Nanoparticle Depot for Intraperitoneal Chemotherapy of Ovarian Cancer

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ABSTRACT SUMMARY
Paclitaxel (PTX) nanocrystals (NC), surface-stabilized with human serum albumin (HSA), were prepared to improve PTX dissolution in a hyaluronic acid (HA)-based hydrogel as a local drug delivery system for intraperitoneal (IP) chemotherapy. We report the properties of NCs in comparison with Taxol and micrometer-scale precipitates (PPT) and discuss their potentials and limitations in sustained local delivery of PTX.

INTRODUCTION
Ovarian cancer is currently managed by cytoreductive surgery followed by intravenous administration of platinum and taxane. On the other hand, a growing number of preclinical and clinical studies have shown that IP chemotherapy is more effective than systemic therapy in post-surgical management of ovarian cancer1. Therefore, a new drug delivery system optimized for IP drug delivery is highly desired.

In an attempt to develop a drug delivery system for sustained IP delivery of PTX, we previously used a HA-based hydrogel as a carrier of PTX in the form of micrometer-scale (~4 µm) PPT and tested its anti-tumor efficacy in IP tumor model2. While PPT (but not the 14 nm Taxol micelles used as a reference product) delivered using hydrogel was well retained in the peritoneal cavity due to the large size, the prolonged retention did not improve anti-tumor effect compared to Taxol, likely due to the limited dissolution of PTX3.

To enhance dissolution of PTX while maintaining sufficient size for retention in hydrogel, we developed PTX NC, optionally stabilized with serum albumin (HSA-NC). Here we report properties of NCs, comparing with those of PPT. PTX release kinetics from hydrogels containing the particles was studied in vitro using a condition mimicking the peritoneal cavity.

EXPERIMENTAL METHODS
NC was produced by the antisolvent precipitation method3. To further stabilize NC, the NC suspension (PTX concentration: 1 mg/mL) was mixed with HSA solution (2 mg/mL) and incubated for 1.5h. The HSA-coated NC (HSA-NC) was collected by centrifugation and washed with water twice. The NCs were visualized by scanning electron microscopy (SEM), and their size and zeta potential were measured by Dynamic Light Scattering (DLS).

PTX stability was tested in the form of Taxol, NC and PPT. These PTX formulations were incubated in the phosphate-buffered saline (PBS, pH 7.4) containing 25% of fetal bovine serum (FBS) at 37°C. The suspensions were sampled after 10 days, PTX extracted with ethyl acetate and analyzed with HPLC. Cabamazepine was added to each sample as an internal standard.

Cytotoxicity of NCs, PPT, and Taxol was tested with luciferase-expressing SKOV3 human ovarian cancer cell line (SKOV3-luc). Cells were plated in a 96-well plate at a density of 5000 cells per well in 200 µL of RPMI1640 complete medium. Treatments were added to the culture in the final PTX concentration ranging from 1 to 10,000 nM. After 2 day incubation, cells were lysed, and luciferin was added to the cell lysate to measure luminescence. The luminescence intensity of SKOV3-luc cells was proportional to the density of live cells (r² = 0.9959).

In vitro PTX release kinetics was studied using HA hydrogel (gel) containing NCs, Taxol, or PPT. HA derivatives (HA-adipic acid dihydrazide and HA-aldehyde) were dissolved in PTX suspensions in 40 mg/mL and mixed together to form NC-gel, HSA-NC gel, Taxol-gel, PPT-gel, respectively. To mimic the peritoneal cavity environment, which contains a limited amount of fluid with amphiphilic solutes, PBS containing 25% FBS and 10 U/mL hyaluronidase (HAase) or PBS with 0.2% Tween-80 and 10 U/mL HAase were used as a release medium, and total PTX concentration in the medium was kept at 10 µg/mL, slightly above the PTX solubility determined in 0.2% Tween-80 (8.7 µg/mL).

RESULTS AND DISCUSSION
NC had a rod-shape with a length of ~300 nm and a width of ~100 nm (Fig. 1A). The average diameters of particles are shown in Table 1. Surface adsorption of HSA helped NC to maintain particle size. When subjected to repeated centrifugation, bare NC aggregated to form 3089 nm particles, whereas HSA-NC maintained the size at ~300 nm.

Table 1. Size and zeta potential of PTX formulations (n=3)

<table>
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<tr>
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<th>PPT</th>
<th>Taxol</th>
<th>NC</th>
<th>HSA-NC</th>
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<tbody>
<tr>
<td>Size (d, nm)</td>
<td>7538.7 ± 1430.0</td>
<td>13.8 ± 0.1</td>
<td>289.6 ± 14.0</td>
<td>324.9 ± 8.9</td>
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PTX was least stable when solubilized as Taxol and more stable when stored as solid (NC and PPT) (Fig. 1B). Between NC and PPT, the recovery of NC
was relatively low, most likely due to faster initial dissolution of NC than PPT due to the small size.  

Fig. 1. (A) SEM picture of NC. (B) Stability of PTX formulations (0.1 mg/mL) in 25% FBS for 10 days (n=6). *: p<0.05.

Cytotoxicity study shows that the efficacy of PTX decreased in the order of Taxol, NCs, and PPT (Fig. 2). NC and HSA-NC showed higher toxicity than PPT at 10000 nM (85.4 µg/mL), suggesting better PTX dissolution from NC than PPT due to the higher surface area to volume ratio of NC. There was no difference between NC and HSA-NC, likely due to in-situ surface stabilization of NC by serum proteins in the complete culture medium.

Taxol-gel released 85.7% in 24h followed by no further release. PPT-gel and NC-gel continuously released 35.2% and 29.5% over 10 days, respectively (Fig. 3A). This release profile indicates that NC may have quickly aggregated over time within HA gel, as indicated by the presence of significant non-released fraction, facing similar challenge in dissolution as PPT. The incomplete total PTX recovery (Fig. 3B), inconsistent with stability results shown in Fig. 1B, is not fully understood, but it is possible that NC and PPT were separated from the degrading gel before dissolution and partially removed from the medium during the repeated sampling. The release kinetics study shows that simple reduction of size did not avoid the dissolution challenge faced by PPT-gel and it is necessary to make further effort to prevent aggregation of NC in gel during the release period.

On the other hand, PTX release or total recovery did not significantly improve by the use of HSA-NC (Fig. 4), which indicates that HSA-NC still underwent aggregation in the degrading gel during incubation. It is possible that the coverage of NC surface by HSA (8.6 ng/mm²) was incomplete and/or the difference in hydrophilicity between HSA-NC and HA gel caused segregation of HSA-NC and accelerated the aggregation.

Fig. 2. Cytotoxicity of PTX formulations (n=3).

Fig. 3. (A) In-vitro PTX release kinetics performed in PBS with 25% serum and 10U/mL HAse. (B) Total PTX recovery (n=3).

CONCLUSION

Cytotoxicity test shows the potential of NCs to improve PTX dissolution in aqueous medium; however, release kinetics suggests that their segregation/aggregation in HA gel was not effectively prevented. While NC will be further stabilized by greater coverage of HSA, hydrogels with a denser network and hydrophobic domain will also be pursued to reduce the mobility of NCs in the gel and subsequent aggregation.

REFERENCES

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