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Cytotoxicity of Hypo-osmolar Lavage Fluid on Ovarian Cancer Cells *In vitro*

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Abstract

Objective: Ovarian cancer is difficult to diagnose early and many patients present with advanced disease. The presence of exfoliated ovarian cancer cells in the peritoneal cavity after debulking surgery is a poor prognostic indicator. Sterilization of the peritoneum during surgery may have clinical benefit in reducing tumor burden. Several studies have evaluated osmotic cytotoxicity in gastrointestinal and genitourinary cancers with varied results. We studied the cytotoxic effect of lavage fluids of differing osmolarities against multiple ovarian cancer cell lines (SKOV3, OV90, and OVCAR3) *in vitro*.

Methods: Cells were treated for either 10 minutes or 30 minutes with water, or 5 mOsm, 10 mOsm, 50 mOsm, 100 mOsm, 200 mOsm, 280 mOsm NaCl (dilutions in water), and PBS. After 24 hours, surviving cells were enumerated in a Coulter Counter.

Results: All 3 ovarian cancer cell lines were lysed progressively as lavage osmolarity was reduced ($p<0.01$). For the OVCAR3 ovarian cancer cells, lavage for 30 min resulted in greater cell cytotoxicity for lavage with the water, 5, 10, 50, and 200 mOsm saline when compared to lavage for 10 min. For the OV90 cancer cell line, lavage for 30 min resulted in greater cell cytotoxicity for the water, 5, and 10 mOsm saline lavages. In the SKOV3 ovarian cancer cell line, only lavage for the 30 min water had cytotoxicity.

Conclusions: Since ovarian cancer uniquely resides in the peritoneal cavity, this anatomic feature allows concentrated washing to directly target these cancer cells residing in the peritoneal cavity. Hypo-osmolar treatment was found to be most effective in lysing ovarian cancer cell lines *in vitro*.

Keywords: Ovarian cancer; Hydrocytolotic lavage; *in vitro*

Introduction

Ovarian cancer is the leading cause of death from gynecologic cancer in the United States and is the country's fifth most common cause of cancer mortality in women. Ovarian cancer is difficult to diagnose at an earlier, more curable stage. Initial patient symptoms are often vague and include bloating, abdominal or pelvic pain, early satiety, and urinary symptoms. In fact, more than 70% of patients present with advanced disease at diagnosis. Optimal debulking of the tumor during initial surgery has been shown to improve survival rates [1-6]. Initial surgery should be a comprehensive staging laparotomy, including abdominal hysterectomy and bilateral salpingo-oophorectomy with extensive cytoreduction. The presence of exfoliated ovarian cancer cells in the peritoneal cavity after ovarian cancer debulking surgery is well recognized as a poor prognostic indicator. These cells are viable *in vitro* and tumorigenic *in vivo*. Theoretically, sterilization of the peritoneum at the time of cancer surgery may be clinically significant in reducing tumor burden in cancer patients. This tumor reduction by hydrocytolytic peritoneal lavage may translate to improved survival.

Several studies have evaluated osmotic cytotoxicity in gastrointestinal and genitourinary cancers with varied results [7-12]. Different lavage fluids that have been studied include normal saline, water, chlorhexidine and betadine. Providone iodine has been known to injure fibroblasts and cause peritonitis in colorectal models [13]. Chlorhexidine toxicity is still not well characterized. In this study, we studied the cytotoxic of hypoosmolar peritoneal irrigation against three ovarian cancer cell lines *in vitro*.

Methods

Three human ovarian cancer cell lines, SKOV3, OV90, and OVCAR3, were obtained from American Type Culture Collection (ATCC). For these experiments, SKOV3, OV90, and OVCAR3 were cultured as monolayers at 37°C with 5% CO_2 in RPMI 1640 medium with glutamine supplemented with 10% bovine calf serum, penicillin, and streptomycin. Sub culturing occurred every 3 days at 10%-30% confluence. For experiments, cells were seeded in 48-well plates at a density of 2.0×10^4 cells per well. The following day, cells were treated

for either 10 minutes or 30 minutes with the following treatments: 0 mOsm, 5 mOsm, 10 mOsm, 50 mOsm, 100 mOsm, 200 mOsm and 280 mOsm NaCl (dilutions in water), or PBS. Treatments were performed in triplicate and two independent experiments were performed. After 24 hours, cells were washed with PBS, trypsinized, and then counted in PBS using a Z1 Beckman Coulter Particle Counter. Statistical analysis was performed using an unpaired student t test on continuous variables. p values of <0.05(*), <0.01(**), <0.001(***) were considered significant.

Results

All 3 human ovarian cancer cell lines, SKOV3, OV90, and OVCAR3, were lysed progressively ($p<0.01$) as the osmolarity of the lavage solution was reduced (Figures 1-5) with 0 mOsm, 5 mOsm, 10 mOsm, 50 mOsm, 100 mOsm, 200 mOsm, 280 mOsm NaCl (dilutions in water), or PBS. In addition, there was marked variation in the number of ovarian cancer cells killed between the 3 cell lines when lavage for 10 versus 30 min was compared (Figures 4-5).

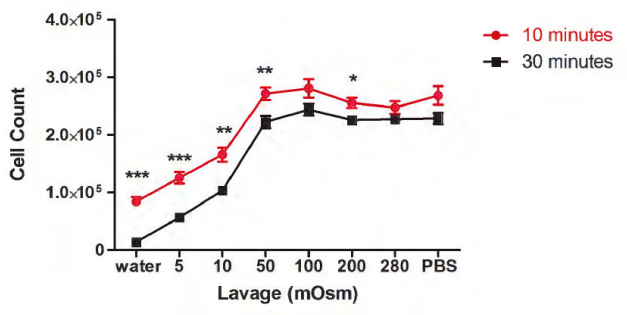

Figure 1: The effect of lavage osmolarity and time of treatment on the OVCAR3 cell line. The cells were treated with various concentrations saline for 10 minutes (red) and 30 minutes (black) using water, 5 mOsm, 10 mOsm, 50 mOsm, 100 mOsm, 200 mOsm, 280 mOsm NaCl (dilutions in water), and PBS. After 24 hours, cells were counted in a Coulter Counter.

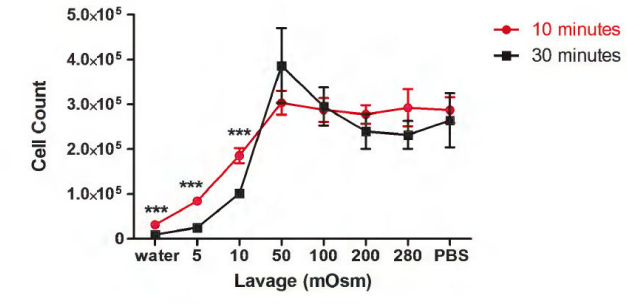

Figure 2: The effect of lavage osmolarity and time of treatment on the OV90 cell line. The cells were treated with various concentrations saline for 10 minutes (red) and 30 minutes (black) using water, 5 mOsm, 10 mOsm, 50 mOsm, 100 mOsm, 200 mOsm, 280 mOsm NaCl (dilutions in water), and PBS. After 24 hours, cells were counted in a Coulter Counter.

SKOV3 cells were more resistant to hypo-osmolar cell death overall than the OVCAR3 and OV90 ovarian cancer cell lines. For the OVCAR3 cells, lavage for 30 minutes resulted in a statistically significant lower cell count for the water ($p<0.001$), 5 ($p<0.001$), 10 ($p<0.01$), 50 ($p<0.01$), and 200 ($p<0.05$) mOsm saline lavages when compared to a 10 minute lavage (Figure 1).

For the OV90 cell line, 30 minutes of lavage resulted in a statistically significant lower cell count for the water ($p<0.001$), 5 ($p<0.001$), and 10 ($p<0.001$) mOsm saline lavages when compared to a 10 minute lavage (Figure 2).

In the SKOV3 cell line, only the 30 minute water lavage was statistically lower ($p<0.001$) than the 10 minute lavage (Figure 3).

In the 10 min lavage, there was significantly lower cells in the 10 mOsm than the 50 mOsm saline lavages for both the OVCAR3 ($p<0.001$) and OV90 ($p<0.01$) cell lines (Figure 4). In the 30 minute lavage, there was significantly lower cells in the 10 mOsm than the 50 mOsm saline lavages for both the OVCAR3 ($p<0.001$) and OV90 ($p<0.05$) cell lines (Figure 5).

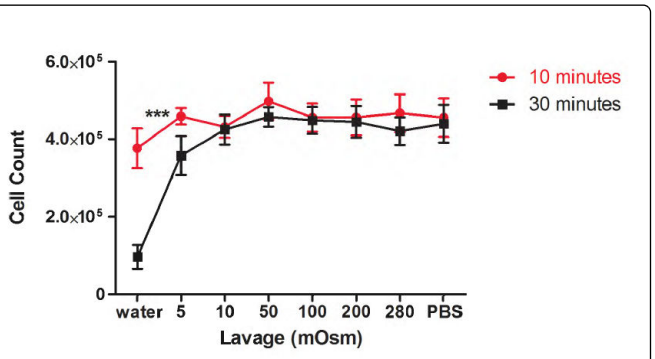

Figure 3: The effect of lavage osmolarity and time of treatment on the SKOV3 cell line. The cells were treated with various concentrations saline for 10 minutes (red) and 30 minutes (black) using water, 5 mOsm, 10 mOsm, 50 mOsm, 100 mOsm, 200 mOsm, 280 mOsm NaCl (dilutions in water), and PBS. After 24 hours, cells were counted in a Coulter Counter.

Discussion

Ovarian cancer provides an excellent model for hydrocytolysis of residual and exfoliated cancer cells after tumor debulking. Studies have confirmed that less residual tumor after surgery leads to better patient prognosis [1-5]. Thus, lysing exfoliated cancer cells with lavage fluids hypothetically should increase the survival of ovarian cancer patients. Furthermore, ovarian cancer uniquely resides in the abdominopelvic cavity and rarely metastases outside of this cavity. This unique characteristic allows targeted washing to the peritoneal cavity. The abdominopelvic cavity provides a natural basin to instill fluid for hydrocytolysis of the residual cancer cells. In contrast, another study in head and neck cancer depicted their difficulty in soaking residual tumor cells due to the lack of an anatomical basin in the head and neck region [14].

The practice of abdominopelvic lavage and the fluid used varies widely. In a survey of 118 surgeons, most surgeons lavaged until the abdominal fluid was clear while some used a fixed amount of irrigation fluid. In a contaminated abdomen, 47% of the surgeons used saline

lavage fluid, 38% aqueous betadine, 9% water and 3% antibiotic lavage [12]. Most gynecologic oncologists perform saline or water lavage into the abdominopelvic cavity after completion of ovarian cancer debulking surgery to detect bleeders and to remove residual exfoliated tumors.

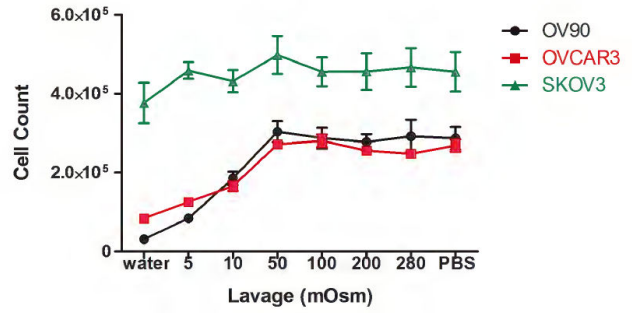

Figure 4: The effect of 10 min of lavage on ovarian cancer cell lines. OV90 (black), OVCAR3 (red), and SKOV3 (green) cells were treated with various concentrations saline for 10 minutes using water, 5 mOsm, 10 mOsm, 50 mOsm, 100 mOsm, 200 mOsm, 280 mOsm NaCl (dilutions in water), and PBS. After 24 hours, cells were counted in a Coulter Counter.

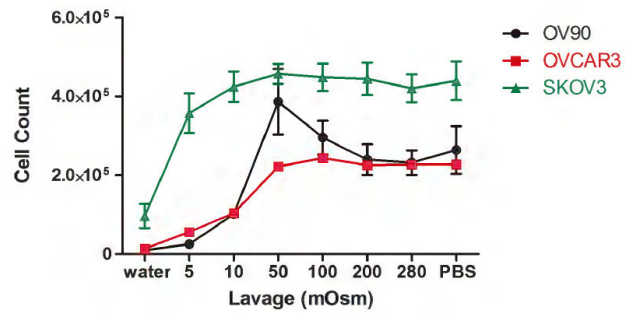

Figure 5: The effect of 30 min of lavage on ovarian cancer cell lines. OV90 (black), OVCAR3 (red), and SKOV3 (green) cells were treated with various concentrations saline for 30 minutes using water, 5 mOsm, 10 mOsm, 50 mOsm, 100 mOsm, 200 mOsm, 280 mOsm NaCl (dilutions in water), and PBS. After 24 hours, cells were counted in a Coulter Counter.

To our best knowledge, no study has been done with human ovarian cancer cells to show the effect of abdominopelvic lavage on the residual cancer cells. Our *in vitro* studies demonstrate several important points. First, hypo-osmotic lavage solution such as water is more tumoricidal to ovarian cancer cell lines than iso-osmotic solutions such as normal saline. This observation is consistent with the physiology of the cells. Water diffuses through cell membranes freely following the osmotic gradient. Under normal conditions, the intracellular osmolarity is approximately equal to plasma osmolarity. With water peritoneal irrigation, cancer cells are hyperosmotic relative to the water lavage. Water thus diffuses into the ovarian cancer cells, increases their volume, and subsequently lyses them. Secondly, results of this study show a minimal of 30 minutes of lavage is required to obtain maximal tumor kills. Indeed, a higher rate of cytotoxicity is observed when the cancer cell lines are treated with longer durations of hypo- osmotic solution. The current peritoneal lavage duration at the end of tumor debulking surgery ranges between five and ten minutes. The above results suggest that this duration is inadequate because complete cell lysis requires lavage for a longer time period of time than is currently practiced. Thirdly, the varying ovarian cancer cell lines appear to be affected differently against water lavage, but all of them were almost completely lysed after 30 minutes of exposure to hypo- osmotic solution.

We foresee potential hurdles and opportunities with our findings. First, lavaging the abdominopelvic cavity with water in patients for 30 minutes may lead to lysing not only of cancer cells, but also of normal peritoneal cells. Furthermore, free water may be absorbed into the vasculature resulting in hyponatremia. Also, most patients continue to produce isotonic lymphatic fluid even after optimal debulking surgery. This lymphatic fluid increases the osmolality of water lavage and may reduce water's effectiveness in lysing residual ovarian cancer cells. Furthermore, an additional 30 minutes of operating room time in intubated patients poses additional problems such as increasing operative cost or raising other operative-related complications such as hypothermia and pulmonary atelectasis. Next, this water lavage probably will not able to lyse larger tumor implants. Thus, optimal surgical debulking continues to be the corner stone of ovarian cancer treatment. Once the patient has minimal residual tumor, water lavage would further increase the effectiveness of the initial tumor debulking. Subsequently, the patient would still benefit from post-surgical intra-peritoneal chemotherapy. Finally, our *in vitro* study needs further validation *with in vivo* studies.

The potential solution to prolongation of surgery time was addressed in a previous study, which suggested continuing peritoneal lavage postoperatively [15]. We propose placement of an intraperitoneal dialysis catheter allowing the patient to be lavaged with water for an additional 30 minutes in the recovery room. A chemistry panel, including plasma sodium, can be monitored periodically in the recovery room. The osmolarity of the lavage solution exiting the abdomen should be tested to assure that the lavage solution hypoosmolarity is maintained during the 30 minute period. The intraperitoneal dialysis catheter could then be discontinued at the bedside postoperatively. Most gynecologic oncologists are already familiar with placing intraperitoneal catheters as preparation for intraperitoneal chemotherapy. We simply need to slightly modify the catheter to allow fluid instillation and removal from the abdominal cavity.

Conclusion

This study suggests that abdominopelvic lavage with water for 30 minutes killed a significant amount of human ovarian cancer cells *in vitro*. We plan to carry this investigation *in vivo* and, hopefully, to human study in the future.

Acknowledgments

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Author Contributions

Paulus-literature review, study design, data collection, data analysis, data interpretation, writing, critical revisions; Santoso-literature review, study design, data analysis, data interpretation, writing, critical revisions; Sims-data collection, data analysis, data interpretation; Patel-literature review, study design, critical revisions; Pfeffer-literature review, study design, data analysis, data interpretation, writing, critical revisions.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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