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An injectable depot system for sustained intraperitoneal chemotherapy of ovarian cancer results in favorable drug distribution at the whole body, peritoneal and intratumoral levels

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ABSTRACT

The current study characterizes the impact of docetaxel (DTX) distribution on efficacy following sustained intraperitoneal (IP) chemotherapy in murine models of ovarian cancer. A polymer-lipid biodegradable depot (PoLigel) was used to deliver DTX in a sustained manner over 21-days following IP administration. Distribution and efficacy studies were carried out in SCID mice bearing SKOV3 IP solid tumors or C57BL/6 mice with ID8 IP ascites fluid. In addition, a subcutaneous (SC) SKOV3 model was used to determine whether systemic drug levels that result from IP administration of the PoLigel influence antitumor efficacy. Immunostained IP and SC SKOV3 tumor sections were used to study cell death, intratumoral drug distribution and tumor penetration. Sustained concentrations of DTX were observed in plasma, tissue, tumor and ascites over the entire study period. Drug accumulation was several fold greater in tumors and ascites when compared to plasma levels. Sustained chemotherapy resulted in significant reduction in tumor burden and ascites volume. IP tumors showed greater cell death compared to the SC tumors as seen by higher TUNEL and caspase-3 expression. At the intratumoral level, DTX distributed more towards the core of IP tumors compared to the SC tumors. Tumor penetration of drug from nearest blood vessel was 1.5 fold greater in the IP tumors than the SC tumors. Overall, favorable drug distribution at the whole-body, peritoneal and intratumoral levels in combination with local and systemic sustained drug exposure contribute to the high efficacy observed. These results encourage the clinical use of IP sustained chemotherapy for ovarian cancer.

1. Introduction

The peritoneal cavity is the principal site of ovarian cancer both at diagnosis and relapse [1]. The delivery of chemotherapeutics through intraperitoneal (IP) administration into the peritoneal space has the potential to expose the cancer to higher concentrations of drug over longer periods of time while minimizing systemic toxicity [2]. Phase III trials have demonstrated a substantial survival advantage following IP chemotherapy in patients with optimally debulked ovarian cancer when compared to intravenous chemotherapy [3–5]. Nevertheless, the use of IP chemotherapy has remained limited due to local toxicities and complications associated with prolonged use of indwelling catheters required to deliver the chemotherapeutics [6].

Drug delivery systems including liposomes, nanoparticles and microparticles have been explored for IP chemotherapy in an effort to overcome catheter-related problems. However, liposomes and nanoparticles are rapidly cleared from the peritoneal cavity by absorption into the lymphatic circulation [7–9], thus requiring frequent administration. Microparticles are retained longer in the peritoneal cavity [8–10], although local toxicities caused by material components have been documented [11]. Another approach to increase drug retention and minimize frequent dosing has been the use of IP implantable drug delivery systems. Grant et al. have developed a paclitaxel (PTX) loaded implant for IP chemotherapy of ovarian cancer [12]. This implant was shown to be biocompatible, provide sustained drug levels in the peritoneal cavity and lead to greater efficacy compared to standard intermittent chemotherapy [13–16].

Sustained IP chemotherapy with docetaxel (DTX), a semi-synthetic analogue of PTX, holds much promise for ovarian cancer therapy. Firstly, high peritoneal DTX levels are attainable upon IP administration, as clinical studies have shown 152–207 fold greater DTX concentrations in the peritoneal cavity when compared to systemic drug levels [17]. Furthermore, DTX is a cell cycle specific drug [18] and only a small percentage of cancer cells are found at vulnerable phases (i.e. G_2/M) at any given time [19]. Achieving sustained DTX levels ensures the drug is present when more cells cycle through these phases, potentially resulting in greater cell death. Recently an injectable depot (PoLigel) was developed in

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order to achieve sustained delivery of DTX in the peritoneal cavity [20]. The physicochemical properties of the PoLigel formulation as well as molecular interactions and organization of materials within PoLigel have been evaluated and published elsewhere [20]. Sustained release of DTX from the PoLigel delivery system was confirmed in mice with 4% of the total DTX loaded being released per day [20]. The PoLigel formulation does not induce local toxicity or result in a systemic inflammatory response and is progressively degraded *in vivo*, with $7.4 \pm 5.0\%$ of the original injected mass remaining four weeks following IP administration [20,21]. The PoLigel resulted in a significant decrease in tumor burden and ascites fluid accumulation when compared to intermittent IP administration of Taxotere®, the clinically used formulation of DTX [22]. Additionally, studies evaluating the impact of sustained versus intermittent chemotherapy at the cellular and molecular levels revealed that sustained DTX resulted in greater cell death, less angiogenesis, less cell proliferation and a reduction in the development of drug resistance [22,23].

Another limitation that has hindered widespread clinical use of IP chemotherapy, administered using an indwelling catheter, has been the heterogeneous drug distribution that results within the peritoneal cavity [24–26]. In order to improve drug distribution a large volume of fluid is instilled into the peritoneal cavity at time of treatment, which has been shown to result in significant pain and patient discomfort [24]. Alternatively, the PoLigel has been shown to result in a homogeneous distribution of drug within the peritoneal cavity, of healthy CD-1 mice, without the need for fluid co-administration [20]. As well, compared to IP administration of Taxotere® in healthy CD-1 mice, the PoLigel results in sustained peritoneal drug levels [27]. The present study investigated DTX distribution at the whole-body, peritoneal and intratumoral levels in disease bearing mice in an effort to further understand the improved efficacy that results from sustained IP delivery of DTX. Furthermore, as the PoLigel provides sustained plasma levels of DTX, the influence of sustained systemic drug exposure on antitumor efficacy was explored. This was accomplished by evaluating efficacy following IP administration of PoLigel in a subcutaneous (SC) murine model.

2. Materials and methods

2.1. Materials

DTX was purchased from Jari Pharmaceutical Co. (Jiangsu, China). Tritium labeled DTX (^{3}H-DTX) was purchased from American Radiolabeled Chemicals (St. Louis, USA). Chitosan was purchased from Marinard Biotech Inc. (Quebec City, Canada). Egg phosphatidylcholine (ePC) and lauric aldehyde (LA) were purchased from Sigma-Aldrich Chemical Co. (Oakville, Canada). All other chemicals were reagent grade and used as received.

2.2. Cell culture

The SKOV3 human ovarian adenocarcinoma cell line was obtained from the American Type Culture Collection (Rockville, USA). The murine epithelia ovarian cancer cell line ID8 was a gift from Dr. Jim Petrik (University of Guelph, Canada) [28]. Both cell lines were maintained in RPMI 1640 medium supplemented with 10% (v/v) fetal bovine serum and 1% (v/v) penicillin-streptomycin (100 U/mL penicillin G and 100 mg/mL streptomycin), grown as a monolayer at 37 °C in 5% CO_2 and 90% relative humidity.

2.3. Preparation of PoLigel

The PoLigel was prepared as outlined elsewhere [20]. In brief, a water-soluble chitosan derivative (WSC), synthesized by conjugating glycidyltrimethylammonium chloride onto the chitosan backbone [29], was dissolved in distilled deionized water to prepare a 4.2% (w/v) solution. DTX was dissolved in anhydrous ethanol and dried under nitrogen and then placed under vacuum for 24 h to remove any residual solvent. To the DTX containing vial, ePC and LA (1:4 w/w) were added and vortexed until the drug film dissolved. Finally the WSC solution was mixed with the ePC-LA-DTX solution and vortexed for 1 min (drug to material ratio of 1:8 w/w). Samples were sterilized under UV-light for 3 h prior to use in animals.

2.4. Biodistribution

All studies were conducted in 6–8 week old female SCID or C57BL/6 mice (Charles River, Canada) using sterile techniques and in accordance with the guidelines of the University of Toronto Animal Care Committee and the Canadian Animal Care Council. Two IP models and one SC model were used. For the IP models, SCID mice ($n = 4$) received 1×10^6 SKOV3 cells and C57BL/6 mice ($n = 4$) received 2.5×10^6 ID8 cells suspended in 200 μL PBS via IP injection into the peritoneal cavity. Seven and fourteen days post inoculation with SKOV3 and ID8 cells, respectively, mice were injected IP with the PoLigel (DTX dose: 32 mg/kg, 0.002% ^{3}H-DTX) in the lower left quadrant. For the SC model, SCID mice ($n = 4$) were inoculated SC in the right flank with 2×10^6 SKOV3 cells suspended in 200 μL PBS. Seven days post inoculation (tumors ~2 mm), mice were injected IP with the PoLigel (DTX dose: 32 mg/kg) in the lower left quadrant. Control animals for each model ($n = 4$) were injected IP with 20 μL sterile saline solution post cancer cell inoculation. All mice were monitored daily for signs of lethargy, peritonitis, weight loss, and abdominal distention. Endpoints requiring humane euthanasia included excessive muscle wasting, abdominal girth, hypothermia, inactivity, and weight loss in excess of 20%. Plasma, tumors, ascites, liver, spleen, stomach, intestine, heart, kidney and peritoneal layer were collected from the IP models at various time points and analyzed using scintillation counting as described previously [27]. Plasma and tumors were collected from the SC model on day 21 post treatment and analyzed using high performance liquid chromatography as detailed elsewhere [20].

2.5. Immunohistochemistry

IP and SC SKOV3 tumors were processed and immunostained for terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), caspase-3 and CD-31 as previously described [16]. In brief, after initial processing tumor sections were incubated with active caspase-3 antibody (1/50 dilution overnight), CD-31 primary antibody (1/50 dilution overnight), or with biotin-nucleotide cocktail and DNA polymerase 1 for 1 h at 37 °C for TUNEL. Sections were counterstained with Mayer's haematoxylin. IP SKOV3 tumors were immunostained during a previous study [22], but re-analyzed using an improved image analysis technique as outlined below. Each tumor section was digitized using a bright-field scanner (ScanScope XT, Aperio Technologies Inc.) at 20x magnification with a resolution of 0.5 microns/pixel.

2.6. Intratumoral distribution

Semiquantitative analysis of the intratumoral distribution of DTX in IP and SC SKOV3 tumor sections immunostained for caspase-3 was carried out using a customized image analysis program written in MATLAB® (version 7.9.0.529, R2009b). The border of each tumor section was first delineated by thresholding the image in order to remove any background noise. Next, the caspase-3 stained regions in the original immunostained tumor section were determined based on an empirically chosen threshold of the color intensity. Finally, the minimum Euclidean distance between each caspase-3 region to the periphery of the tumor section was calculated and pooled into three separate regions (i.e. edge, inner and core) with equidistant thicknesses.