# Title and Subtitle
Evaluation of Interacavitary Chemotherapy Delivery for Treatment of Mammary Carcinoma

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## Abstract
This project evaluated paclitaxel chemotherapy delivery from a gel polymer system placed into a wound bed following conservative (marginal) surgical removal of human breast cancers grown in nude mice. This delivery method was shown to control local tumor disease as well as assist in control of systemic metastasis. We established 5 human breast cancer cell lines within our laboratory. We elected purchase and implement a unique (luciferase) imaging system which allows in vivo imaging of tumor growth and metastasis (and subsequently decrease animal use). Tumor cell lines were transfected with the luciferase gene. In vitro testing of cell lines established paclitaxel sensitivity and showed a synergistic effect of delivering paclitaxel by the poloxamer polymer, especially for the chemotherapy resistant cell line, MCF-7-ADR. We completed the simultaneous evaluation of local and systemic toxicity, local, regional and systemic distribution and local and systemic efficacy of locally delivered paclitaxel chemotherapy following tumor removal using the MCF-7-ADR cell line in nude mice. Intracavitary administration of taxol in poloxamer was well tolerated (locally and systemically) and resulted in significantly improved control of local tumor regrowth and comparable control of metastasis following marginal tumor removal as compared to intravenous paclitaxel (parent drug). Sustained drug levels (from polymer delivery) were seen in plasma and liver tissue at 60 days.

## Subject Terms
Breast Cancer, Chemotherapy, Delivery, Local Control, Mouse Model, Pre-clinical Evaluation, Toxicity, Efficacy
William S. Dernell DVM, MS

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Introduction: This proposal evaluated paclitaxel (taxol) chemotherapy delivery from a gel polymer system (poloxamer 407, Pluronic F127, BASF) placed into a wound bed following conservative surgical removal of human breast cancers grown in nude mice. This novel delivery method was shown to control local tumor disease as well as assist in control of metastasis and may offer a cost-effective alternative to adjuvant radiation therapy.

Background: Death rates in the United States from breast tumor (carcinoma) in 1998 were greater than 41,000 women. It is estimated that in the year 2001, over 192,000 new cases of breast carcinoma will be diagnosed in the United States alone, making it the leading cancer disease in women.(1) Treatment failure is due to local tumor recurrence, distant metastasis, or both (2). Breast conserving surgery is gaining popularity with the more frequent detection of smaller diameter breast cancers.(3-5) Because larger excisional margins often result in a poorer cosmetic result and the ability to achieve initially clear histologic margins intraoperatively is often difficult, adjuvant therapy has gained popularity and has shown benefit in both survival and local recurrence rates in a number of studies.(6) Therefore, conservative surgery is frequently followed by adjuvant radiation to the affected breast and often the draining lymph nodes.(2)

Local recurrence rates with breast conserving surgery alone are approximately 25.7% and with the addition of radiation, local recurrence rates are approximately 5.5%.(7) Most believe that recurrence occurring in the same quadrant as the index lesion is the result of residual disease left in the tumor bed after excision.(4,6,8-9) Most local recurrence in the absence of radiation is in the same quadrant and in close proximity to the original tumor bed.(10) Local recurrence has been associated with an increased risk of death.(11) Because local recurrence is a distressing event that dramatically affects quality of life, and is associated with an increased risk of distant metastasis and death, improved local-regional control is an important goal. There is a need to investigate novel ways of preventing local recurrence, especially for those patients for whom radiation therapy may not be suitable or readily available. Intracavitary chemotherapy; drug delivery into a wound cavity following conservative (microscopically incomplete) surgery may offer a novel adjuvant option.

In this day and age of cost consciousness in health care delivery, breast sparing with an adjuvant local chemotherapy that reduced or eliminated the need for follow-up radiation therapy has the potential to profoundly reduce financial burden on the health care system.(12,13) The selection of women with breast cancer who are in stage 0 (no measurable disease or 1 (local disease only) and fall within the grouping of Tis (small, in situ tumor) or T1 (small local tumor) would be appropriate for treatment with intracavitary chemotherapy after lumpectomy.(14) These women are most likely to benefit from conservative therapy as opposed to a more aggressive resection.(15-18) In addition to reducing the overall cost of therapy for women with breast cancer, the use of intracavitary chemotherapy can reduce the duration of the treatment process. Radiation is often delivered Monday through Friday over a six-week period whereas the polymer is implanted only once.

Local delivery of chemotherapy in people has been utilized for intraperitoneal...
carcinomatosis (19) and for brain tumors. (20, 21) Local drug delivery into a marginally resected tumor bed can dramatically increase local wound concentration of chemotherapy and has the potential to decrease local recurrence rates while eliminating standard whole body toxicities. Simplistically, if the tumor cells are close to the wound edges it is desirable to put the drug in maximal concentration at that site. Through the use of polymer delivery, high local and systemic drug concentrations, when compared to that achievable by standard intravenous administration, may be achieved. (22, 23) Other benefits of this approach include: 1. High local drug delivery may allow preferential accumulation of drug in regional lymph nodes. 2. Polymer delivery allows higher drug doses without systemic toxicity when compared to systemically administered drug. 3. Liquid polymer could be injected intraresionally in a percutaneous fashion much like interstitial radiation implants. 4. Various polymers can act as tissue conductive matrices and result in cosmetic breast prostheses for tissue ingrowth.

Hypothesis: Local delivery of paclitaxel chemotherapy following conservative surgery for breast tumor will be efficacious in controlling local tumor disease as well as impacting metastasis.

Body: Task (objective) 1 (proposed to be completed in year 1): To evaluate the efficacy of polymer delivered paclitaxel (taxol) chemotherapy against human breast tumor cell lines. As per task 1, we established 5 human breast cancer cell lines within our laboratory; MCF-7, MCF-7 AL, MDA-MB-435, MDA-MB-231 and MX-1. Sensitivity (LC5) to taxol as well as poloxamer 407 - taxol mixture (polotax) was determined in vitro in four human breast tumor cell lines: MCF-7, MCF-7/Adr, MDA-MB-231, and MDA-MB-435. MTS-assays (Celltiter 96 Aqueous one solution cell proliferation assay - Promega) in 96 well plates were used, each well received 100 ul of cell culture medium and 5,000 cells of the respective cell lines (Figure 1).
MCF-7 Cell Line

Paclitaxel Concentration (uM)

% Survival

MCF-7/Adr Cell Line

Paclitaxel Concentration (uM)

% Survival

Figure 1A (above)

Figure 1B (above)
Figure 1 (above).
Cell survival curves for 4 human breast tumor cell lines exposed to various concentrations of taxol and poloxamer-taxol (polotax) combination (24).

Figure 2 (above). Cell survival curves for 4 human breast cancer cell lines exposed to various concentrations of poloxamer 407 (Pluronic F127)(24).

Results of In Vitro Studies.
a) Taxol treatment alone produced LC50 values of 20μM, 10μM, 0.01μM, and 0.01μM for MCF-7, MCF-7/Adr, MDA-MB-231, and MDA-MB-435 respectively.
b) Treatment with polotax resulted in LC50 values of 0.1 µM, 0.000001 µM, 0.000001 µM, and 0.1 µM, respectively.

- Three of the four cell lines studied clearly demonstrate that paclitaxel delivered via Pluronic F-127 is more effective than the drug delivered alone.
- We hypothesize that the polotax data is the result of a synergistic relationship between the paclitaxel chemotherapy and the polymer delivery system.
- The pluronic F-127 itself had inherent cytotoxic effects, supporting previous investigator findings. This polymer also appears to sensitize cells to chemotherapeutics (Figure 2).
- Pluronic F-127 appears to sensitize the multidrug resistant (MDR) MCF7-Adr cell line to paclitaxel relative to the parental MCF7 cell line.
- Potential mechanisms of MDR cell sensitization by polymers could include:
  - Alteration of membrane viscosity
  - Abolish sequestration of drug in cytoplasmic vesicles
  - Depletion of ATP

To test the hypothesis of ATP depletion, luciferase transfected MCF-7-Adr cells were incubated with Pluronic F127, then exposed to luciferin. The reaction of luciferine with luciferase to yield light photons is ATP dependent. If Pluronic inhibited ATP, then a decrease in luciferin reaction should occur, dependent on the amount of pluronic exposure. Figure 3, demonstrates this relationship.

![ATP Depletion in MCF-7/Adr Cell Line](image)

**Figure 3** (above). Photon emission from luciferase transfected MCF-7-Adr cells exposed to luciferin after 24 hrs exposure to pluronic F-127 results in reduced photon emission at higher concentrations, suggesting the ATP depleting effect of pluronic F-127 (24).

We have elected to purchase and implement a unique luciferase imaging system (not in original proposal and not paid for using grant monies from this award) which allows in vivo imaging of tumor growth and metastasis. The following paragraph describes how this system is implemented:

Tumor growth is evaluated by IVIS technology. Briefly, animals are anesthetized by i.p. injection of 40 ul of a ketamine and xylazine (4:1) solution. An aqueous solution of the substrate luciferin (the substrate for luciferase, Molecular Probes, 50mM, 126mg/kg) is administered by intraperitoneal injection 5 min before imaging (25). Supine mice are then placed into a light-
tight specimen chamber mounted with the charge-coupled device (CCD)-camera cooled to -120°C. A gray-scale body-surface reference image is collected first followed by acquisition of the photons transmitted from the luciferase transfected cells in the mice. Using LIVINGIMAGE software (Xenogen), overlay of the pseudocolor image represents the spatial distribution of photon counts. Signal intensity is quantified as the sum of all detected photon counts within the region of interest after subtraction of background luminescence measured at shoulder level (26-28).

Use of this system allows evaluation of disease progression (and response to treatment) without the need for animal sacrifice until the final endpoints of the study. This will significantly decrease animal use. Use of this system requires transfection of the breast tumor cell lines with the luciferase gene. We successfully transfected the 5 breast tumor cell lines mentioned above with the luciferase gene prior to moving on to task 2.

**Tasks (objectives) 2-4:** To evaluate the local and systemic toxicity, the local, regional and systemic distribution and the local and systemic efficacy of locally delivered paclitaxel chemotherapy following tumor removal. These objectives are to be evaluated simultaneously in the in vivo portion of the study. Methods and preliminary results relating to tasks 2 and 3 will be discussed following the discussion of the methods and preliminary results of task 4.

Four cell lines (MCF-7, MCF-7/Adr, MDA-MB-231, and MDA-MB-435), identified as sensitive to taxol, were stably transfected with the luciferase reporter gene (Figure 4). When these luciferase transfected cancer cells are exposed to the substrate luciferin, they emit light photons. With the ultrasensitive CCD-camera of our new In Vivo Imaging System (IVIS, Xenogen), we can detect these bioluminescent signals emitted from the cancer cells inside the mouse on the outside of the body of a live mouse. With this technology we are able to detect quantitatively small numbers of tumor cells and follow these over several logs of cell growth. Data is integrated with LIVINGIMAGE software (Xenogen), and tumor size signal is expressed as total photons emitted.

**Figure 4 (below).** Luciferase imaging of cultured MCF-7-Adr (-luc) breast tumor cells.
MCF-7/Adr is a human breast adenocarcinoma cell line, in vitro selected for adriamycin resistance. MCF-7/Adr's express a high level of P-glycoprotein pumps. In vivo tumor growth displays a highly vascularized, aggressive pattern. In vitro data on MCF-7/Adr taxol exposure (cfr supra), indicated a remarkable synergistic effect with a $10^6$-fold increase in sensitivity of the cell line to taxol when delivered in combination with the poloxamer 407. We subcutaneously inoculated 70 nude mice with 1 million MCF-7/Adr cells in the left inguinal mammary fat pad. Tumor growth was monitored weekly by imaging of luciferase activity with a CCD camera and LIVINGIMAGE software and by caliper measurements. Animals with immediate peritoneal metastasis (3/70) were removed from the project. Only animals with localized primary tumor growth were considered. Primary tumors were allowed to grow to 3 different size ranges: 500-800 mm$^3$, 400-500 mm$^3$ and 300-400 mm$^3$. At time of primary (marginal) tumor resection, animals were randomly assigned to one of 5 treatments: a) polotax (200 ul of 22% poloxamer/5.4mg/ml taxol suspension) in wound, b) 200 ul polotax remote (between 2 scapulae), c) 200 ul 22% poloxamer in wound, d) 20 mg/kg taxol IV (200 ul of 400ug taxol, 1:2 dilution of 6mg/ml Taxol in cremophor in saline (Paclitaxel, Bristol-Myers Squibb) and e) no drug control. Mice were imaged for luciferase after surgery to evaluate for tumor remnant. Subsequently, mice were imaged on a weekly schedule to evaluate tumor regrowth and tumor metastasis. By means of this highly sensitive, non-invasive imaging method, one can image tumor dynamics over time within the same animal. In the initial proposal we planned to assess tumor regrowth at two time points (14 and 60 days), however with this new imaging modality we can assess tumor behavior on a weekly basis within the same animal, reducing the animal number for this study to half (Figure 5).
Figure 5.
Images of local tumor growth and pulmonary metastasis from mice implanted with MCF-7-Adr human breast tumor cells.

Images from Zenogen IVIS Camera

Mice injected with 3 mg luciferin (0.1 ml of 30 mg/ml) 15 minutes prior to image

Results of In Vivo Experiments.

Figures 6-9 show results of local tumor regrowth and metastasis as evaluated using the luciferase imaging system. Zero of 9 and one of 9 mice treated with polotax placed within the wound (intracavitary) following marginal tumor removal has shown local regrowth by 40 and 60 days post treatment, respectively (Figures 6 and 7). Five of 8 and Six of 9 mice treated with intravenous taxol (parent drug) have shown tumor regrowth by 40 and 60 days post treatment, respectively (Figures 6 and 7). Zero of 9 and one of 9 mice treated with polotax placed within the wound (intracavitary) following marginal tumor removal have shown distant metastasis at 40
and 60 days, respectively (Figure 8 and 9). Two of 9 and 5 of 8 mice treated with intravenous taxol have shown metastatic failure at 40 and 60 days, respectfully (Figure 8 and 9). This would support a benefit to local treatment with polotax over intravenous taxol. Polotax placed at a distant sight (not within the tumor wound) showed similar control of local tumor regrowth to systemically administered taxol (Figures 6 and 7). These effects were seen even with large primary tumors (prior to removal) as well as tumors removed at an earlier, smaller stage. (Figure 7)

In an attempt to repeat the in vivo efficacy experiments with additional cell lines, MCF-7-luc (non-Adriamycin resistant MCF cells, luciferase transfected) and MDA-MB-231-luc cells were orthotopically implanted in mice and growth monitored using luciferase imaging. Successful growth was established for both cell lines. Unfortunately, consistent growth of MCF-7 cells requires implantation of estrogen pellets which were not budgeted for. Additional funding is presently being sought to complete efficacy testing in these additional cell lines.

**Figure 6 (below).**
Overall Local tumor regrowth of MCF-7-Adr human breast tumor cells following marginal removal of primary tumor in nude mice after postoperative treatments (to 60 days following treatment).

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**Percent Tumor Regrowth in Mice**
**Post Surgery and Taxol Treatment**

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12
Figure 7A-C (below).
Local tumor regrowth of MCF-7-Adr human breast tumor cells following marginal removal of primary tumor in nude mice after postoperative treatments based on size of the tumor at the time of surgical removal (to 60 days following treatment).

Figure 7A (below)
Tumor Regrowth Post Surgery and Taxol Treatment in Mice with 300-400mm$^3$ Tumors

[Graph showing tumor regrowth post surgery and taxol treatment with data points at 14, 40, and 60 days post treatment for control, poloxamer only, polotax wound, polotax remote, and IV Taxol treatments.]
Figure 7B (below)

Tumor Regrowth Post Surgery and Taxol Treatment in Mice with 400-500mm³ Tumors

![Graph showing tumor regrowth percentages for different treatments and time points.]

- Control
- Poloxamer Only
- Polotax Wound
- Polotax Remote
- IV Taxol

Legend:
- 14 days post treatment
- 40 days post treatment
- 60 days post treatment

Figure 7C (below)

Tumor Regrowth Post Surgery and Taxol Treatment in Mice with >500mm³ Tumors

![Graph showing tumor regrowth percentages for different treatments and time points.]

- Control
- Poloxamer Only
- Polotax Wound
- Polotax Remote
- IV Taxol

Legend:
- 14 days post treatment
- 40 days post treatment
- 60 days post treatment
Figure 8 (below)
Tumor metastasis of MCF-7-Adr human breast tumor cells following marginal resection of local tumors in nude mice after postoperative treatments (to 60 days following treatment).

Percent Metastasis in Mice
Post Surgery and Taxol Treatment

Figure 9A-C (below)
Tumor metastasis of MCF-7-Adr human breast tumor cells following marginal resection of local tumors in nude mice after postoperative treatments based on size of the tumor at the time of surgical removal (to 60 days following treatment).
Figure 9A (below)

Percent Metastasis in Mice Over Time with Tumors 300-400mm³ at Time of Surgery and Treatment

- 14 days post treatment
- 40 days post treatment
- 60 days post treatment

Figure 9B (below)

Percent Metastasis in Mice Over Time with Tumors 400-500mm³ at Time of Surgery and Treatment

- 14 days post treatment
- 40 days post treatment
- 60 days post treatment
Figure 9C (below)

Percent Metastasis in Mice Over Time with Tumors > 500mm$^3$
at Time of Surgery and Treatment

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Experiment: test cell line
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Comment: 98 days post inj
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Figure 10A (above). Bioluminescence of orthotopic growth of MCF-7-luc breast tumor cells in nude mice.
Figure 10B (above). Bioluminescence of orthotopic growth of MDA-MB-231-luc breast tumor cells in nude mice.

Task 2

To evaluate the local and systemic toxicity of locally delivered (intracavitary; within the wound bed) paclitaxel chemotherapy following tumor removal.

Three of 12 mice treated with intracavitary polotax developed wound dehiscence and due to wound healing complications were sacrificed. Tissues have been obtained from all mice that have died or have been sacrificed and will be evaluated during year three for evidence of organ toxicity. Three of 12 mice treated with intravenous taxol died following injection from what appeared to be an anaphylactic reaction. This has been reported for taxol, specifically related to the carrier cremafor EL. Subsequent mice were pretreated with steroid and antihistamine to counteract this reaction. Histologic evaluation showed only mild to moderate inflammation at sites of polotax injection (Figure 11) or implantation (wound cavity). No organ toxicity was noted.
Task 3
To evaluate the local (wound bed), regional (lymphatic) and systemic (organ system) distribution of paclitaxel following intracavitary polymer delivery

Tissues (and serum) was obtained from sacrificed, treated mice and banked for analysis of drug levels. Paclitaxel was analyzed using LCMS (mass spectroscopy) following (tissue) extraction using established techniques. Figures 12 and 13 graphically demonstrate tissue and plasma levels at 60 days post treatment. Only these samples were shown to have measureable paclitaxel levels at this time point. Measurement of tissue and plasma levels at additional time points was not performed in this trial once the decision was made to utilize the luciferase imaging and decrease the need for animal sacrifice. Previous studies, however, using poloxamer delivery of paclitaxel have been performed and demonstrate the sustained nature of paclitaxel release and systemic distribution (Figure 14)
Figure 12 (above). Paclitaxel concentrations in the plasma of mice 60 days after no treatment (control), treatment with intravenous paclitaxel, treatment with polymer (poloxamer) delivered paclitaxel (polotax) or treatment with intracavitary paclitaxel in poloxamer polymer (polotax, placed in the wound following orthotopic breast tumor removal).
**Paclitaxel Concentration in Liver 60 Days Post Treatment**

(MCF7B-AdrL Tumors)

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<th>Polotax Wound</th>
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Significant difference between groups \( p = .013 \) (ANOVA)

**Figure 13** (above). Paclitaxel concentration in the liver of mice 60 days after no treatment (control), treatment with intravenous paclitaxel, treatment with polymer (poloxamer) delivered paclitaxel (polotax) or treatment with intracavitary paclitaxel in poloxamer polymer (polotax, placed in the wound following orthotopic breast tumor removal).

**Figure 14** (below). A. Plasma paclitaxel concentration over time following subcutaneous injection of paclitaxel suspended in poloxamer at 2 different dosages. B. Injection paclitaxel concentration over time following subcutaneous injection of paclitaxel suspended in poloxamer at 2 different dosages.
Key Research Accomplishments:

1. Establishment of 5 (commercially available) human breast tumor cell lines within our laboratory.
   a. MCF-7
   b. MCF-7 Adr
   c. MDA-MB-435
   d. MDA-MB-231
   e. MX1

2. In vitro cytotoxicity testing of these 5 cell lines for sensitivity to paclitaxel and polymer delivered paclitaxel. A synergistic effect was seen between paclitaxel and poloxamer polymer, supporting this delivery method to increase therapeutic advantage.

3. Transfection of cell lines with the luciferase gene to facilitate in vivo imaging.

4. In vivo growth of MCF-7 Adr cell line in nude mice. This represents a very stringent model for the evaluation of therapy as this cell line demonstrates chemotherapy (multiple) drug resistance.

5. In vivo growth of MCF-7 and MDA-MB-231 cell lines in nude mice.

6. Establishment of tolerability of poloxamer (locally and systemically) delivered paclitaxel in nude mice.

7. Establishment of efficacy of poloxamer locally delivered paclitaxel against local regrowth (following surgery) and distant metastasis of MCF-7-Adr breast tumors in nude mice.

8. Measurement of plasma and organ levels of paclitaxel following polymer delivery. Sustained release and systemic distribution of paclitaxel was shown following polymer (poloxamer) delivery.
Reportable Outcomes:


Dernell, WS. *Evaluation of lymphatic drainage and uptake following intracavitary chemotherapy administration for mammary carcinoma*. 7/1/03-6/30/04. Grant submitted to the **US Army Medical Research and Materiel Command (Concept Grant)**. $284,724 (direct costs). Not funded.

Dernell, WS. *Evaluation of lymphatic drainage and uptake following intracavitary chemotherapy administration for mammary carcinoma*. 7/1/03-6/30/04. Grant submitted to the **Pardee Foundation**. $284,724 (direct costs). Not funded.

Dernell, WS. *Evaluation of lymphatic drainage and uptake following intracavitary chemotherapy administration for mammary carcinoma*. 7/1/03-6/30/04. Grant submitted to the **US Army Medical Research and Materiel Command (Idea Grant)**. $75,000 (direct costs). Not funded.
Conclusions:
1. Four of the five established human breast cancer cell lines established in our laboratory have shown sensitivity to paclitaxel.
2. Paclitaxel delivered through poloxamer has shown a synergistic effect on cytotoxicity for the chemotherapy resistant cell line, MCF-7-Adr cell line. This synergism appears to be ATP dependent. Further research is warranted to evaluate (and substantiate) this mechanism.
3. Consistent growth has been established for the MCF-7-AdR cell line in nude mice, establishing this as a viable (yet stringent, drug resistant) model for chemotherapy testing. In vivo growth of additional cell lines has been established (MCF-7 and MDA-MB-231) as well.
4. Poloxamer delivered paclitaxel has shown significantly better control of local tumor regrowth and comparable control of metastasis following intracavitary treatment of marginally removed MCF-7-ADR tumors as compared to intravenous paclitaxel. This appears true for very large, established tumors as well as for early, smaller growth. This tumor control was more complete at 40 days and was somewhat reduced (increased failures) by 60 days post treatment.
5. Minimal control of local regrowth or systemic metastasis is seen following traditional intravenous administration of the parent drug, paclitaxel. The improved tumor control using poloxamer delivered paclitaxel (see 4 above) may reflect high local concentrations of drug as well as preferential uptake of drug/polymer within lymphatics; the first direction of metastatic spread. Funding for further research on lymphatic uptake of poloxamer delivered paclitaxel is presently being sought (see reportable outcomes above).
6. Mild to moderate local tissue reaction is seen following intracavitary treatment of poloxamer delivered paclitaxel, without clinical evidence of systemic toxicity. Histologically.....Mechanisms to improve tissue tolerance of the paclitaxel/polymer warrant further investigation.
7. Marked systemic toxicity can be seen following intravenous administration of the parent drug, paclitaxel. The reduced systemic toxicity using the poloxamer delivered paclitaxel offers a potential treatment advantage, especially if comparable or improved control of metastasis can be achieved.
8. Sustained levels of paclitaxel were evident in plasma and organs (liver specific) following polymer (poloxamer) delivered paclitaxel when compared to intravenous delivery. This sustained release and systemic distribution of drug may be responsible for the local and systemic efficacy seen using this treatment method in this model.
9. The decision to obtain and utilize the in vivo luciferase imaging system has resulted the decreased use of animals and an increase in sensitivity of monitoring tumor progression and metastasis.
Bibliography


**Personnel Supported by the Grant:**

William S. Dernell  
Principle Investigator

Annette Bachand  
Statistician

Laura Chubb  
Laboratory Supervisor

Susan Hudachek  
Graduate Student

Jeremy Cantlon  
Laboratory Assistant (hourly)

Gina Kwong  
Laboratory Assistant (hourly)

Scott Rizzo  
Laboratory Assistant (hourly)

Bamboo Dong  
Laboratory Assistant (hourly)
References:


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Appendices


In vitro evaluation of the efficacy of polymer delivered paclitaxel chemotherapy against human breast tumor cell lines. Scott Rizzo, Susan Hudachek, Alexandra De Lille, Bamboo Dong, William Dernell*. Animal Cancer Center, Colorado State University.

Despite innovative medical therapies and advances in the treatment of breast cancer, local tumor control is still problematic. The current standard of care calls for surgical resection followed by systemic chemotherapeutics and/or radiation therapy. Despite these treatments, local recurrence ranges from 5 to 26%. Maximal chemotherapy dosage is restricted by the toxic effects that can be tolerated systemically. Local delivery of chemotherapy following conservative surgery precludes systemic toxicity while still providing high local drug levels. Previous investigators have shown that controlled delivery of chemotherapeutic agents from polymeric materials directly into wounds after surgical resection provides for higher local tissue drug levels while avoiding systemic toxicity. The present experiment evaluates the efficacy of paclitaxel suspended in a 22% pluronic (poloxamer)F-127 gel (polotax) against human breast tumor cell lines in vitro. The LC50 of paclitaxel, and polotax was determined in three human breast tumor cell lines: MCF-7, MCF-7/Adr, and MDA-MB-231. Percentages of viable cells after treatment were determined relative to drug free controls, using the colorimetric CellTiter96® AQueous One Solution Cell Proliferation Assay (Promega). Paclitaxel treatment alone produced LC50 values of 20μM, 10μM, 0.01μM for MCF-7, MCF-7/Adr and MDA-MB-231 respectively. Treatment of the cell lines with polotax resulted in LC50 values of 0.1μM, 0.000001μM and 0.000001μM respectively. These results clearly indicate that paclitaxel delivered via a polymer is more effective than the drug delivered alone. In addition, the pluronic F-127 itself had inherent cytotoxic effects, supporting previous investigator findings. We hypothesize that the polotax data is the result of a synergistic relationship between the paclitaxel chemotherapy and the polymer delivery system. Current efforts are focused on exploring the possible synergistic mechanism between the pluronic F-127 and paclitaxel using flow cytometry. Future studies are planned to assess local and systemic efficacy of intracavitary, polymer-delivered paclitaxel for the treatment of the above mentioned tumor cell lines xenografted into nude mice. Cell lines are to be transfected with the luciferase gene. Using the IVISTM Imaging System (Xenogen), we are able to monitor the growth of tumors expressing the luciferase gene and the response to treatment of these tumors following conservative surgical resection and local polotax therapy. This innovative technology allows a non-invasive, quantitative method for evaluating local tumor growth and metastasis in vivo over time, ultimately allowing us to assess the local and systemic efficacy of paclitaxel chemotherapy delivered using the pluronic F-127 gel polymer system.

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EVALUATION OF INTRACAVITARY CHEMOTHERAPY DELIVERY FOR THE TREATMENT OF MAMMARY CARCINOMA

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Introduction: The purpose of this study was to evaluate tolerability and efficacy of local (intracavitary) delivery of paclitaxel from a gel polymer (poloxamer 407) following marginal (histologically incomplete) resection of mammary carcinoma in a mouse model.

Methods: In vitro sensitivity to paclitaxel as well as poloxamer-taxol mixture (polotax) was determined for 4 human breast tumor cell lines using the MTS-assay (Promega). Nude mice were then injected with MCF-7/ADR (Adriamycin\textsuperscript{R} resistant) cells. Tumor growth was monitored by imaging of luciferase activity with a CCD camera (IVIS system, Xenogen). Primary tumors were allowed to grow to 3 different size ranges. At the time of primary tumor resection, animals were randomly assigned to treatment groups comparing intracavitary (in the wound) polotax to intravenous paclitaxel. Mice were imaged weekly to evaluate tumor regrowth and metastasis.

Results: All cells lines demonstrated sensitivity to paclitaxel and three of the four cell lines demonstrated improved cytotoxicity with polotax compared to drug delivered alone. One of 9 mice treated with polotax had local regrowth (by 60 days) compared to 6 of 9 mice treated with intravenous paclitaxel. One of 9 mice treated with polotax had distant metastasis at 60 days compared to 5 of 8 mice treated with intravenous paclitaxel. These effects were seen with tumors of all sizes.

Discussion/Conclusions: Poloxamer delivery of paclitaxel appears to result in improved efficacy compared to paclitaxel alone. Improved local and systemic control of mammary carcinoma is seen following intracavitary poloxamer delivery of paclitaxel compared to paclitaxel alone in this mouse model.
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Objective/Introduction: The purpose of this study was to evaluate tolerability and efficacy in controlling local tumor regrowth and metastasis using local (intracavitary) delivery of paclitaxel from a gel polymer (poloxamer 407) following marginal (histologically incomplete) resection of mammary carcinoma in a mouse model.

Study Design/Procedure/Sample Population: In vitro sensitivity to paclitaxel as well as poloxamer-taxol mixture (polotax) was determined for 4 human breast tumor cell lines using the MTS-assay (Promega). Nude mice were then injected with MCF-7/ADR (Adriamycin® resistant) cells. Tumor growth was monitored by imaging of luciferase activity with a CCD camera (IVIS system, Xenogen). Primary tumors were allowed to grow to 3 different size ranges. At the time of primary tumor resection, animals were randomly assigned to treatment groups comparing intracavitary (in the wound) polotax to intravenous paclitaxel. Mice were imaged weekly to evaluate tumor regrowth and metastasis.

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Discussion/Conclusions: Poloxamer delivery of paclitaxel appears to result in improved efficacy compared to paclitaxel alone. Intracavitary polymer delivery of paclitaxel appears to be clinically tolerated. Improved local and equivalent systemic control of mammary carcinoma is seen following intracavitary poloxamer delivery of paclitaxel compared to paclitaxel alone in this mouse model.

Relevance to Breast Cancer: Breast-sparing surgery with an adjunctive local chemotherapy that shows improved local tumor control may reduce the need for follow-up radiation therapy and has the potential to profoundly reduce care costs as well as decrease patient morbidity. Follow-up studies may be warranted to compare intracavitary chemotherapy to radiation following conservative surgery.
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