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Intraperitoneal delivery of platinum with in-situ crosslinkable hyaluronic acid gel for local therapy of ovarian cancer

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ABSTRACT

Intraperitoneal (IP) chemotherapy is a promising post-surgical therapy of solid carcinomas confined within the peritoneal cavity, with potential benefits in locoregional and systemic management of residual tumors. In this study, we intended to increase local retention of platinum in the peritoneal cavity over a prolonged period of time using a nanoparticle form of platinum and an in-situ crosslinkable hyaluronic acid gel. Hyaluronic acid was chosen as a carrier due to the biocompatibility and biodegradability. We confirmed a sustained release of platinum from the nanoparticles (PtNPs) and nanoparticle/gel hybrid (PtNP/gel), receptor-mediated endocytosis of PtNPs, and retention of the gel in the peritoneal cavity over 4 weeks: conditions desirable for a prolonged local delivery of platinum. However, PtNPs and PtNP/gel did not show a greater anti-tumor efficacy than CDDP solution administered at the same dose but rather caused a slight increase in tumor burdens at later time points, which suggests a potential involvement of empty carriers and degradation products in the growth of residual tumors. This study alerts that although several materials considered biocompatible and safe are used as drug carriers, they may have unwanted biological effects on the residual targets once the drug is exhausted; therefore, more attention should be paid to the selection of drug carriers.

1. Introduction

Intraperitoneal (IP) chemotherapy has been pursued as a promising post-surgical therapy of solid carcinomas confined within the peritoneal cavity, such as ovarian cancer (OC) and peritoneal carcinomatosis. The benefit of IP chemotherapy is multifaceted. A drug delivered IP can achieve a higher concentration and a longer half-life in the peritoneal cavity compared to those observed with intravenous (IV) administration [1–3] and, thus, has a greater opportunity for locoregional effects [4–6]. Moreover, the IP-administered drug is partly absorbed to systemic circulation, getting access to regions of a tumor that are not in direct contact with peritoneal fluid or tumors remote from the peritoneal cavity via blood [7,8]. The systemic absorption of an IP-administered drug occurs more slowly than IV drug; therefore, IP administration also brings the benefits of sustained drug delivery (a prolonged blood half-life and lower C_{max}) [9–11]. Finally, if delivered as nanoparticles with a specific size, a drug can be trafficked through lymph nodes [3,12,13], which provides an opportunity to treat cancer cells spreading via the lymphatics.

With increasing awareness of the potential benefits, several drugs have been delivered IP for the therapy of peritoneal malignancies [14–16]; however, most of them are simple repurposing of IV drugs, not necessarily designed with special constraints for IP delivery in mind. Those requirements include (i) the biocompatibility of the material system– an important feature given the sensitivity of the peritoneal cavity to foreign insults [17], (ii) an optimum rate of degradation and absorption for an extended retention in the peritoneal cavity, and (iii) the ability to control the drug release for prolonging the local effect and attenuating systemic drug absorption. Considering these needs, we have used an

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in-situ crosslinkable hydrogel based on hyaluronic acid (HA) derivatives as a drug carrier to the peritoneal cavity [18–21]. The gel is composed of adipic dihydrazide modified HA (HA-ADH) and oxidized HA (HA-CHO), which can be applied as liquid and instantly crosslink via the hydrazone link to form a gel as they mix. Of several advantages of the HA gel, relevant to the IP therapy are the biodegradability, biocompatibility, and the in-situ crosslinkability, which allows for flexible and broad coverage of the peritoneal cavity [22,23]. Moreover, HA has abundant carboxyl groups, which can form a complex with platinum (Pt) [24] and attenuate its release over several days [25–27].

Based on these advantages, we hypothesize that the in-situ crosslinkable HA gel as a carrier of Pt will localize and release the drug in the peritoneal cavity over a prolonged period, thereby more effectively reducing the tumor burden than a free drug solution. Pt compounds are an important arsenal in post-surgical chemotherapy of OC, but they present significant systemic side effects such as nephro- and neurotoxicity [28,29]; therefore, a new local delivery system for Pt compounds is well justified. Here, we prepare a nanoparticle (NP) form of Pt and deliver the NP with the in-situ crosslinkable HA gel IP (Fig. 1) to test the utility of the Pt gel system in the local chemotherapy of tumors established in the peritoneal cavity.

2. Materials and methods

2.1. Materials

Hyaluronic acid (HA, 35 kDa) was purchased from Lifecore Biomedical, LLC (Chaska, MN, USA). FPR-648 dye was a gift from BioActs (Incheon, Korea). Cell culture medium and supplements were purchased from Invitrogen (Carlsbad, CA, USA). All other reagents including cis-dichloro-diamine-platinum (CDDP, cisplatin) were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Preparation and characterization of Pt-incorporated HA nanoparticles (PtNPs)

PtNPs were produced as described in the literature [25–27]. Briefly, 25 mg HA and 5 mg CDDP were dissolved in 5 mL of deionized water. The mixture was stirred gently for 4 days in darkness. Non-encapsulated CDDP was removed by dialysis (molecular weight cut-off: 3.5 kDa) against deionized water for 1 day. The purified PtNPs were lyophilized for 2 days with trehalose as a lyoprotectant. For determination of Pt content, PtNPs were dissolved in 0.3 M NaCl at 37 °C for 3 days [30] and analyzed with the Atomic Absorption Spectrometry (AAS) using a Perkin–Elmer 3110 Spectrometer (Waltham, MA, USA) equipped with a Pt lumina hollow cathode lamp (Perkin–Elmer). The Pt content and encapsulation efficiency were calculated as the weight ratio of Pt to PtNPs and the ratio of the measured Pt content to theoretical Pt content, respectively. The particle size and zeta potential of PtNPs were measured with Malvern Zetasizer Nano ZS90 (Worcestershire, UK).

2.3. Preparation of in-situ crosslinkable HA gel loaded with PtNPs (PtNP/gel)

In-situ crosslinkable HA derivatives, HA-ADH and HA-CHO, and a crosslinked HA gel were prepared as described previously [21]. PtNP/gel was prepared by suspending PtNPs in solutions of HA derivatives in PBS and extruding them through a common outlet using a double-barreled syringe. The HA concentration in gel was 40 mg/mL unless specified otherwise.

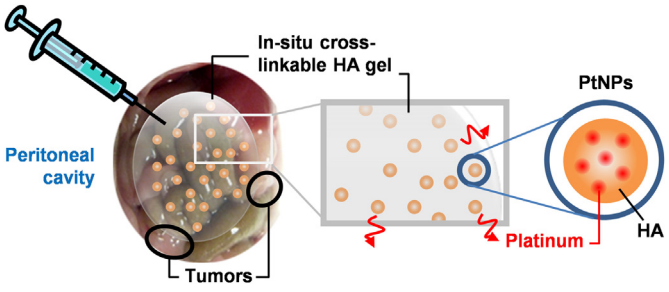

Fig. 1. Schematic diagram of intraperitoneal delivery of platinum with an in-situ crosslinkable hyaluronic acid gel and nanoparticles.

2.4. In vitro release kinetics

20 or 40 mg/mL of HA derivative solutions containing PtNPs were co-extruded into a Float -A-Lyzer® bag (1 mL capacity, Spectra/Por®). The bag was perforated with a 27 gauge needle 12 times. In the case of PtNPs or CDDP, the samples were suspended in PBS and loaded in a dialysis bag. In all cases, 1 mL of sample contained 0.65 mg Pt. Each bag was placed in 15 mL of PBS or PBS containing 10 U/mL hyaluronidase (HAse) and incubated at 37 °C for 5 days under constant agitation. At regular intervals, 5 mL of the release medium was sampled for AAS analysis and replaced with fresh PBS.

2.5. Cytotoxicity of PtHA NPs

SKOV3 human ovarian cancer cells (ATCC, Manassas, VA, USA) were maintained in RPMI-1640 medium supplemented with 10% FBS and 1% penicillin-streptomycin. Cells were plated in a 96-well plate at a density of 10,000 cells per well in 200 μL of complete medium. After 24 h, 20 μL of the concentrated PtNP suspension or CDDP solution was added to each well to provide Pt in the final concentration ranging from 0.065 to 65 μg/mL. A control group was treated with 20 μL of PBS. Cells were either incubated for 1 day in each treatment with a 2 day recovery period in treatment-free medium or incubated for 3 days with the treatment prior to the cell assay. The cell viability was estimated by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. At the end of each treatment, the medium was replaced with 100 μL of fresh medium and 15 μL of 5 mg/mL MTT solution and incubated for 3.5 h. One hundred microliters of solubilization/stop solution was then added, and the plate was left in darkness overnight. The absorbance of the solubilized formazan was read with a SpectraMax M3 microplate reader (Molecular Devices, Sunnyvale, CA, USA) at a wavelength of 562 nm. The measured absorbance was normalized to the absorbance of PBS-treated cells.

2.6. Confocal microscopy

Fluorescently labeled PtNPs (PtNPs*) were produced by labeling a portion of HA with a pre-activated FPR-648 dye (Ex/Em = 648/672 nm). Briefly, HA-ADH was labeled with FPR-648 according to the manufacturer's instruction and used to replace 20% of HA in PtNPs. SKOV3 cells were plated at a density of 80,000 cells/cm^2 in 35 mm dish with a glass window (MatTek) and incubated for 24 h. The medium was replaced with 0.3 mg/mL of PtNPs* suspended in the serum-free medium with or without 10 mg/mL of HA. After 1 h incubation, the NP containing media was replaced with the fresh serum-free media, and the dishes were washed with media twice to remove free PtNPs*. Hoechst 33342 was added to 2 μg/mL 30 min prior to imaging to stain the nuclei. Confocal microscopy was performed using a Nikon A1R confocal microscope equipped with a Spectra Physics 163C argon ion laser and a Coherent CUBE diode laser. PtNPs* were excited with a 488 nm laser, and the emission was read from 500 to 600 nm. Cell nuclei were excited with a 633 nm laser, and the emission was read from 650 to 750 nm.

2.7. Determination of the maximum tolerated doses (MTDs) of treatments

All animal procedures were approved by Purdue Animal Care and Use Committee, in conformity with the NIH guidelines for the care and use of laboratory animals. The MTD of each treatment was determined according to the method published by the National Cancer Institute's Developmental Therapeutics Program [31]. Healthy female Balb/c wild-type mice (17–20 g, Harlan Laboratories, Indianapolis, IN), were randomly assigned to CDDP, PtNP, and PtNP/gel groups and given a single IP injection of each formulation at different dose levels (one mouse per dose). The mice were observed over a period of 2 weeks after the injection. The highest dose tolerated without >20% weight loss or other signs of significant toxicity was designated as the MTD of each treatment.

2.8. Anti-tumor effectiveness of treatments in an IP tumor model

A mouse model of IP tumor was prepared as described in our previous study [21]. SKOV3 or luciferase-expressing SKOV3 cells (SKOV3-luc, donated by Prof. Glen Kwon at University of Wisconsin-Madison) [32] were maintained in a complete RPMI-1640 medium. Ten million cells were suspended in 1 mL PBS and injected to a female Balb/c nude mouse (8–10 week old, ~20 g, Harlan Laboratories). Two sets of experiments were performed independently.

In the first set (Supporting Fig. 1a), animals were injected with SKOV3 cells 2 weeks before the treatment. Animals were randomly assigned to three Pt formulations (CDDP, PtNPs, PtNP/gel: 4.8 mg Pt/kg as a single dose) and two negative control treatments (PBS and blank gel) (n = 10 per group). The treatments were administered as 1 mL IP injection. At 2 or 4 weeks after the treatment, 5 animals per group were sacrificed by ketamine anesthesia followed by cervical dislocation, and the weight and location of tumors in the peritoneal cavity were recorded. Blood was collected by cardiac puncture and submitted to Animal Disease and Diagnostic Laboratory for blood chemistry analysis. Peritoneal lavage samples were collected as previously described [21], and the level of Pt remaining in the lavage fluid was determined by AAS.

In the second set (Supporting Fig. 1b), animals were injected with SKOV3-luc cells, and tumor burdens were monitored weekly using the whole body imaging