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Evaluation of Cisplatin in Combination with a Biologic Response Modifier in a Murine Mammary Carcinoma Model

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ABSTRACT

The purpose of this study was to determine the efficacy of intracavitary cisplatin against local regrowth and metastasis after resection of a murine mammary carcinoma and the ability of a biologic response modifier (Virulizin®) to enhance chemotherapy. C3H-HeJ mice were injected with Gollin-B tumor cells. Once growth reached 8 mm, tumors underwent marginal resection and the mice were assigned randomly to intraperitoneal (IP) cisplatin, Virulizin, a controlled release cisplatin-impregnated sponge (OPLA–Pt), a combination of treatments or no treatment and were evaluated for local regrowth, metastasis, and toxicity at 14 or 60 days after surgery.

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A possible beneficial interaction was seen between OPLA–Pt and Virulizin at 14 days. All cisplatin groups had significant advantages over controls in all variables measured with OPLA–Pt displaying significant advantages over IP cisplatin in local recurrence rate, tumor score, survival time, and delay in regrowth at 60 days. No toxicity related to either cisplatin or Virulizin was observed.

Key Words: *Carcinoma; Virulizin; Cisplatin; Controlled release*

INTRODUCTION

Randomized clinical trials have demonstrated that breast-conserving surgery (BCT) plus radiation therapy (RT) is an alternative to mastectomy for most women with early stage breast cancer (clinical stage I and II), and that survival and recurrence rates are comparable (1–4). Furthermore, other randomized clinical trials have clearly demonstrated that the use of RT following BCT substantially reduced the risk of local recurrence (4,5). Most of the local recurrences in the ispilateral breast, either after mastectomy or BCT, seem to be related to the presence of residual cancer or positive axillary lymph nodes (6).

The rate of local relapse with BCT and RT is still considerable, with values reported from 2 to 21% for five years (1,4,5,7–12). 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 (13), 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 RT may not be suitable or readily available.

We have evaluated an intracavitary chemotherapy system comprising a biodegradable polymer containing 8% cisplatin by weight (open cell polylactic acid–platinum, OPLA–Pt). The OPLA–Pt system has been demonstrated in dogs to have lower systemic toxicity than seen with an equivalent dose of intravenous cisplatin, while simultaneously achieving a local, intracavitary concentration (in the tumor bed) as much as 50 times that achieved by the peak intravenous level (14). This cisplatin delivery system was used in clinical patients in the treatment of canine nasal tumors (as a radiation sensitizer) (15) and soft tissue sarcomas (16). In addition, it has been used in a preliminary study on the efficacy of OPLA–Pt in a murine mammary carcinoma model (17). A decrease in local recurrence rates was observed when OPLA–Pt was placed in the wound cavity following incomplete resection of mammary tumor. Control mice that received no treatment following incomplete resection had 100% local tumor regrowth. No clinically significant renal, bone marrow, or neural toxicity was noted. In addition, OPLA–Pt has also been used as an adjuvant to RT in the treatment of canine nasal tumors as low sustained doses of cisplatin were shown to enhance the radiation-induced cell killing in several tumor types (15,18,19).

Considering the numerous advantages of OPLA–Pt, it is possible that this method of local drug delivery would be effective in decreasing the local recurrence rate after BCT. Rodent models continue to be important in defining both the etiology of breast cancer and in generating new prevention and treatment strategies against this prevalent tumor type. Although cisplatin is not commonly used for the treatment of breast cancer in women, it was chosen in this study due to it's presence within the polymer (OPLA–Pt) system. In addition, the goal of this study was to evaluate the principle of local (intracavitary) chemotherapy on local tumor regrowth. Pilot work demonstrated efficacy of the OPLA–Pt following intracavitary use within this mouse tumor model (17). Furthermore, the potential of immunotherapy, using a biological response modifier, to enhance the efficacy of either OPLA–Pt or cisplatin given intraperitoneally was examined. The biological response modifier (Virulizin®, Imutec Pharma, Scarborough, Ontario, Canada), is a bovine bile-derived extract that appears to be capable of stimulating the immune system to eliminate cancer cells. It is a potent activator of macrophages, as measured by cytokine release and tumoricidal activity against various cell lines in culture. In addition, Virulizin demonstrated inhibition of pancreatic tumor xenografts in mice and appeared to augment gemcitabine chemotherapy (20). Moreover, it is now approved for use in Mexico for the treatment of pancreatic cancer. The product is also being evaluated in human clinical trials in the United States and Canada (21).

METHODS

Animals

Test animals included 180 C3H-HeJ virgin female mice, 4–6 weeks old with a mean weight of 21 g. They were housed in an AALAC-approved facility in cages of five animals each and kept in a temperature-controlled environment with 12 hr cycles of light and darkness. They were fed with standard rodent chow and water *ad libitum*. All the procedures were approved by the Colorado State University Animal Care and Use Committee.

Cell Line

The mice were inoculated with murine mammary carcinoma (MTG-B) by subcutaneous injection into the mammary fat pad of the left flank. The MTG-B cell line is a murine mammary adenocarcinoma characterized by being locally aggressive (volume doubling time less than 48 hr) and metastatic (22–25). This tumor cell line is easily transplantable in non-immunocompromised mice, such as the C3H-HeJ strain. Previous work demonstrated a 100% rate of regrowth following intracapsular (marginal) tumor removal (17). The MTG-B cell suspension was derived from tumors excised from passage mice. The tumors were digested using collagenase, filtered, supported in tissue culture media, and a viability stain (trypan blue) was used to determine an approximate number of live cells. A saline cell suspension was created to result in an inoculation of 1.0×10^6 of MTG-B tumor cells per mouse in a volume of 0.125 mL. An equal volume of a mouse sarcoma derived basement membrane growth promoter (Matrigel®, Becton Dickinson Labware, Bedford, MA) was mixed with the tumor cell suspension to ensure optimal tumor growth (26). Previous work demonstrated consistent growth with 5×10^5 to 1×10^6 tumor cells injected in saline alone, however, the addition of the Matrigel resulted in a narrower growth time frame (unpublished data).

A flow cytometry evaluation was performed on mammary mouse tumors after they were surgically excised from control mice in order to evaluate the tumor cell kinetic parameters. These parameters included the DNA index, labeling index, the duration of the S phase, and the potential doubling time. Bromodeoxyuridine was injected intravenously 2–4 hr prior to excision of the tumor. Single cell suspensions were obtained by mincing the tumor samples and disaggregating the cells in phosphate-buffered saline using a Stomacher® (VWR, Denver, CO). The cells were then filtered, counted, fixed in 50% ethanol/citric acid-buffered saline, and stained. Flow cytometry was performed on an EPICS V cytometer (Coulter, Hialeah, FL).

Once inoculated, the mice were examined daily, the injection site palpated, and any growth measured. Three standard measurements were taken using a Vernier caliper. When the tumor growth reached 8 mm in any direction, surgery was performed. Typically, this occurred 5–7 days after inoculation. General anesthesia was induced and maintained with isoflurane and oxygen. Surgical sites were clipped and aseptically prepared. The tumors were marginally excised (histologically incomplete). However, an attempt was made to remove all grossly evident tumor. The tumors were palpably circumscribed, and resection on the pseudocapsule was generally straightforward. Previous work demonstrated a 100% rate of regrowth following similar surgical removal. The skin was closed in all mice with 3–0 nylon in a continuous pattern.

At the time of the surgery the mice were randomized into six different treatment groups of 30 animals each. *Group 1* (cisplatin) received intraperitoneal (IP) cisplatin (Platinol® Bristol Myers-Squibb, New Brunswick, NJ) at 5 mg/kg (17–19); *group 2* (OPLA–Pt) was implanted with the OPLA–Pt at 5 mg/kg within the wound bed; *group 3* (OPLA–Pt/Virulizin) received OPLA–Pt once and Virulizin injected IP at a dose of 0.5 mL, once at the time of surgery and the second time 7 days, post-operatively; *group 4* (cisplatin/Virulizin) was injected with IP cisplatin and IP Virulizin, given under the same dosing regimen; *group 5* (Virulizin) received Virulizin alone (given under the same dosing regimen); and *group 6* (control) received no treatment. The redosing schedule was chosen to mimic the clinical situation of induction chemotherapy without maintenance therapy, which is commonly employed in the treatment of solid tumors.

The mice were evaluated daily and the regrowth was measured and recorded. Since differences in treatment effects might not be seen early in the trial, the mice were randomized into two follow-up groups (short term—14 days and long term—60 days) of 15 animals from each group. Following sacrifice, a complete necropsy was performed to evaluate histologically the presence of metastasis and local tumor recurrence as well as any sign of toxicity due to cisplatin, Virulizin, or OPLA–Pt. The evidence of tumor (macroscopic and microscopic) in any organ (liver, lung, spleen, heart, abdomen, surgical site, kidney) was recorded and described for each mouse.

Therapeutic Agents

Biodegradable Delivery System

The OPLA–Pt devices used in this study were composed of homogeneous D,L-polylactic acid, within which cisplatin is incorporated at a concentration of 2.0% (w/w). The polymer/drug construct is a porous body (apparent density $\sim 0.0504–0.0552\,\text{g/cm}^3$), with an internal architecture of partially closed, randomly sized, shaped, and positioned intercommunicating interstices. All structural partitions are incomplete, providing (i) unrestricted communication between all internal void chambers and (ii) multiple avenues of communication from any void within the device to any location on the unit's surface. The void partitions are fenestrated with additional porosities that are $1–5\,\mu\text{m}$ in diameter. All partition surfaces are rendered hydrophilic to encourage free flow of blood and interstitial fluids throughout the unit's internal network of spaces. The OPLA–Pt devices were sterilized by exposure to $2.5–3.0\,\text{Mrads}$ of gamma radiation. Post-radiation polymer profiles were determined by gel permeation chromatography, with $M_\text{w} = 160,480$ and a polydispersity of 1.51 (average of two determinations).

Biological Response Modifier

Virulizin was obtained from Imutec Pharma (now Lorus Therapeutics). Virulizin is a bovine bile-derived extract that appears to be capable of stimulating the immune system to eliminate cancer cells. It is a potent activator of macrophages, as measured by cytokine release and tumoricidal activity against various cell lines in culture. In addition, Virulizin demonstrated inhibition of pancreatic tumor zenografts in mice and appeared to augment gemcitabine chemotherapy (20) and is now approved for use in Mexico for the treatment of pancreatic cancer. The product is also being evaluated in human clinical trials in the United States and Canada. It has been evaluated in over 450 patients for treatment of pancreatic cancer and Kaposi's sarcoma, with a very low toxicity profile (21).

Statistical Analyses

Separate analyses were performed for the short- and long-term group. Tumor regrowth, metastasis, and histological tumor score were observed. The scoring system used to classify the tumor regrowth was based on histological criteria. A score of 0 meant no recurrence of tumors, a score of 1 was given to tumors that were characterized by scattered, individual cancerous cells. When a small mass (2 mm) of tumor cells was identified, a score of 2 was given, and a score of 3 indicates a large mass (3–5 mm) with necrosis. Survival time (time from the surgery until the sacrifice) and delay time (days from treatment until regrowth) were also statistically analyzed.

Significance of the effect of the treatment and Virulizin on the Kaplan–Meier product limit estimates of delay and survival time were assessed using the Lifetest procedure in SAS. Strata were defined by the treatment and Virulizin groups. Significance tests included the log-rank, Wilcoxon, and likelihood ratio tests. These three significance tests (the log-rank, Wilcoxon, and likelihood ratio tests) are reported in this study because they emphasize different parts of the survival curve. The Wilcoxon test puts greater emphasis on differences earlier in the event history. The log-rank test puts greater emphasis on differences later in the event history. The likelihood ration test has an even stronger emphasis on the latter part of the event history. The significance of the effects of the treatment (cisplatin or OPLA–Pt) and Virulizin on the percentage of occurrence of regrowth, metastasis, and histological tumor score were assessed using logistic regression via the CATMOD procedure in the SAS software package.

Toxicity and Pharmacokinetic Evaluation

Following the efficacy trial, two groups of 21 four to six week old female C3H-HeJ mice each were implanted with 1.0×10^6 MTG-B mouse carcinoma cells within the inguinal fat pad. Following the growth of 8 mm diameter (in any plane), 10 and 15 mg/kg of 2% (w/w) cisplatin within an open cell polylactic acid polymer (OPLA®, THM Biomedical, Duluth, MN) was implanted within the wound bed of each group, respectively, following marginal resection of the tumors. Seven mice each were sacrificed at 7, 14 and 21 days, and blood and serum were obtained for complete blood count, serum urea nitrogen (SUN), and platinum concentration by atomic absorption spectroscopy. A full necropsy was performed and tissues submitted for histological evaluation, following standard preparation and staining with hematoxylin and eosin. All the tissues were reviewed by a single pathologist (BEP).

RESULTS

Flow cytometry studies of the tumors showed that all of the tumors studied were aneuploid, with a DNA index

of 1.6–1.63. The labeling index varied from 0.15 to 0.27, with a mean of 0.2. Duration of the S phase ranged from 7.4 to 12.3 hr, with a mean value of 9 hr. Finally, the potential doubling time ranged from 1.8 to 2.7 days, with a mean of 2.1 days.

Short-Term Treatment Groups

The results of the short-term group (14 days) are summarized in Table 1. Four of the mice were not included in the study due to injection of tumor into the body wall. No death was associated with the surgical procedure. Furthermore, no clinical or histological toxicity to cisplatin or Virulizin was seen. Thirteen animals (15%) were sacrificed before the predetermined endpoint because of weight loss presumed to be due to cancer cachexia. All mice from groups 2 and 3 had local microscopic reactions to the OPLA–Pt implant. Six of the 29 in these two groups (21%) also showed local reactions (inflammation, swelling) that did not affect the outcome of the treatment. The results observed in the short-term group suggested possible interaction between Virulizin and OPLA–Pt. In many instances, the frequency of the events of interest (regrowth, metastasis, etc.) was low. However, with such a short duration for this arm of the study, differences between groups may not have been fully expressed.

Local Recurrence

The application of significance tests among all groups was not possible because all of group 5 displayed local recurrence within 14 days. However, an empirical observation suggested that the administration of Virulizin had some positive effects on chemotherapy. Most notable were the differences in percentage of regrowth for group 3 vs. group 2. These results suggest a possible beneficial interaction between OPLA–Pt and

Virulizin early in the course of the disease, where immunotherapy was given on a weekly basis.

Overall, differences among treatments (cisplatin, OPLA–Pt, and control) were significant ($p < 0.001$). Groups 1 and 2 (cisplatin and OPLA–Pt groups) had a significantly smaller number of cases of regrowth than the control animals ($p < 0.001$), but were not different from each other ($p = 0.436$). The difference between the OPLA–Pt groups containing Virulizin and those without Virulizin approached significance ($p = 0.170$), whereas, significant differences between IP cisplatin, with and without Virulizin, were not observed.

Metastasis

The overall rate of metastasis was 4.65%. Therefore, the low expected frequencies invalidated tests of significance. Numeric differences between all groups were small, which suggested that they were likely due to chance alone.

Tumor Score

For this histological variable, the application of the significance tests was not possible because of zero frequencies in some groups. However, the data suggests that Virulizin administration may have interacted with cisplatin. Of particular interest is the difference in tumor scores for group 3 vs. group 2. These results suggested a beneficial interaction between OPLA–Pt and Virulizin early in the course of the disease, especially since the duration of the study coincided with the length of Virulizin treatment.

Overall, differences between the treatment groups were significant ($p < 0.001$) with the cisplatin and OPLA–Pt groups having significantly smaller tumors than control ($p < 0.001$), but were not significantly different from each other ($p = 0.316$). Differences

Table 1

Local Tumor Regrowth and Metastasis Rates for the Short-Term Treatment Groups

Treatment Groups	Group 1 Cisplatin (IP), n = 14	Group 2 OPLA–Pt, n = 14	Group 3 OPLA–Pt/Virulizin, n = 15	Group 4 Cisplatin/Virulizin, n = 15	Group 5 Virulizin, n = 15	Group 6 Control, n = 15
Local regrowth	8	10	3	7	15	13
Metastasis	0	1	1	0	2	1
Median survival time (days)	14	14	14	14	12	14
Median tumor score	1	1	0	1	3	3

between groups with and without Virulizin were not statistically significant ($p = 0.379$).

Survival Time

Survival time is defined as the time from surgery until the planned sacrifice date. Eighty-four percent of all subjects survived for 14-days duration of the short-term study. All subjects survived for at least 11 days. This minimal variation in response meant that differences between animals in each of the groups were minimal. Therefore, comparisons between groups were not informative.

Delay Times in Tumor Regrowth

Differences in regrowth times between treatment groups were significant ($p < 0.001$ for log-rank, Wilcoxon, and likelihood ratio tests). The control group had a tumor-regrowth delay time shorter than for either the cisplatin or OPLA–Pt groups. More notably, delay time was significantly longer for OPLA–Pt than for cisplatin ($p < 0.001$ for each of the log-rank, Wilcoxon, and likelihood ratio tests). Differences between the control group and the one given Virulizin alone were likely due to chance alone ($p = 0.375$, 0.283, 0.409 for the log-rank, Wilcoxon, and Likelihood ration tests, respectively).

Long-Term Treatment Groups

The same number of animals were used in the long-term study as in the 14 day groups described above. However, two mice were not included in the long-term study due to injection of tumor cells into the body wall, as determined by post-mortem examination. One death was associated with hemorrhage after surgery. No clinical or histological toxicity due to IP cisplatin

(0.5 mg/kg) or IP Virulizin was seen. Sixty-three mice (72.4%) were sacrificed before the end of the study because of probable cancer cachexia. Most of them belonged to groups 5 and 6. All the mice from group 2 and 3 had histological local reactions to the OPLA–Pt implant. Seven of the 30 mice (23%) in the OPLA–Pt group also displayed evidence of local reactions (infection, swelling). The data for the long-term group are summarized in Table 2.

Local Recurrence

The interaction of Virulizin with chemotherapy was not significant ($p = 0.947$). However, note that the Virulizin treatment was discontinued after the second week of the study. It was not possible to obtain the p values for significance of the differences between all the three treatments (OPLA–Pt, cisplatin, and control) because of the 100% regrowth rate for the control animals. This meant there was no variation in response for that category, making it impossible to compute standard errors and p values. The difference in the rate of recurrence between OPLA–Pt and the cisplatin treatment groups was highly significant ($p < 0.001$), with OPLA–Pt being more effective at preventing local recurrence. The recurrence rate in OPLA–Pt treated subjects was half that of the cisplatin-treated subjects.

Metastasis

The interaction of Virulizin with cisplatin (either cisplatin or OPLA–Pt) was not significant ($p = 0.195$). Both the OPLA–Pt and cisplatin groups had significantly lower metastasis rates than control ($p < 0.001$). Although the rate of metastasis was lower in cisplatin than in OPLA–Pt groups, this difference was not significant ($p = 0.404$).

Table 2

Local Tumor Regrowth and Metastasis Rates for the Long-Term Treatment Groups

Treatment Groups	Group 1 Cisplatin (IP), n = 14	Group 2 OPLA–Pt, n = 15	Group 3 OPLA–Pt/Virulizin, n = 15	Group 4 Cisplatin/Virulizin, n = 15	Group 5 Virulizin, n = 13	Group 6 Control, n = 15
Local regrowth	10	5	7	13	13	15
Metastasis	2	2	6	3	7	10
Median survival time (days)	41	60	43	37	18	16
Median tumor score	3	0	0	3	3	3

Tumor Score

The interaction of Virulizin with either chemotherapy was not significant ($p = 0.998$). Two of the treatment methods (OPLA–Pt and OPLA–Pt/Virulizin) markedly reduced the median tumor score. In addition, animals in the OPLA–Pt-treated group had significantly greater frequencies of lower tumor scores than did those treated with IP cisplatin ($p < 0.001$).

Survival Time

Differences between the treatment groups were significant ($p < 0.001$ for log-rank, Wilcoxon, and likelihood ratio tests). The control group had shorter survival times than either the cisplatin or OPLA–Pt groups. More notably, survival times were significantly longer for OPLA–Pt than for cisplatin groups ($p = 0.012$, 0.042, and 0.020 for the log-rank, Wilcoxon, and likelihood ratio tests, respectively). Differences in survival time for groups containing Virulizin were not significant ($p = 0.285$, 0.356, and 0.358 for the log-rank, Wilcoxon, and likelihood ratio tests, respectively). Figure 1 shows Kaplan–Meier survival curves of survival time estimated by the treatment group. They indicate that not only is there a clear advantage of treatment over control groups, but that OPLA–Pt is superior to IP cisplatin in extending survival time.

Delay Times in Tumor Regrowth

Differences between treatment groups were significant ($p < 0.001$ for log-rank, Wilcoxon, and likelihood ratio
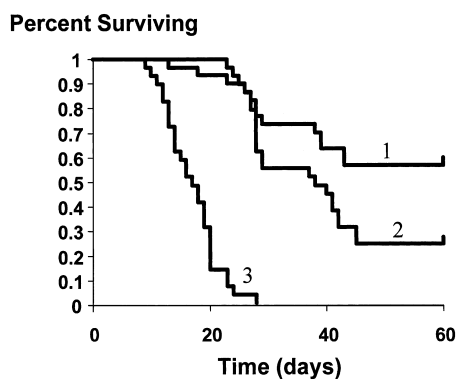

Percent Surviving

Figure 1. Kaplan–Meier survival function estimates for survival time by treatment in the long-term (60 days) group. Significantly prolonged survival was evident in mice treated with OPLA–Pt within the resection wound (1) and mice treated with intraperitoneal cisplatin (2) compared to mice not treated (3) following marginal tumor removal.
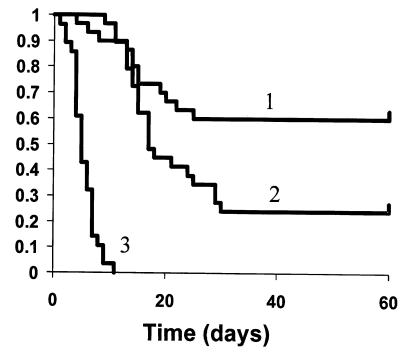

Percent Growth Delay

Figure 2. Kaplan–Meier survival function estimates for tumor growth delay time by treatment in the long-term (60 days) group. Significantly delayed tumor regrowth was evident in mice treated with OPLA–Pt within the resection wound (1) compared to mice treated with intraperitoneal cisplatin (2) or the mice not treated (3) following marginal tumor removal.

tests). The control group had shorter delay than cisplatin or OPLA–Pt groups. More notably, delay times were significantly longer for OPLA–Pt than for cisplatin ($p = 0.014$, 0.041, and 0.002 for the log-rank, Wilcoxon, and likelihood ratio tests, respectively). Differences between groups given Virulizin were not statistically significant ($p = 0.392$, 0.542, 0.417 for log-rank, Wilcoxon, and likelihood ratio tests, respectively). Figure 2 shows Kaplan–Meier survival curves of tumor regrowth-delay time estimated by the treatment group. Again, OPLA–Pt exhibits a significantly greater delay than cisplatin, which is better than the control group.

Toxicity and Pharmacokinetic Evaluation

Clinical toxicity from any of the therapies was not observed, in either the short- or long-term groups, as all the mice continued to eat, drink, and behave normally. Following this initial trial, blood analysis (Pt levels, complete blood count, and blood urea nitrogen) was done in a separate group of mice implanted with OPLA–Pt at 10 and 15 mg/kg. In these two groups, neutropenia was seen, yet changes in kidney-associated values were not observed. The platinum serum levels were similar, independent of dose. Peak serum platinum levels of 0.7 and 0.3 μg/mL appeared at day 7 and tapered to day 21; 0.3 and 0.1 for the 15 and 10 mg/kg groups, respectively. Kidney toxicity was also histologically evaluated after the mice were sacrificed and no evidence of damage was seen.

DISCUSSION

The aim of this study was to investigate alternative methods of decreasing local recurrence and metastasis rates following conservative breast cancer surgery in a rodent model. Most recurrences in breast tissue after conservative surgery and RT are at or near the site of the primary tumor (23). Patients appear to have a better prognosis when the incidence of local recurrence is less after mastectomy (27). The reason may be due to the fact that local relapse after BCT usually develops from residual foci of primary disease rather than from metastatic spread (27). An intracavitary delivery system of chemotherapy placed directly in the wound bed could be effective against microscopic tumor disease either when used alone or in combination with immunotherapy or even radiation.

The OPLA–Pt polymer system used in this study is able to release high concentrations of the chemotherapeutic agent (cisplatin) over time in the tumor excision bed (14). Furthermore, there is systemic absorption of cisplatin, so that the serum levels are high enough to be effective against micrometastatic disease, at least in dogs with osteosarcoma (28). In the murine mammary carcinoma model used in this study, the tumors were excised marginally so that microscopic tumor foci were left in the wound bed to simulate a conservative surgical procedure with residual disease. The high recurrence rate seen in most of the mice after surgery demonstrates that tumor excision was marginal. Moreover, this adenocarcinoma cell line has been shown to be locally aggressive (18–20). Flow cytometry data indicate that it has a potential doubling time of 2 days and displays a high-labeling index. These results imply that the tumor was obtained from the same clonogen and that there was a consistent cell line implanted in each mouse.

The results of this study showed that the OPLA–Pt intracavitary chemotherapy system has a positive effect against local tumor recurrence, tumor metastasis, and survival time. It is also successful in delaying the time of tumor regrowth. Both cisplatin given IP and local OPLA–Pt treatments result in significantly greater efficacy over the control group in all variables measured. In addition, OPLA–Pt had significant advantage over IP cisplatin in recurrence rate, tumor score, survival time, and tumor regrowth-delay.

Interestingly, the tumor recurrence rate in mice that received OPLA–Pt was higher in the short-term group than in the long-term group. This marked difference in tumor recurrence rate (71 vs. 33%) and the median tumor score (which was 1 in the short-term group vs. 0 in the long-term group) could be due to a delay in the rate of tumor death, with histological evidence of tumors being observed at 14 days that would eventually succumb to cisplatin. Conversely, since OPLA–Pt is left at the surgical site, it may result in a delayed increase in the local platinum concentration in the tumor bed, resulting in more effective control of regrowth that manifests itself over a longer time course (>14 days).

Overall, the results from the short-term group were less informative than those from the long-term group. In many instances, differences in events of interest (local recurrence, metastasis, etc.) were small, and might not have been fully expressed. However, the short-term group was quite informative regarding the interaction of Virulizin and cisplatin. While there was little evidence that Virulizin given by itself was effective in this model, there was a marked decrease in local recurrence in the short-term group by administering Virulizin along with OPLA–Pt (decrease from 71 to 20%; $p = 0.017$). This effect was lost in the long-term group (Table 2). This may be due to discontinuance of the Virulizin therapy after the second week of the study. In a previous study with pancreatic tumor zenografts, significant growth inhibition was noted with Virulizin alone when given daily for 14 days. Similarly, in that study, Virulizin potentiated the chemotherapy agent gemcitabine (20). These data indicate that immunotherapy with Virulizin may be a useful adjuvant to intracavitary chemotherapy. However, no such effect was observed with IP cisplatin and Virulizin, suggesting that systemic administration of cisplatin may interfere with the effects of Virulizin.

In both the short- and long-term groups, cisplatin was effective at diminishing local regrowth and preventing metastasis, with OPLA–Pt being superior to IP cisplatin. Coupled with the lower incidence of adverse reactions and toxicity, this work indicates that OPLA–Pt may be a viable treatment option following tumor excision.

The choice of using cisplatin in this study was made because of previous experience with OPLA–Pt (14,16,17,28). It is recognized that this particular chemotherapeutic agent is not the most effective systemically administered drug against breast cancer. In addition, the OPLA–Pt was not used at the maximum tolerated dose based on the subsequent dose escalation study. The results may have been different if higher dosages of the OPLA–Pt had been used. However, the goal of the study was to evaluate whether an intracavitary or local approach to chemotherapy could be effective against local tumor relapse in a breast tumor model. It suggests that such a delivery system using different chemotherapy agents, such as paclitaxel, may be even

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more effective against mammary tumors. Such studies are currently under investigation.

In addition, the evaluation of intracavitary chemotherapy in rodent models that more closely mimic human breast carcinoma or large animal spontaneous mammary tumor may be indicated. Dogs are considered to be a valid tumor model system for a comparison with humans (29). They develop tumors twice as frequently as in women and some canine malignancies (i.e., mammary tumors) have histopathological and biological behavior similar to human tumors (30). Most canine tumors progress at a more rapid rate than in people, making it easier to accumulate experimental data in a relatively short period. Moreover, one could determine whether significant platinum levels could be reached in regional lymph nodes after OPLA–Pt implantation, providing the additional benefit of treating tumor cells that have spread to regional lymph nodes. It has been observed that local recurrence is seen in about 25% of patients if there is involvement of axillary nodes; whereas if there is not, local recurrence is seen in only about 5% of patients (23).

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