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Ovarian cancer spheroid shrinkage following continuous exposure to cisplatin is a function of spheroid diameter

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HIGHLIGHTS

- Sustained, low drug doses are most effective against small ovarian cancer spheroids.
- Treatment of ovarian cancer spheroids may be diffusion-controlled.
- Data stresses importance of surgical debulking prior to intraperitoneal therapy.

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ABSTRACT

Objective. Most ovarian cancer patients present with advanced-stage disease, disseminated in the peritoneal cavity. Standard treatment involves surgical resection of all visible tumor, followed by delivery of systemic therapy. Patients with advanced-stage disease may be candidates for intraperitoneal (IP) chemotherapy following surgical debulking. Recent clinical trials have created controversy regarding the benefits of this approach. Previous clinical trials report that patients with microscopic residual disease respond best to IP therapy. The goal of this study was to determine the relationship between tumor size and the efficacy of continuous chemotherapy.

Methods. Small and large ovarian cancer spheroids (derived from UCI101 and A2780 cell lines) were exposed to short-term high (modeling an IP injection, "IP") or prolonged, low cisplatin concentrations (modeling an implanted device, "device"), which have been previously shown to be less toxic. Spheroid diameter was measured at various time points via image analysis and used to quantify tumor shrinkage over the course of treatment.

Results. We show that "IP" doses more effectively shrink large spheroids when the same cumulative dose is administered with both treatments, but that both regimens similarly treat small spheroids. We also demonstrate that higher cumulative "device" doses are most effective at shrinking large spheroids.

Conclusions. These results support the hypothesis that intratumoral drug distribution following IP treatment is diffusion-controlled. An implanted device that continuously delivers low doses of IP chemotherapy would, therefore, be maximally effective against microscopic tumors.

1. Introduction

Approximately 22,000 women in the United States are diagnosed with ovarian cancer each year, with annual death rates of over 14,000 [1]. The presence of ovarian cancer tumors throughout the abdominal cavity at the time of diagnosis has made tumor-debulking surgery the clinical standard of treatment prior to adjuvant chemotherapy. Administration of intraperitoneal (IP) chemotherapy has also been shown to significantly improve survival over systemic treatment due to the localized nature of the disease [2]. The ability of surgical debulking to lengthen patient survival following treatment depends on a number of factors. Optimal resection is defined by no residual tumors >1 cm in diameter following surgery and is technically difficult to achieve. Only 20–40% of ovarian cancer patients have access to surgeons skilled in surgical cytoreduction, such as gynecologic oncologists, who can improve rates of optimal resection in patient cohorts from <25% to 75% [3]. Access to

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a skilled surgeon was shown to increase average overall survival (OS) from 23 to 36.8 months [3]. There is debate over the extent of surgical debulking required for good prognosis, despite the Gynecologic Oncology Group recommendation for optimal debulking to <1 cm. Data published in 2006 demonstrated significant differences in OS between patients with microscopic residual disease, gross disease ≤1 cm in diameter, and gross disease >1 cm in diameter after debulking surgery [4]. Another study published in 2008, however, found no difference in progression-free survival (PFS) or OS between patients with 0.1–1 or 1.1–5 cm residual disease [5]. Despite disagreement among the exact diameters of tumors required for "optimal" debulking, it has been shown that achieving microscopic residual disease results in the best outcomes for patients in the long-term, particularly for patients receiving IP chemotherapy [4–6].

Recent preliminary results of GOG trial 252 underscore the importance of surgical debulking on the efficacy of adjuvant IP treatment. These results, presented at the 2016 Annual Meeting on Women's Cancer, revealed no significant difference in PFS among patients receiving IV or IP chemotherapy, nor was there a significant increase in PFS in women who had microscopic residual disease following surgical debulking [7]. These new results are in stark contrast to three previous phase III clinical trials, including GOG 172, GOG 114, and GOG 104, which showed that women with no visible residual disease who received IP chemotherapy had longer PFS and OS than those receiving IV therapy or IP therapy with gross residual but optimally debulked disease [2,6,8,9]. GOG 172 showed a 16-month improvement in OS benefitting women treated with IP chemotherapy on the trial, compared to patients on study treated with IV chemotherapy alone [2]. Two factors likely contributed to the limited efficacy in GOG 252: (1) almost 500 clinical trial sites were used to recruit the large number of eligible patients, compared to only 40 sites used to recruit patients for GOG 172, and (2) the dose of IP cisplatin was reduced from prior doses of 100 mg/m^2 to 75 mg/m^2 [2,7,10]. The large number of clinical trial sites implies a large degree of variability in the standards used to evaluate the degree of surgical debulking achieved. These measures are subjective and surgeon-specific and have been criticized as both biased and frequently inaccurate [11]. The unsuccessful reduction in the IP cisplatin dose also indicates that high doses are required to maximize treatment efficacy, despite the significant incidence of high-grade toxicity associated with IP therapy [2,7].

These clinical findings suggest that IP chemotherapy is more efficacious against smaller tumor nodules. Jandial et al. supported this hypothesis in mouse models of human ovarian cancer by showing that platinum levels in IP tumor nodules following IP cisplatin administration decrease significantly with increasing tumor size [12]. Helland et al. reached a similar conclusion by demonstrating that mice treated with debulking surgery followed by chemotherapy lived longer on average than those treated with either intervention alone [13]. The advantage of IP over IV therapy comes from the direct penetration of agents into tumor tissues [14]. Drug penetration into tumor nodules is controlled by both diffusion and convection, although small molecules are primarily diffusion-controlled [15]. Diffusion of drugs through tissues depends on both the partitioning of drug, length scale for diffusion, and its diffusivity in the tissue. Studies have shown that for cisplatin, this benefit is limited to the outer 1.5–3 mm of tumor nodules, in which drug concentrations in the tumor tissue are higher following IP than IV therapy [14,16]. Brenner hypothesizes that multiple IP administrations may kill tumor nodules up to roughly 0.5 cm in diameter in a layer-by-layer fashion, with each cycle of therapy killing the outermost cell layers [16]. Sustained tumor exposure to chemotherapy may lead to continuous cell kill from the outer to the innermost layers, offering an advantage over bolus IP doses of cisplatin that are rapidly cleared from the peritoneal cavity. There may, however, be a maximum nodule diameter for which this is true.

Despite the significant evidence suggesting that small molecule penetration into tumor nodules is diffusion-controlled, this topic remains controversial within the clinical community. We have previously shown that continuous IP delivery of cisplatin *via* implanted devices can match the efficacy of intermittent, high-dose injections with lower associated toxicity [17]. An average of 4.7 times more total cisplatin dose was, however, administered with the devices to achieve the same tumor reduction as observed with IP bolus injections. The concentration gradient between the peritoneal fluid and the center of a tumor nodule is smaller during continuous drug delivery compared with high-dose IP injection. It is, therefore, expected that low doses of drug administered *via* devices will penetrate less deeply into tumor nodules of the same size and that treatment efficacy is a function of average tumor diameter.

Drug distribution within tumors is controlled by a number of factors, categorized by Minchinton et al. into supply, flux, and consumption [15]. These phenomena can be difficult to evaluate *in vivo*. The development of *in vitro* models of drug penetration has enabled a better understanding of the role of intratumoral distribution in treatment efficacy and drug resistance [18–21]. Two commonly employed models are multicellular cell layers (MCLs) and multicellular spheroids (MCSs). Multicellular tumor aggregates are shed from the primary tumor and are disseminated by peritoneal fluid flow throughout the abdominal cavity, where they form metastases [22,23]. This process requires remodeling of surface proteins (*e.g.* integrins, cadherins) that is not recapitulated in traditional 2D cultures of metastatic ovarian cancer cells [22,24]. Cells grown as spherical aggregates *in vitro* not only express similar surface proteins as metastatic nodules *in vivo*, but also develop extracellular matrix and upregulate ovarian cancer biomarkers not observed in 2D culture [15,24–26]. It has been shown that tumor aggregates within malignant ascites are the greatest impediment to effectively treating late stage epithelial ovarian cancer [23,27]. It is believed that unresected nodules respond poorly to chemotherapy due to poor drug penetration and the ability of cells at the core of these aggregates to develop resistance [27]. Multiple studies have shown that both *in vitro* and *in vivo* ovarian cancer MCSs require higher chemotherapy doses than those same cells grown in a monolayer [22,23,25,27]. Loessner et al. found that for the same treatment regimen with paclitaxel, there was a 50% greater survival of ovarian cancer cell spheroids compared to their monolayer counterparts [24]. These results demonstrate that MCSs better model the structure and biological markers of multicellular metastatic tumor aggregates as well as the predicted *in vivo* therapeutic response [21,28].

Tumor spheroids each composed of two different cell lines were used in the following *in vitro* experiments to establish preliminary conclusions about the relative efficacy of continuous low doses and high bolus doses of cisplatin against differently sized spheroids. Spheroid diameter was used to quantify tumor shrinkage as a physiologically relevant measure of antitumor efficacy.

2. Materials and methods

2.1. Experimental design

The objective of this research was to investigate the relationship between tumor size and the efficacy of continuous chemotherapy. We hypothesized that sustained low doses of cisplatin would be more effective against smaller ovarian tumor nodules.

Multiple human ovarian cancer cell lines were used to generate spheroids *in vitro*. UCI101 and A2780 cells formed spheroids most readily and were therefore used for subsequent experiments.

Small and large UCI101 and A2780 spheroids grown *in vitro* were exposed to short-term high (modeling an IP injection, "IP") or prolonged low (modeling an implanted device, "device") cisplatin concentrations and nodule shrinkage was quantified over time using image analysis. Treated spheroids were compared to untreated controls at various time points. Spheroids from the same suspension were transferred to random wells of a non-tissue cultured treated 24-well plate to allow

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