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Nanoscopic tumor tissue distribution of platinum after intraperitoneal administration in a xenograft model of ovarian cancer

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ABSTRACT

There is increasing interest in the treatment of advanced stage ovarian cancer (OC) using intraperitoneal (IP) delivery of platinum (Pt)-based chemotherapy. The antitumor efficacy of IP chemotherapy is determined by efficient tumor tissue penetration. Although it is assumed that Pt penetration is limited to a few millimeters after IP delivery, little is known on the distribution of Pt in different tumor compartments at the ultrastructural level following IP administration.

Here, using synchrotron radiation X-ray fluorescence spectrometry (SR-XRF) and laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS), Pt distribution and penetration in OC peritoneal xenografts were determined at nanometer scale after IP chemoperfusion of cisplatin at 37–38 °C or 40–41 °C (hyperthermic). Using principal component analysis (PCA) the presence of phosphorus, manganese, calcium, zinc, iron, bromine, and sulfur was correlated with the distribution of Pt, while k-means analysis was used to quantify the amount of Pt in weight% in tumor stroma and in tumor cells.

The results showed a heterogeneous distribution of Pt throughout the tumor, with an accumulation in the extracellular matrix. LA-ICP-MS mappings indicated significantly higher concentrations of Pt ($P = 0.0062$) after hyperthermic chemoperfusion of cisplatin, while SR-XRF demonstrated a deeper tissue Pt penetration after hyperthermic treatment. Using PCA, it was showed that Pt co-localizes with bromine and sulfur. No differences were observed in Pt distribution regarding tumor cells and stroma, when comparing normo- *vs*. hyperthermic treatment.

In conclusion, SR-XRF and LA-ICP-MS are suitable and highly sensitive techniques to analyze the penetration depth and distribution of Pt-based drugs after IP administration. To the best of our knowledge, this is the first experiment in which the distribution of Pt is analyzed at the cellular level after IP administration of cisplatin.

Abbreviations: Ar, argon; Br, bromine; Ca, calcium; CPS, counts per second; CRS, cytoreductive surgery; Cu, copper; ECM, extracellular matri; GAG, glycosaminoglycans; H&E, haematoxylin and eosin; HIPEC, hyperthermic intraperitoneal chemoperfusion; IP, intraperitoneal; IPC, intraperitoneal chemoperfusion; IQR, interquartile range; LA-ICP-MS, laser ablation – inductively coupled plasma – mass spectrometry; MC, Monte Carlo; Mn, manganese; MT, metallothioneins; OC, ovarian cancer; P, phosphorus; PC, principal component; PCA, principal component analysis; Ppm, parts per million; Pt, platinum; RGB, red green blue; ROI, region of interest; S, sulfur; SR-XRF, synchrotron-radiation x-ray fluorescence; Zn, zinc.

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1. Introduction

In Europe, about 65.500 women are diagnosed with ovarian cancer (OC) every year, often at an advanced stage [1]. The current standard of care in advanced stage OC consists of cytoreductive surgery (CRS) combined with platinum (Pt) and taxane-based chemotherapy. Despite clinical remission in the majority of patients, recurrence in common, leading to a 5-year survival rate of less than 30% [2]. Specific to OC is that it generally remains contained to the abdominal cavity over a long time period. Recently, intraperitoneal chemotherapy (IPC) has been added to the arsenal of possible therapies [3]. Bathing the abdominal cavity in a concentrated solution of chemotherapeutic drugs is expected to increase local drug exposure, while minimizing systemic toxicity. Data from the literature indicate improved overall survival after CRS with IPC in varying stages and settings of the disease [4].

Despite promising clinical results, the efficacy of Pt-based IPC is limited by poor tissue Pt penetration, which is limited to a few millimeters. Tissue transport after IP delivery is affected by numerous variables including drug type and concentration, exposure time, and treatment temperature [5]. Unfortunately, little is known on the detailed transport mechanisms and tissue distribution of Pt on an ultrastructural level after IP delivery. Previous studies reported Pt distribution and sensitivity in single tumor cells [6,7]. Such *in vitro* studies are unable to study interactions between the tumor cells and stroma, or the role of the extracellular matrix (ECM) in the transport and biodistribution of Pt [8]. Additionally, it is known that some trace elements such as Cu are important in the pathology of biological systems for angiogenesis and Pt resistance. However, their distribution and correlation with Pt are not known at ultrastructural level.

Here, it was studied for the first time Pt distribution in relevant compartments (intracellular, ECM) after IPC with cisplatin at a nanoscopic scale in an OC xenograft model using advanced analytical techniques including synchrotron radiation X ray-fluorescence (XRF) imaging and laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS). In addition, since IPC is often administered in clinical practice under hyperthermic conditions (40–41° C) the effect of treatment temperature on the Pt biodistribution and penetration was also evaluated. Furthermore, the distribution of phosphorus (P), sulfur (S), calcium (Ca), zinc (Zn), and copper (Cu) were visualized and correlated at the ultrastructural level using principal component analysis (PCA) in order to investigate their possible role in the distribution of Pt. In addition, k-means analysis was performed to study the distribution and concentration in weight% (wt%) of Pt between interstitial tumor stroma and tumor cells.

2. Materials and methods

2.1. Compliance with ethical standards

All animal experiments were approved by the Animal Ethics Committee of the Faculty of Medicine at Ghent University, Belgium (ECD 15/51) and were performed according to the national and European legislature on animal welfare. Mice were examined daily for pain or discomfort and "The Guidelines for the welfare and use of animals in cancer research" were strictly followed for distention of the abdomen, physical condition and other clinical signs that would require intervention, as prescribed by The National Cancer Research Institute [9]. Food and water was given ad libitum. All procedures were performed under general anesthesia (Isoflurane, Forene®, AbbVie, Waver, Belgium).

2.2. Cell line

The human ovarian cancer cell line SK-OV-3 Luc IP1 was provided by the Laboratory of experimental cancer research (Ghent University, Ghent, Belgium). SK-OV-3 cells from the American Type Culture Collection were transfected with luciferase and subjected to an *in vivo* selection, to create a more aggressive cell line, SK-OV-3 Luc IP1. Cells were cultured in Dulbecco's Modified Eagle's Medium (Life technologies/ThermoFisher, Ghent, Belgium) and supplemented with 2% penicillin/streptomycin + 0.005% fungizone (Bristol-Myers-Squib B.V., Utrecht, The Netherlands) and 10% fetal calf serum (Sigma-Aldrich, Diegem, Belgium).

2.3. Subperitoneal xenograft mouse model and intraperitoneal chemoperfusion

Female athymic, *nude-foxn1nu* mice (ENVIGO, NM horst, the Netherlands) of 8 weeks old and an average weight of 25 g were conditioned one week before the start of the study. After conditioning, 11 mice underwent a laparotomie under general anesthesia and were subsequently injected bilaterally, immediately beneath the peritoneum (subperitoneal) with 5.0×10^5 SK-OV-3 Luc IP1 cells dissolved in 50 μl of BD matrigel® (Life Sciences, Antwerp, Belgium). After two weeks, mice underwent intraperitoneal continuous chemoperfusion (IPC) during 60 min with cisplatin (70 mg/m^2, Hospira Benelux BVBA, Antwerp, Belgium). The dose of cisplatin given to the mice was calculated as 1/100th of that in human relative to the body surface area. The 'open' chemoperfusion circuit consisted of 1 in- and outlet drain (3.2 mm bore; 1.6 mm wall; Watson-Marlow NV, Zwijnaarde, Belgium), 2 temperature probes (TM 9604; Ellab A/S, Hilleroed, Denmark), a 520 U process pump (Watson-Marlow NV, Zwijnaarde, Belgium) and a M3 LAUDA heat exchanger (LAUDA-Brinkmann, New Jersey, USA).

Chemoperfusion was conducted at 37–38 °C (normothermic, N = 5) or at 40–41 °C (hyperthermic intraperitoneal chemoperfusion or HIPEC, N = 5). One control mouse underwent HIPEC with saline solution during 60 min.

2.4. Sample preparation and histological analysis

All mice were immediately euthanized after (H)IPEC using cervical dislocation. Tumor nodules were excised, fixed in 4% formaldehyde (Klinipath, Olen, Belgium) for 24 h, and embedded under orientation in the paraffin block (Klinipath, Olen, Belgium). For synchrotron based X-ray fluorescence (SR-XRF) analyis, three consecutive tumor sections of 2 μm thick were cut using an HM 355S microtome (Thermo Fisher scientific, Ghent, Belgium). The first and third section were placed onto glass slides, air-dried, and kept at room temperature before haematoxylin-eosin (H&E) staining. The second section was placed on Ultralene® foil (SPEX® SamplePrep, New Jersey, United States), subsequently fixed onto Polyether ether ketone slides with a central opening, specifically made for analysis using the ESRF ID16B-NA SR beam. For the mapping of ^{195}Pt in tumor sections with LA-ICP-MS (Thermo Scientific, Bremen, Germany), three 5 μm consecutive paraffin-embedded sections were cut and placed onto glass slides. Sections 1 and 3 were air-dried and kept at room temperature before H&E staining, while Section 2 was air-dried and was intended for LA-ICP-MS imaging.

2.5. Micro- and nanoscopic distribution of Pt using SXRF

The tumor sections were analyzed using the ID16B-NA beamline of the ESRF (Grenoble, France) [10]. The beamline used X-rays with an energy of 17.5 keV, focused down to a 50×50 nm^2 beam spot size in the focal point and a photon flux of 2.93×10^{11} photons/s.