyzed with HPLC, as described previously [17]. The HPLC stationary phase comprised a cleanup column (Nova-Pak C_8; Waters Assoc. Ireland) and an analytical column (Bakersfield C_{18}; Mallinkrodt Baker, Phillipsburg, NJ). The cleanup mobile phase was 37.5% acetonitrile in water and the analytical mobile phase was 49% acetonitrile. Paclitaxel was detected at 229 nm. The

standard curve was linear at concentrations between 10 and 10,000 ng per injection.

2.6. Animal protocol

Female athymic mice, 5–7 weeks old, were obtained from Charles River/NCI Laboratories (Wilmington, MD). Mice had free access to food and water. Studies were performed at the Ohio State University Animal Facilities, and animal treatment and maintenance were in accordance with Institutional Animal Care and Use Committee guidelines. Human pancreatic Hs766T tumor cells were a gift from Dr. Byoungwoo Ryu (John Hopkins University, Baltimore, MD) and maintained in Dulbecco's modified Eagle's Medium containing 9% fetal bovine serum, 2 mM L-glutamine, 90 μg/ml gentamicin, and 90 μg/ml cefotaxime sodium at 37 °C in a humidified atmosphere of 5% CO_2 in air. Metastatic sublines were established as described earlier; serial re-implantation of cells collected from peritoneal washings of mice implanted with the parent cells yielded multiple tumor nodules in 100% of the mice after 10 days [17]. Hence, treatments were initiated 10 days after tumor implantation; tumor-bearing mice were given IP injection of either physiological saline or blank microspheres (controls) or one of the nine drug-containing treatments. The drug treatments comprised either single agents or combinations of the following four paclitaxel delivery systems: Cremophor micelles (referred to as paclitaxel/Cremophor), small PLGA microparticles that released the drug rapidly, small microparticles that released the drug slowly, and large microparticles that released the drug at a medium rate (see Results for details).

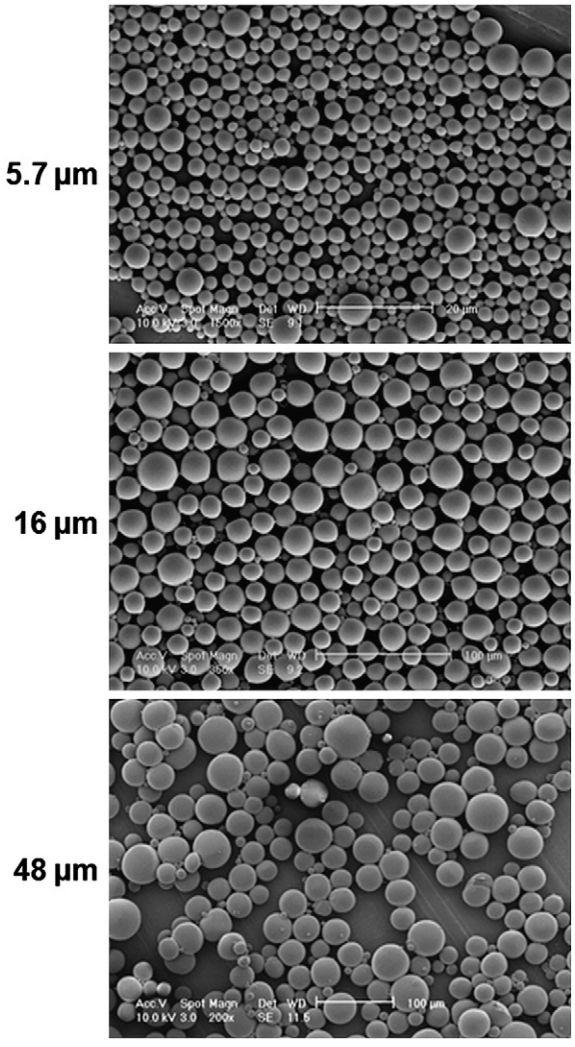

Fig. 1. Scanning electron microscopy of paclitaxel-loaded PLGA microparticles.

Mice were euthanized when they showed >30% body weight gain, visible abdominal swelling (due to ascites fluid build-up), or loss of righting reflex. Post-mortem autopsies were performed to determine the cause of death. Mice that died within 10 days post-treatment and showed a body weight loss of >15% were considered drug toxicity-related deaths. Mice that died after 10 days and presented with tumor nodules and/or tumor infiltration into organs were considered disease-induced deaths. Two mice (one in MP40SF and one in three weekly Paclitaxel/Cremophor group) died of complications from IP injections and were excluded in data analysis.

Animals were monitored for at least 110 days after the initiation of drug treatments or 8-times the MST of controls; mice with no visible tumors at that time were considered cured. Because the drug release was calculated from the day of treatment, MST was also expressed in post-treatment day. Increase in lifespan (ILS) was calculated as ($MST_{treated\ group} - MST_{control\ group}$) divided by $MST_{control\ group} \times 100\%$.

2.7. Statistical analysis

The difference in drug release rates between different PLGA microparticle preparations was evaluated using Student's *t*-test. The relationship between the amount of released drug and efficacy (MST) was fitted by linear regression and further evaluated using F test. The level of significance in the differences in survival times was analyzed using the log-rank tests using SAS (Cary, NC). For a group size of 8 animals and with the coefficient of variation of about 33% in survival times between individual animals as observed in the current study, the log rank test has a 80% power to detect a 1.85-fold difference in MST between two treatments at α of 0.05, *i.e.*, differences of less than 1.85-fold would not reach 5% statistical significance.

3. Results

3.1. Characterization of paclitaxel-loaded PLGA microparticles

Fig. 1 shows the scanning electron micro-photographs of three representative paclitaxel-loaded PLGA microparticle preparations with volume-averaged diameters of 5.7, 16 and 48 μm. Microparticles were spherical in shape with a smooth exterior surface without visible paclitaxel crystals. Minimal drug degradation (<0.1%) was detected under storage at 4 °C for 3 months, indicating paclitaxel was stable within the polymer matrix. The blank microparticles and drug-loaded microparticles showed comparable Tg, suggesting no drug–polymer interactions in the matrix.

Seven paclitaxel-loaded PLGA microparticles were prepared. Table 1 summarizes their properties. All except one showed mass balance of between 90 and 100%. The yield and entrapment efficiency were generally high, averaging >85%, which corresponded to a mean drug loading ranging from 4.0 to 4.7% across the different microparticle formulations.

3.2. In vitro drug release

Fig. 2 shows the *in vitro* paclitaxel release from PLGA microparticles and Table 1 summarizes the data. Drug release from microparticles was multi-phasic, with an initial burst release on the first day, followed by a second phase with slower sustained release for about 3 weeks. The microparticles composed of 50:50 PLGA exhibited a third phase with a more accelerated release. Results from a separate experiment showed different morphology between microparticles composed from 50:50 PLGA (Fig. 3A) and those composed from 75:25 PLGA (Fig. 3B) following continued aqueous exposure. This suggests that the additional release observed in the 50:50 PLGA microparticles may be due to enhanced release from polymer erosion as 50:50 PLGA is more amorphous in nature compared to 75:25 PLGA.

Table 1
Characterization of paclitaxel-loaded PLGA microparticles. Volume-averaged particle size was calculated by measuring the diameters of individual particles with scanning electron microscopy (>300 particles/sample). Drug release was obtained from three separate experiments; results are expressed in Mean ± SD.

LA:GA ratio	VIS dl/g	Diameter μm	*In vitro* release, % dose		Selected for *in vivo* studies (abbreviation)
			1 day	28 days	
50:50	0.17	5.7	48 ± 2.5*,**	71 ± 3.8*,***	Yes (SF)
50:50	0.17	16	25 ± 5.4	56 ± 7.9	No
50:50	0.17	48	20 ± 4.3	44 ± 6.3	Yes (LM)
50:50	0.39	5.8	10 ± 0.2**	36 ± 2.7***	No
50:50	0.39	19	3.1 ± 0.3	36 ± 11	No
50:50	0.39	66	0.9 ± 0.1	24 ± 11	No
75:25	0.67	5.6	2.7 ± 0.2	12 ± 2.4	Yes (SS)

* $p < 0.001$ compared to 75:25 PLGA microparticles.
** $p < 0.001$ compared to large microparticles prepared with the same polymer.
*** $p > 0.05$ compared to large microparticles prepared with the same polymer.

Microparticles comprising PLGA of low VIS (0.17 dl/g) showed significantly greater initial burst and 28-day cumulative drug release compared to microparticles of similar size but with higher VIS ($p < 0.001$ for both initial and cumulative release). At comparable size (5–6 μm), the 50:50 LA:GA microparticles showed significantly greater burst and cumulative release compared to 75:25 LA:GA particles ($p < 0.001$). At the same polymer VIS (0.17 or 0.39 dl/g), the smaller microparticles generally showed higher cumulative release compared to the larger particles; however, the difference was mainly due to the higher initial burst on day 1 from the small particles ($p < 0.01$) with no significant difference in release over the subsequent 28 days ($p > 0.05$).

The seven microparticles were categorized into three groups based on their drug release rates, *i.e.*, fast, medium and slow, and further categorized by their particle sizes. Microparticle with varying but appreciable drug release within the duration of the 28-day *in vitro* release study were selected for the *in vivo* pharmacodynamic studies; these included the small particles with fast release (SF, corresponding to 50:50 LA:GA, 0.17 dl/g VIS, 5–6 μm diameter), large particles with medium release (LM, corresponding to 50:50 LA:GA, 0.17 dl/g VIS, 50–60 μm), and small particles with slow release (SS, corresponding to 75:25 LA:GA, 0.67 dl/g VIS, 5–6 μm) to attain therapeutic drug levels for disease control and avoid delayed dose-dumping. The selected microparticles were given to tumor-bearing mice as single agents or in combinations; a total of seven microparticle treatments were evaluated and compared with two dose schedules of paclitaxel/Cremophor micelles (single dose or 3-weekly doses).

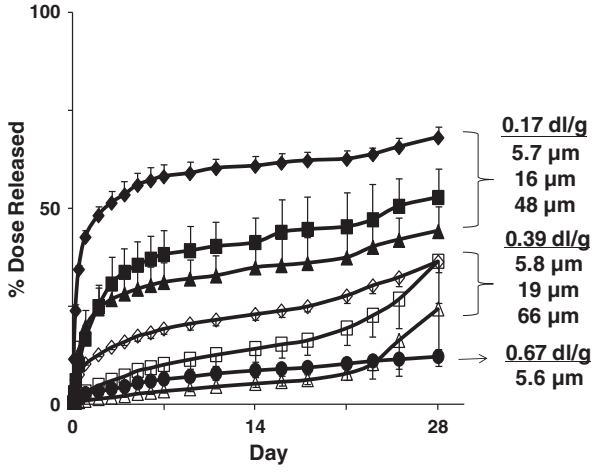

Fig. 2. Relationship between PLGA microparticle properties and drug release. Bar: 1 SD (n = 3).

3.3. Simulated in vivo drug release

Analysis of the *in vitro* paclitaxel release profiles from SF, LM and SS microparticles using Eqs. (1)–(3) showed that Eq. (1) yielded poor description of the data, with underestimation of data at early times and overestimation after one-half of the study duration (*i.e.* 14 days). Eqs. (2) and (3) showed similar performance, with <2% difference in AIC values and <3% difference in simulated drug release data; Eq. (2) yielded lower AIC values for two of the three microparticles. The release parameters for SF, LM, and SS microparticles are presented in Table 2.

Examples of data fitting by Eq. (2) are shown in Fig. 4A; the fitted curves superimposed most of the data points. The release parameters (α, β, R1) obtained from data analysis using Eq. (2) were used to simulate the dosing rate and the amount released from microparticles in the peritoneal cavity up to 76 days, which was when the last disease-related death occurred.

The results for seven microparticle treatment groups and the two paclitaxel/Cremophor groups are shown in Fig. 4B and Table 3; these 9 treatment groups covered a wide range of cumulative drug dose (ranged from 19 mg/kg for MP80SS to 120 mg/kg for paclitaxel/Cremophor) and dosing rate (average daily rate ranged from 0.3 mg/kg/day for MP80SS to 1.8 mg/kg/day for paclitaxel/Cremophor).

Among the seven microparticle groups, two combinations of 2 or 3 different microparticles (MP40SF40LM40SS, MP60SF60SS) yielded nearly identical profiles, whereas the remaining five combinations resulted in different burst release or cumulative release. As discussed below, these two microparticles, due to their different sizes, yielded different spatial distribution in IP cavity and different *in vivo* pharmacodynamics, indicating that the efficacy of paclitaxel-loaded microparticles depended on the movement and localization of the microparticles within the cavity, in addition to the dosing rate and the total dose.

3.4. Effect of delivery system on toxicity

The control group treated with blank PLGA microparticles showed minimal body weight loss (≤5%), indicating microparticles had no discernible toxicity. Seven treatments (MP40SF, MP80SS, MP40SF80SS, MP40SF40LM40SS, MP40SF80SSx2, single dose and 3-weekly doses of paclitaxel/Cremophor) resulted in minor body weight loss (<15%) and animals typically recovered to their pre-treatment weight after 1 week. The remaining two treatments (MP60SF60LM, MP60SF60SS) caused >20% body weight loss and resulted in toxicity-death (Table 3).

The toxicity of microparticles differed from the toxicity of paclitaxel/Cremophor in several ways. First, comparison of drug release and toxicity-death within the seven microparticle treatments showed that toxicity-death occurred when the 1-day paclitaxel release exceeded 30 mg/kg, but was not related to the cumulative release as several microparticles with total release exceeding the two toxic treatments were not lethal (*e.g.*, MP40SF80SS × 2 *vs.* MP60SF60LM and MP40SF40LM40SS *vs.* MP60SF60SS); this finding indicates for IP paclitaxel-loaded microparticles, the toxicity from rapid dose presentation was severe and fatal whereas sustained dosing over days or weeks was better tolerated presumably due to recovery from non-lethal damage. In contrast, the instantaneous delivery of a single 40 mg/kg paclitaxel/Cremophor dose, which rapidly clears from the peritoneal cavity (>99% within 12 h; [17]), was not lethal. Further, in the repeated treatment groups, all animals given MP40SF80SS showed full recovery within 1 week and no enhanced toxicity after the second dose, whereas repeated paclitaxel/Cremophor treatment (3 weekly treatments) caused continuous weight loss until death from disease progression in nearly one-half of the animals (3/7). Taken together, these results indicate (a) different toxicity profiles for microparticles and paclitaxel/Cremophor micellar solution, (b) local toxicity of IP paclitaxel is determined by the dosing rate and residence time in the peritoneal cavity, and (c) cumulative toxicity for weekly paclitaxel/Cremophor but not every-3-week microparticles.

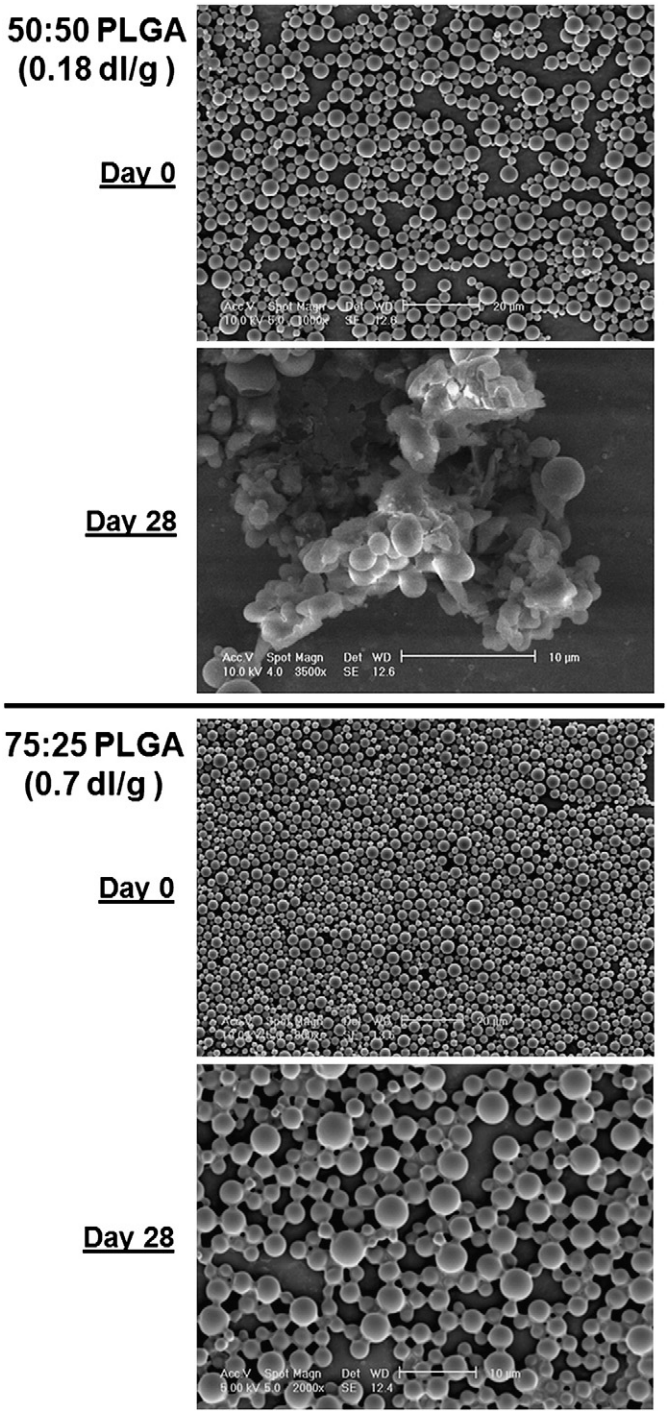

Fig. 3. Morphological change of microparticles in the release medium.

3.5. Effect of delivery system on antitumor activity

Fig. 5 and Table 3 summarize the treatment outcomes. The controls treated with physiological saline or blank particles showed overall survival times of 14–15 days, equivalent to 24–25 days post-tumor implantation. All nine drug-treated groups yielded survival advantage.

Among single dose drug treatments, compared to paclitaxel/Cremophor, two microparticle groups (MP40SF80SS, MP60SF60SS) showed significantly higher ILS ($p < 0.05$), three groups (MP40SF, MP60SF60LM, MP40SF40LM40SS) yielded higher but not statistically significant ILS, and the remaining group (MP80SS) showed a trend of lower ILS ($p = 0.06$).

Repeated treatment with microparticles (MP40SF80SS, 2 doses) or paclitaxel/Cremophor (3 doses of 40 mg/kg) yielded 40–100% higher ILS compared to single treatment with the respective delivery system, and repeated microparticle treatment yielded >2-times higher ILS compared to repeated paclitaxel/Cremophor treatment. But the differences in both comparisons were <1.85-fold and hence did not reach statistical significance.

Table 2
Model parameters for paclitaxel release from PLGA microparticles. Mean (SE) estimates are based on fitting Eq. (2) to the mean *in vitro* release profile.

Microparticles	α (1/day)	β (1/day)	R1 (%)
SF	4.37 (0.34)	0.0052 (0.00046)	51.46 (0.94)
LM	1.38 (0.082)	0.0030 (0.00012)	25.52 (0.42)
SS	1.05 (0.26)	0.0011 (0.000056)	3.75 (0.26)

3.6. Correlation between simulated drug release and in vivo pharmacodynamics

Table 3 compares the treatment efficacy (expressed in MST) with the simulated dose released in the peritoneal cavity. Fig. 6A shows the plots of efficacy *vs.* 1-day drug release from respective treatment groups and Fig. 6B shows the plots for drug release at the MST; the efficacy of the seven microparticle treatments plus the vehicle controls was linearly correlated with the drug release. Similar results were obtained between efficacy and drug release over 76 days (not shown).

Comparison of the linearly regressed lines for microparticles and paclitaxel/Cremophor indicated different quantitative relationships for the two delivery systems; the slope of the best-fit regressed line for microparticles was >2-times that for paclitaxel/Cremophor (0.68 *vs.* 0.33 for 1 day-release and 0.39 *vs.* 0.15 for MST-release), indicating greater survival benefits for a given dose delivered *via* microparticles.

Another important pharmacodynamic consideration was tumor-free cures. Among the 7 microparticle groups, tumor-free cures were observed only in groups comprising combinations of fast release and medium/slow release microparticles but not in the two groups comprising either fast or slow release microparticles, indicating benefit of using combination microparticles with different drug release rates. Comparison of the cumulative drug amount in the peritoneal cavity at MST indicated that cure was achieved only when the total drug amount was ≥40 mg/kg.

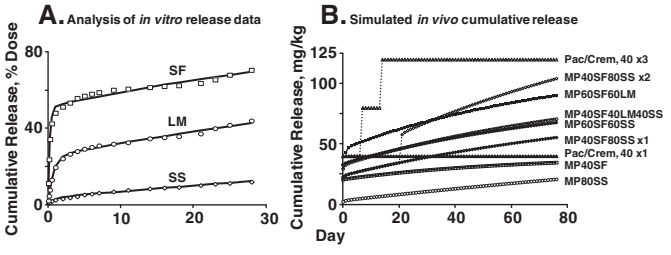

Fig. 4. Drug release-time profiles. (A) *In vitro* drug release from three PLGA microparticles, *i.e.*, SF (50:50 LA:GA, 0.17 dl/g, 5–6 μm diameter, fast release), LM (50:50 LA:GA, 0.17 dl/g, about 50 μm, slow release), SS (75:25 LA:GA, 0.67 dl/g, 5–6 μm, slow release), were analyzed. Symbols, experimental data. Lines, best-fitted curves using Eq. (2). (B) Simulated amount of dose released in peritoneal cavity for the nine drug treatment groups. Pac/Crem 40 × 1 refers to a single treatment of paclitaxel/Cremophor at 40 mg/kg. Abbreviations for single type microparticles are: MP(dose)(type of microparticles). For example, MP40SF is small, fast release microparticles at 40 mg/kg dose. Abbreviations for combination microparticles are: MP(dose of first microparticles)(type of first microparticles)(dose of second microparticles)(type of second microparticles). For example, MP40SF80SS is a combination of small, fast release microparticles at 40 mg/kg dose and small, slow release microparticles at 80 mg/kg. Abbreviations for repeated treatments are: Pac/Crem 40 × 3 is 3 weekly treatments of 40 mg/kg on day 0, 7, and 14 days post-treatment (equivalent to 10, 17 and 24 days post-tumor-implantation). MP40SF80SS × 2 is 2 treatments of MP40SF plus MP80SS given on day 0 and 21 days post-treatment (equivalent to 10 and 31 days post-tumor-implantation).

Table 3
In vivo pharmacodynamics: Correlation with simulated *in vivo* drug release/dose. Treatments were initiated 10 days after tumor implantation. Pac/Crem 40 × 1 refers to a single treatment of paclitaxel/Cremophor at 40 mg/kg. Abbreviations for single type microparticles are: MP(dose)(type of microparticles). For example, MP40SF is small, fast release microparticles at 40 mg/kg dose. Abbreviations for combination microparticles are: MP(dose of first microparticles)(type of first microparticles)(dose of second microparticles)(type of second microparticles). For example, MP40SF80SS is a combination of small, fast release microparticles at 40 mg/kg dose and small, slow release microparticles at 80 mg/kg. Abbreviations for repeated treatments are: Pac/Crem 40 × 3 is 3 weekly treatments of 40 mg/kg on 0, 7, and 14 days post-treatment (equivalent to 10, 17 and 24 days post-tumor-implantation). MP40SF80SS × 2 is 2 treatments of MP40SF plus MP80SS given on day 0 and 21 days post-treatment (equivalent to 10 and 31 days post-tumor-implantation). MST is the duration of animal survival in days post-treatment. Cure is defined as no residual tumors at 110+ days, or 8 times the MST of controls. Dose efficiency, defined as the extent of benefit normalized to the administered dose, was calculated as % ILS and % cure per mg drug released.

Group (n)	Biological activities				Simulated dose presented in peritoneal cavity		Dose efficiency	
	Toxicity death %	Cure %	MST Days	ILS %	mg/kg released in 1 day	mg/kg released in MST	% ILS per mg released in MST	% cure per mg released in MST
Saline control (6)	0	0	14	0	NA	NA	NA	NA
Blank particle control (6)	0	0	15	7	NA	NA	NA	NA
Pac/Crem 40 × 1 (16)	0	6	27	93	40	40	2.3	0.15
MP40SF (7)	0	0	36	157	20.5	29.5	5.3	0
MP80SS (8)	0	0	21	54	2.25	8.54	6.3	0
MP40SF80SS × 1 (8)	0	25	41*	193	22.8	43.6	4.4	0.57
MP60SF60SS (8)	13	25	47*	236	32.5	57.9	4.1	0.43
MP60SF60LM (8)	25	25	42	200	42.7	76.0	2.6	0.33
MP40SF40LM40SS (8)	0	13	36	157	29.6	54.6	2.9	0.24
Pac/Crem 40 × 3 (7)	0	14	33	136	40	120	1.1	0.12
MP40SF80SS × 2 (8)	0	25	55*	293	22.8	89.6	3.3	0.28

* $p < 0.05$ compared to single dose paclitaxel/Cremophor (40 mg/kg).

For the microparticles, the correlations between treatment efficacy and 1 day-release or MST-release were about equal (r^2 of 0.82 and 0.86), suggesting that both instantaneous and sustained drug exposure are needed for disease control. Accordingly, the lack of appreciable antitumor activity of the single agent slow release microparticle MP80SS was likely due to inadequate early drug release (<3 mg/kg for day 1).

3.7. Relative dose efficiency of treatments with different drug release rates

The dose efficiency of the different treatments, defined as extent of survival benefit per administered dose, was calculated as % ILS and % cure per mg drug delivered/released (Table 3). In general, the microparticles had higher dose efficiency for ILS and cures compared to single or repeated paclitaxel/Cremophor treatments.

Among the seven microparticle groups, the combination of fast and slow release microparticle (MP40SF80SS, single dose) was the most dose efficient for disease-free cure. Note that the dose efficiency measurement was mainly for comparing the mg potency at different drug delivery rates. For example, the slow release microparticle (MP80SS) and the fast release microparticles (MP40SF) were the most dose efficient for ILS, but neither produced cures. In addition, MP80SS showed the shortest ILS. These results indicate that while either bolus dose input or slow input had higher dose efficiency, their combination yielded greater disease control, albeit at the expense of lower mg potency.

Among the four microparticle groups that received a total of 120 mg/kg paclitaxel-equivalents, the two groups comprising the large microparticles (48 μm diameter) yielded inferior antitumor activity compared to the other two groups treated with smaller microparticles (5–6 μm diameter). Similarly, among the seven microparticle groups, the two large microparticle groups had the lowest dose efficiency. This data indicates greater activity for the small microparticles.

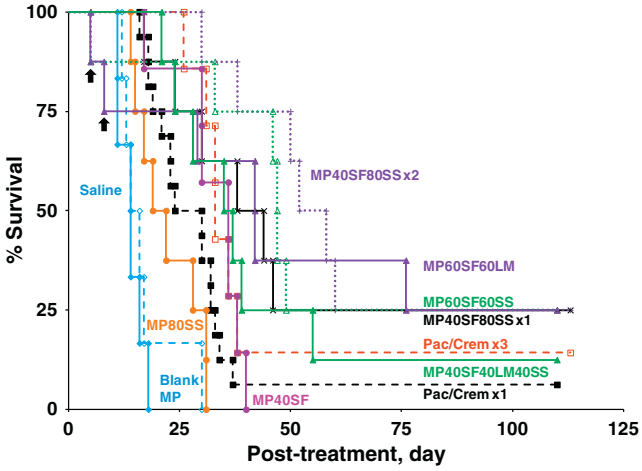

Fig. 5. *In vivo* biological activity. Mice were given IP injections of physiological saline, blank microparticles, or one of the nine treatments as described in Fig. 4. Day 0 represents the day of treatment initiation, which corresponded to 10 days post-tumor implantation (about 40% of the MST of controls). Survival over time is shown in Kaplan–Meier curves. Toxicity-related death events are indicated by the arrows.

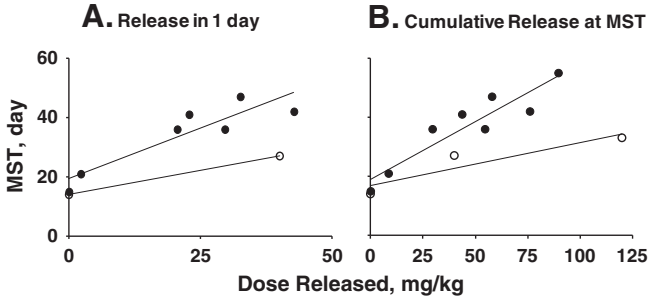

Fig. 6. Correlation of simulated drug release in peritoneal cavity and *in vivo* pharmacodynamics. The drug amount released in peritoneal cavity for individual treatments was obtained from Fig. 4. The data points at 0 mg/kg are corresponding to the respective vehicle control for each group (saline and blank microspheres, respectively). Open circle: paclitaxel/Cremophor; solid circle: microparticles. Treatment efficacy was expressed in MST. (A) MST *vs.* dose released in 1 day. Only data from single treatment groups were included. The best-fit linearly regressed lines were: MST = 0.68*(released dose) + 19 for microparticles ($r^2 = 0.82$, $p < 0.01$), and MST = 0.33*(released dose) + 14 for paclitaxel/Cremophor ($r^2 = 1$). (B) MST *vs.* dose released at MST. All single and repeated treatment groups were included. The best-fit linearly regressed lines were MST = 0.39*(released dose) + 19 for microparticles ($r^2 = 0.86$, $p < 0.001$) and MST = 0.15*(released dose) + 17 for paclitaxel/Cremophor ($r^2 = 0.85$, $p = 0.25$).

4. Discussion

The first objective of the present study was to evaluate PLGA microparticles as carriers of paclitaxel. The results, consistent with previous reports [22,29–31], show (a) high encapsulation efficiency as expected for water-insoluble drugs, (b) higher initial burst and more rapid drug release from microparticles with lower VIS polymers due to the greater polymer chain flexibility (resulting in drug deposition on particle surface, plus greater inward water diffusion and consequently greater particle degradation) compared to microparticles comprising polymers with higher VIS, LA content, crystallinity and hydrophobicity, and (c) greater drug release from smaller particles due to the higher surface area-to-volume ratio and shorter radius.

The second objective was to investigate the quantitative relationships between drug delivery in the peritoneal cavity (amount, rate and duration of presentation) and treatment efficacy/toxicity. The *in vivo* release is likely to depend on other factors, *e.g.*, peristalsis, that are difficult to predict or simulate. The present study shows that a simple empirical model was sufficient to describe the *in vitro* release profiles. We extended the model to simulate the initial burst and cumulative drug release *in vivo* using the assumption of similar sink conditions within the peritoneal cavity. The simulated drug release linearly correlated with treatment efficacy in tumor-bearing mice. The results further showed that microparticles generally displayed superior activity and superior dose efficiency relative to paclitaxel/Cremophor. As the major difference between these two delivery systems is the slower drug release rate from microparticles, the difference in their pharmacodynamics indicates that there is a temporal component of drug presentation, in addition to the drug dose, that determines the treatment outcome. In addition, the inferior antitumor activity of the large microparticles (48 μm diameter) relative to the small microparticles (5–6 μm), coupled with our earlier finding that large microparticles are localized in the lower abdomen whereas the smaller microparticles were evenly dispersed throughout the cavity and adhered to the tumor surface [32], indicates the importance of spatial distribution of drug carriers.

The present study provided several potentially useful findings for the development of paclitaxel-loaded microparticles in IP therapy. First, rapid drug release over a short duration (*e.g.*, 1 day) is more toxic compared to fractionated release over days or weeks; the threshold toxic dose in mice was 1-day release of >30 mg/kg paclitaxel. Second, the threshold drug amount in peritoneal cavity (cumulative amount at MST) required for disease-free cure was 40 mg/kg. Third, while either fast or/and slow release microparticles had the highest dose efficiency for ILS, cure was achieved only with their combinations. We propose that this is because the combination provided effective tumor priming and optimal drug release. In tumor priming, the apoptosis-inducing paclitaxel transiently expands the interstitial space and promotes the interstitial drug/microparticle transport (reviewed in [16,33–35]). Hence, combination of two types of microparticles, with one type to release paclitaxel rapidly to promote tumor penetration of the remaining microparticles and the second type to release paclitaxel slowly to sustain the drug levels, enables fractionated dosing to achieve instantaneous and sustained tumor priming and antitumor activity. The two small microparticles SF and SS, in view of their high dose efficiency, are suitable candidates for use in combinations.

In summary, the present study established for IP paclitaxel therapy, the significantly different pharmacodynamics for efficacy and toxicity between drug-loaded PLGA microparticles and the intravenous paclitaxel/Cremophor micellar solution used off-label in previous IP studies. The results further indicated the importance of spatial and temporal factors that in turn are dictated by the characteristics of the drug carrier. Finally, in view of the temporal requirement of instantaneous and sustained drug exposure for favorable pharmacodynamics and disease control, and in view of the well-established difference in tumor growth rates in experimental animals and humans, additional studies to evaluate the drug release-pharmacodynamic relationship as function of tumor growth rate are warranted. Such studies would be useful to identify the suitable dosing schedule (dose intensity and frequency) for the preclinical-to-clinical translational research.

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