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Intraperitoneal delivery of paclitaxel by poly(ether-anhydride) microspheres effectively suppresses tumor growth in a murine metastatic ovarian cancer model

Ming Yang & Tao Yu & Joseph Wood & Ying-Ying Wang & Benjamin C. Tang & Qi Zeng & Brian W. Simons & Jie Fu & Chi-Mu Chuang & Samuel K. Lai & T.-C. Wu & Chien-Fu Hung & Justin Hanes

Abstract Intraperitoneal (IP) chemotherapy is more effective than systemic chemotherapy for treating advanced ovarian cancer, but is typically associated with severe complications due to high dose, frequent administration schedule, and use of non-biocompatible excipients/delivery vehicles. Here, we developed paclitaxel (PTX)-loaded microspheres composed of di-block copolymers of poly(ethylene glycol) and poly(sebacic acid) (PEG-PSA) for safe and sustained IP chemotherapy. PEG-PSA microspheres provided efficient loading (∼13% w/w) and prolonged release (∼13 days) of PTX. In a murine ovarian cancer model, a single dose of IP PTX/PEG-PSA particles effectively suppressed tumor growth for more...
than 40 days and extended the median survival time to 75 days compared to treatments with Taxol® (47 days) or IP placebo particles (34 days). IP PTX/PEG-PSA was well tolerated with only minimal to mild inflammation. Our findings support PTX/PEG-PSA microspheres as a promising drug delivery platform for IP therapy of ovarian cancer and potentially other metastatic peritoneal cancers.

**Keywords** Drug delivery · Controlled release · Chemotherapy · Biodegradable polymers

Ovarian cancer is the fifth leading cause of death from malignancies among women worldwide, with an estimated 275,100 deaths globally in 2011 [1]. It often remains clinically silent until tumors have disseminated beyond the ovaries into the peritoneal cavity, leaving patients with poor prognosis [2]. Intraperitoneal (IP) chemotherapy, which elevates peritoneal drug concentrations, suppresses ovarian tumors more effectively than conventional systemic treatment [3, 4]. Nevertheless, IP chemotherapy can lead to significant side effects due to transient exposure to high levels of chemo drugs and frequent injections [3–6]. In contrast, IP-controlled release systems can maintain therapeutically effective yet moderate levels of chemo in the peritoneal cavity to suppress tumors for a prolonged period of time with reduced side effects [7–9]. While significant advances have been made in the development of IP delivery systems [10–15], new platforms with enhanced efficacy and biocompatibility are still needed. In particular, systems with optimized particle size, surface properties, and degradation kinetics may provide greater particle stability, reduced immunogenicity, and optimal clearance time to improve particle-based IP chemotherapy [7–9].

Biodegradable polymers, including polyesters and polyanhydrides, are widely used to develop drug delivery systems that release therapeutic molecules in a sustained fashion [16–18]. One of the advantages of polyanhydrides is that they can be tailored to degrade at predictable rates and release drug in a surface erosion-driven and tunable manner [16, 19, 20]. A variety of polyanhydride-based copolymers, such as poly (ether-anhydrides) [21–25] and poly (ester-anhydrides) [26–31], have been developed and used for drug delivery applications.

Here, we report the development of a microsphere-based delivery system composed of poly(ethylene glycol)-co-poly(sebacic acid) (PEG-PSA) for IP delivery of paclitaxel (PTX) against ovarian cancer. PSA is a polyanhydride polymer that has been widely studied and is used in Gliadel® wafer, an FDA-approved product [16]. Since sustained release of therapeutic molecules from polyanhydride particles occurs concurrently with the erosion of particles, minimal residual polymer is expected upon depletion of the drug. PEG, a hydrophilic polyether polymer, has a demonstrated history of safe use in FDA-approved pharmaceutical products [32]. During the synthesis of particles composed of PEG-containing amphiphilic co-polymers, PEG partitions to the particle surface, forming a dense coating that improves particle stability and reduces immunogenicity and thus improves the biocompatibility of the particles [32–35].

We first formulated PTX-encapsulated PEG-PSA microspheres (PTX/PEG-PSA) using an oil-in-water emulsion method and characterized their physicochemical properties in vitro. Detailed methods are provided in the Electronic Supplementary Material online. All data represent mean ± standard error of the mean (SEM) unless otherwise specified. Scanning electron micrographs show that PTX/PEG-PSA particles possessed smooth surfaces without drug precipitates (Fig. 1a). The mean diameter of PTX/PEG-PSA particles measured by a Coulter Multisizer was 14.2 μm with a standard deviation of 5.8 μm (Fig. 1b). Submicron particles (e.g., <1 μm) smaller than the openings of peritoneal lymphatic ducts may be cleared rapidly by lymphatic drainage [7, 8]; thus, the relatively large size of PTX/PEG-PSA particles may facilitate particle retention in the peritoneal cavity.

We next tested three different target loading levels of PTX to optimize the drug loading in PEG-PSA particles (Table 1). At a target loading of 20 %, we achieved an optimal PTX loading of 13±1 % with 67±6 % encapsulation efficiency. We further characterized the release of encapsulated PTX from PEG-PSA particles in vitro. As shown on Fig. 1c, PTX was released from PEG-PSA particles for more than 2 weeks with limited burst effects. Further tuning of drug loading and drug release kinetics may be achieved by adjusting the molecular weight and/or hydrophobicity of the polymer [23]. While the system described here is engineered for IP delivery of PTX, we have previously shown that PEG-PSA particles can efficiently encapsulate and provide sustained release of other molecules, such as etoposide [25]. PEG-PSA particles may also be suitable for various types of peritoneal indications other than ovarian cancer, including metastatic cancers in the peritoneal cavity such as pancreatic cancer and peritoneal inflammation such as gastroenteritis.

We next investigated the in vivo release of PTX/PEG-PSA particles injected into the mouse peritoneal cavity. Residual PTX was recovered at different time points by performing a peritoneal lavage using PBS and then quantified by high performance liquid chromatography. Figure 1d shows that Taxol® (the clinical formulation of PTX) was quickly cleared from the peritoneal cavity, with only 14 % of the initial dose recovered by 2 h and no detectable drug level by 24 h. In contrast, ~50 % of the initial dose delivered by PTX/PEG-PSA particles remained in the peritoneal cavity at 24 h and ~8 % was recovered on day 13. The drug retention profile of PTX/PEG-PSA particles in vivo was consistent with the in vitro release kinetics, implying that particles were largely
stable and cleared minimally from the peritoneal cavity. The improved pharmacokinetics of PTX delivered by PEG-PSA microspheres demonstrates the advantage of this particle system for sustained IP drug delivery.

We also measured plasma drug concentrations at predefined time points following treatment (Table 2). In mice receiving 20 mg/kg IP Taxol®, plasma levels of PTX were high (5.2±0.4 μg/mL) at 2 h after treatment and then declined to 1.9±0.3 μg/mL at 4 h consistent with the documented rapid clearance of Taxol® from the peritoneal cavity into systemic circulation [36]. The half-life of IP Taxol® is ~3 h in mice, leading to a rapid decline in plasma PTX concentration to ~0.1 μg/mL at 30 h following IP Taxol® injection at 18 mg/kg [36]. In comparison, the plasma level of PTX in mice receiving IP PTX/PEG-PSA remained relatively constant at ~1 μg/mL from day 1 up to day 14, suggesting the microspheres provided sustained release of PTX within the peritoneal cavity leading to prolonged systemic exposure to PTX but at relatively low levels.

We then evaluated the efficacy of IP PTX/PEG-PSA particles in a previously established murine ovarian tumor model using luciferase-expressing mouse ovarian surface epithelial cells (MOSEC-luc), which allows us to evaluate tumor burden and, thus, the efficacy of new therapies in a noninvasive yet quantitative fashion via bioluminescence measurements [37, 38]. Female C57BL/6 mice were inoculated intraperitoneally with the MOSEC-luc cells. About 4 weeks later, tumor-bearing mice were treated by IP administration of a single dose of PTX/PEG-PSA particles (20 mg/kg), Taxol® (20 mg/kg), or placebo PEG-PSA particles. Only mice receiving Taxol® showed signs of distress immediately upon administration, likely due to the anaphylactic effects of excipients such as Cremophor EL® in Taxol®. As shown in Fig. 2a, tumors in mice receiving IP placebo particles grew steadily,

**Table 1** Drug loading and encapsulation efficiency of PTX/PEG-PSA particles

<table>
<thead>
<tr>
<th>Target loading (% w/w)</th>
<th>Actual loading (% w/w)</th>
<th>Encapsulation efficiency (%)</th>
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<tbody>
<tr>
<td>10</td>
<td>7±1</td>
<td>74±3</td>
</tr>
<tr>
<td>20</td>
<td>13±1</td>
<td>67±6</td>
</tr>
<tr>
<td>30</td>
<td>13±1</td>
<td>43±5</td>
</tr>
</tbody>
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with total bioluminescence signal levels reaching $2 \times 10^8$ p/s by day 34 posttreatment at which point the extremely high tumor load necessitated humane sacrifice. IP Taxol® showed suppression, albeit modest, of tumor growth compared to placebo particles after the first week posttreatment, with average tumor load reaching $2 \times 10^8$ p/s by day 40. In contrast, IP delivery of PTX/PEG-PSA particles effectively inhibited tumor growth over an extended period of time compared to Taxol® and placebo particles. By day 40, the total bioluminescence signals for mice treated with IP PTX/PEG-PSA were still comparable to initial signal levels at day 0 ($1.3 \times 10^7$ p/s). The median survival times of mice receiving placebo and Taxol® were only 34 and 47 days, respectively, with 0% survival on day 60 for both groups (Fig. 2b). In contrast, mice receiving PTX/PEG-PSA particles demonstrated a median survival time of >75 days, with all mice surviving at day 60 (Fig. 2b).

Finally, we evaluated the biocompatibility of IP PTX/PEG-PSA particles. No morphologic anomalies were noted in the major peritoneal organs of mice receiving IP Taxol®, PTX/PEG-PSA particles, or PBS on days 1, 10, and 30 following a single dose administered on day 0. For all groups, minimal to mild inflammatory infiltrates were found in the mesentery but not in other major organs (Fig. 3a). Average pathology scores associated with PTX/PEG-PSA treatment (1.67, 1.29, and 1.00 on days 1, 10, and 30, respectively), with complete return to baseline by day 30 (Fig. 3b). Total peritoneal leukocyte counts for both treatment groups did not increase significantly at any time point compared to the PBS control (Fig. 3c). However, the fraction of neutrophils increased slightly after 1 day in both treatment groups likely due to immediate exposure to PTX, but recovered after 10 and 30 days (Fig. 3d). Throughout the entire course of the study, no signs of gastrointestinal toxicity, such as emesis, diarrhea, or significant weight loss, were observed.

**Table 2** Plasma PTX concentrations following IP Taxol® or PTX/PEG-PSA

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time</th>
<th>Plasma PTX concentration (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taxol®</td>
<td>5 min</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1 h</td>
<td>$3.2 \pm 0.6$</td>
</tr>
<tr>
<td></td>
<td>2 h</td>
<td>$5.2 \pm 0.4$</td>
</tr>
<tr>
<td></td>
<td>4 h</td>
<td>$1.9 \pm 0.3$</td>
</tr>
<tr>
<td>PTX/PEG-PSA</td>
<td>1 day</td>
<td>$0.9 \pm 0.1$</td>
</tr>
<tr>
<td></td>
<td>7 days</td>
<td>$1.2 \pm 0.1$</td>
</tr>
<tr>
<td></td>
<td>14 days</td>
<td>$1.1 \pm 0.1$</td>
</tr>
</tbody>
</table>

**Fig. 2** In vivo efficacy of IP delivered PTX/PEG-PSA microspheres, Taxol®, or blank PEG-PSA microspheres in mice bearing IP MOSEC-luc tumors. **a** Bioluminescence signals from MOSEC-luc tumors. PTX/PEG-PSA particles better suppressed tumor growth than other treatments. *Double asterisks* indicate statistical difference between PTX/PEG-PSA and other treatments starting from day 19 ($p<0.01$). **b** Kaplan-Meier survival curves. PTX/PEG-PSA particles significantly extended animal survival to >75 days compared to blank PEG-PSA particles (34 days) and Taxol® (47 days). *Single asterisk* indicates statistical difference between PTX/PEG-PSA and other groups ($p<0.05$). **c** Representative bioluminescence images of IP tumor burden. Data represent mean ± SEM ($n=5$ per treatment set).
in any of the treatment groups. These results demonstrate that IP therapy using PTX/PEG-PSA particles was well tolerated, with superior local biocompatibility to that of Taxol®.

The overall PTX dose administered in this study (20 mg/kg) is equivalent to ~60 mg/m² in humans [39], which is consistent with the single IP Taxol® dose used previously in a pivotal clinical trial [4]. However, IP Taxol® is commonly combined with intravenous or IP platinum-based chemotherapy (cisplatin or carboplatin) in the clinic, and treatments are often given once every 3 weeks for six cycles [4], which significantly increases overall systemic exposure to chemotherapeutic drugs and likely leads to severe systemic side effects including myelotoxicity. Since PTX/PEG-PSA particles showed markedly higher efficacy than free PTX treatment in our studies, we expect that PTX/PEG-PSA particles may be used at a lower dose and dosing frequency to achieve similar or greater efficacy than the current standard IP Taxol® treatment. In addition, our results suggest that PTX is released from PTX/PEG-PSA particles in a sustained fashion in the peritoneal cavity and gradually absorbed into the systemic circulation, leading to a sustained but relatively low level of plasma PTX. Thus, we anticipate a lower incidence and lower severity of systemic toxicity due to PTX/PEG-PSA treatment compared to that caused by current standard IP chemotherapy.

Our results show that IP PTX/PEG-PSA significantly suppressed the growth of ovarian tumors compared to standard Taxol® treatment with better biocompatibility. The substantial improvement in efficacy is likely due to the improved pharmacokinetics of PTX in the peritoneal cavity when delivered in PEG-PSA particles. Upon IP administration, Taxol® was
cleared by systemic absorption within hours (Fig. 1d). In contrast, the PTX/PEG-PSA microspheres may effectively avoid systemic drainage and last for weeks. Additionally, PEG molecules present on particle surfaces can shield the particles from biological constituents and immune cells in the peritoneal cavity, minimizing particle aggregation and immune elimination. Compared to irritating excipients such as Cremophor EL and ethanol in Taxol®, the components of PEG-PSA microspheres are more biocompatible and less agitating. Overall, PTX/PEG-PSA particles may persist stably and continuously release PTX in the peritoneal cavity, exposing tumors to elevated levels of PTX over longer periods of time with minimal side effects.

In summary, we developed a PEG-PSA-based microsphere delivery system for sustained IP chemotherapy with PTX. We demonstrated that PTX/PEG-PSA particles provided sustained released of PTX in vitro and retention of PTX in the peritoneal cavity over 2 weeks. In a murine model of metastatic ovarian cancer, we demonstrated superior tumor suppression by IP PTX/PEG-PSA particles compared to Taxol®. We also showed that IP PTX/PEG-PSA particles were well tolerated in vivo. The sustained release properties and improved biosafety of PTX/PEG-PSA microspheres may further advance IP chemotherapy for ovarian cancer and potentially other metastatic peritoneal cancers.

Acknowledgments This work was supported in part by NIH grants U54CA151838 (J.H.), P50CA098282 (T.W.), R01CA114425 (T.W.) and a National Science Foundation Graduate Research Fellowship (Y.-Y. W.). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Cancer Institute. We thank M. Koontz, N. Forbes-McBean, and M. Chen for the technical and experimental assistance.

Conflict of interest The authors declare no conflicts of interest.

Declaration of ethical standards The experiments in this work comply with the current laws of the USA.

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