Sustained Release Chemotherapeutic Microspheres Provide Superior Efficacy over Systemic Therapy and Local Bolus Infusions

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Purpose. The present studies evaluated the ability of injectable, biodegradable microspheres releasing carboplatin, doxorubicin, or 5-fluorouracil to suppress the growth of solid tumors implanted subcutaneously or intramuscularly.

Methods. Seven to 10 days after implantation of MATB-III cells, rats received systemic chemotherapy, intratumoral bolus chemotherapy, or injections of chemotherapeutic microspheres into the tumor center or multiple sites along the outer perimeter of the tumor.

Results. A single treatment with carboplatin, doxorubicin, or 5-fluorouracil microspheres along the perimeter of the tumors produced a significant, dose-related suppression in tumor growth, relative to injections directly into the tumor center. Moreover, five temporally-spaced microsphere treatments along the tumor perimeter (with either doxorubicin or 5-fluorouracil microspheres) completely eradicated 100% of the subcutaneous tumors and 40–53% of the intramuscular tumors. Polypharmacy, accomplished by blending doxorubicin- and 5-fluorouracil-loaded microspheres and injecting them into the tumors was even more efficacious than sustained delivery of either drug alone. Comparable doses of systemic chemotherapy or intratumoral bolus chemotherapy were ineffective.

Conclusions. Injectable microspheres might be ideal for local, sustained delivery of chemotherapeutic agents to solid tumors. However, attention must be paid to the placement of the microspheres, for injections around the tumor perimeter may be required for efficacy.

KEY WORDS: sustained release; microspheres; peripheral tumors; carboplatin; doxorubicin; 5-FU.

INTRODUCTION

Despite a growing number of powerful chemotherapeutic drugs, successful pharmacotherapy for solid tumors remains an unrealized goal of cancer therapy. Effective drug delivery to solid tumors is impaired by the unique features of the vasculature supplying and surrounding tumors, where both flow and diffusion of the drug from the vasculature to the tumor interstitium are greatly reduced (1–4). Consequently, tumor cells are not adequately exposed to cytotoxic levels of the chemotherapeutic agent. In an attempt to overcome this problem, chemotherapeutic drugs have been injected as a bolus directly into the tumor mass (5–7). This approach could be beneficial in a number of cancers where the primary tumor negatively impacts morbidity and quality of life (8,9). However, this approach is limited by the brief residence time achieved by local injections because of rapid drug diffusion away from the tumor. Thus, tumor cells are not exposed to cytotoxic drug levels long enough for significant cell killing.

If local concentrations of chemotherapeutic drugs in solid tumors could be elevated for prolonged periods of time, the anti-tumor effects might be significantly greater than those achieved with systemic administration or intratumoral bolus injections, thus improving on the rather modest effects achieved to date. One means of delivering cytotoxic levels of a chemotherapeutic drug in a sustained fashion to solid tumors is to implant drug-loaded polymers directly into or around the tumor. Polymers such as poly (L-lactide co-glycolide) (PLG) can be fabricated into injectable microspheres to deliver high local concentrations of drugs for predefined periods of time ranging from days to months. Microspheres have been used for delivery of a wide range of chemotherapeutics and can be easily injected as a suspension (10–18).

The present studies tested the effects of locally injected polymer microspheres formulated to provide sustained release of carboplatin, doxorubicin, or 5-fluorouracil (5-FU). Two different animal models of peripheral, solid tumors were used. Several issues important to the development and use of chemotherapeutic microspheres were investigated, including: (1) the dose relationship of sustained release chemotherapy on tumor growth, (2) the effect of injecting microspheres directly into the center of the tumor mass vs. along the outer perimeter of the tumor, (3) the differences in single vs. multiple, temporally-spaced injections, and (4) the added benefits of simultaneously administering multiple chemotherapeutic agents.

MATERIALS AND METHODS

Subjects

Male Fischer 344 rats (N = 876; approximately 300 grams; Taconic Farms, Germantown, New York) were used in all studies. Rats were housed as previously reported (12,13) and all studies were approved by Alkermes' Institutional Animal Care and Use Committee and were conducted in compliance with the Guide for the Care and Use of Laboratory Animals.

Fabrication of Microspheres

Three separate microsphere preparations were made with each providing sustained delivery of a single chemotherapeutic agent. Each individual microsphere formulation (PLG, Medisorb 50/50 DL, MW = 10,000, Alkermes, Inc., Wilmington, Ohio) was fabricated to provide 10% (w/w) loading densities of carboplatin (Sigma Chemical, St Louis, Missouri), doxorubicin (Bedford Laboratories, Bedford, Ohio), or 5-FU (Spectrum, Inc., New Brunswick, New Jersey) using a coacervation process as previously reported (12,13). Blank (non-loaded) microspheres were treated in an

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identical manner except that carboplatin, doxorubicin, or 5-FU was omitted.

In Vitro Release of Chemotherapeutic Drugs from Microspheres

In vitro release was determined by incubating the microspheres in phosphate-buffered saline (PBS) at 37°C. At 1 h, 8 h, 1, 3, 7, 10, 14, and 21 days (n = 3/time point), the solution was removed and the amount of drug released was measured using UV spectrometry. For quantification of carboplatin release, a buffer solution was prepared by mixing 50 ml of 4 M NaAc (sodium acetate) with 50 ml of 4 M HCl. A solution of the color developing reagent N,N-dimethyl-p-nitrosoaniline (DMNA) was prepared by dissolving 0.5 g of DMNA in 10 ml of 100% ethanol. These reagents were combined in a mixture of 0.1 ml of the buffer solution, 0.05 ml of the DMNA solution, and 0.2 ml of double distilled water to 15 ml polypropylene tubes containing the release samples. The tubes were heated in a boiling water bath for 10 min and then cooled for 2 min with ice. An additional 1.65 ml of double distilled water was added to each tube. Levels of carboplatin were determined at a wavelength of 520 nm. For doxorubicin and 5-FU, the PBS/drug solution was extracted three times with 2 ml of PBS and the amount of the chemotherapeutic was determined at a wavelength of 490 nm for doxorubicin, and 266 nm for 5-FU. In parallel, the total amount of each drug encapsulated in the microspheres (i.e., percent loading) was measured by dissolving 10 mg of the microspheres in 1 ml of methylene chloride and preparing the samples as described above. In all cases, drug levels were determined by comparisons against known calibration standards.

Cell Culture

A rat ascites mammary adenocarcinoma cell line (MATB-III; ATCC# CRL-1666) was used. Cells were grown and maintained at 37°C in a 95% O_2/5% CO_2 humidified atmosphere using McCoy's 5A Medium supplemented with 10% fetal bovine serum, 20 mM HEPES, and 1/2x penicillin-streptomycin/fungizone. Before implantation, tumor cells were collected and washed briefly in serum-free media followed by PBS. Cells were suspended at a density of 5×10^6 cells/ml in HEPES-buffered serum-free media containing 1.2% methyl cellulose.

Tumor Models

Subcutaneous Tumors

Rats were briefly anesthetized with 1–2% isofluorane and suspensions of MATB-III cells (200 μl containing 1×10^6 cells) were injected, using a 22-gauge needle, subcutaneously into one rear flank. Tumors were palpated and measured daily for 7 days and those animals with tumors that had reached a size of 1250–1500 mm^3 were used in treatment studies. This time point was based on two considerations. First, previously published studies (19,20) demonstrated that 7 days after implantation, the s.c. MATB-III tumors retained a well-defined border making injections into both the tumor center and within the tumor border easy to perform. Second, at 7 days post implantation, the core of the tumor is not necrotic but instead is highly vascularized and contains viable, growing cells. Moreover, in contrast to the s.c. tumors, which were encapsulated, the i.m. tumors were highly infiltrative into the muscle (Fig. 1).

Intramuscular Tumors

To test the generality of the effects reported here, the potential benefits of local sustained release chemotherapy were also determined in animals bearing i.m. tumors. Anesthetized rats received an injection of MATB-III cells (50 μl containing 1×10^6 cells) into a single biceps femoris muscle of the rear leg. To develop and characterize this novel tumor model, animals bearing i.m. tumors were sacrificed 6, 8, 10, 12, 13, 14, or 15 days after implantation. The region through the tumor was embedded in paraffin using routine procedures and sectioned at 10 μm intervals using a cryostat. Representative sections were then stained for visualization of muscle and tumor using Masson's trichrome stain. These pilot studies also revealed that over time, the MATB-III tumors increasingly invaded the surrounding muscle. As early as 6 days after implantation, tumor cells were migrating between muscle fibers and connective tissue. The extent of migration continued with an increasing pattern of invasion and destruction of muscle fibers (Fig. 1). Within 13 to 15 days, the tumor had typically completely invaded the muscle. At this stage, the majority of the muscle had been destroyed and was replaced by the growing tumor mass. Small pockets of digestion of the tibia were also noted in the larger, end-stage tumors. Based on pilot studies that characterized the growth of these tumors, treatments began 10 days after implantation (when the size of the tumors was 1250 to 1500 mm^3). The 10-day-old i.m. tumors did not have a necrotic core and possessed a highly vascularized central core with viable tumor cells.

Effects of Sustained Release Microspheres on Growth of Solid Tumors

Subcutaneous tumors. Seven days following cell implantation, microspheres were suspended in 0.9% saline, 0.1% Tween and 3.0% carboxymethylcellulose (low viscosity) and injected either into the tumor center or along the outer perimeter of the tumor. Injections along the perimeter (25 μl/site) were made approximately 2 mm under the surface of the skin. Pilot studies using microspheres loaded with methylene blue dye confirmed that the microspheres remain confined to the injection site without any leakage out of the injection tract. The ability to restrict the location of the microspheres to the injection site within the tumor maximized the opportunity for the released drug to diffuse within the growing tumor tissue without significant loss outside of the tumor perimeter. Four equidistant injections were made along the greatest extent of the tumor circumference and a single injection was made at each of the two poles of the tumor. In this way, 6 injections uniformly decorated the outer perimeter of the tumor. The microsphere injections contained a total of 0.1, 0.5, 1.0, 5.0, or 10.0 mg of carboplatin, doxorubicin, or 5-FU. Separate sets of animals served as controls and received either blank microspheres or no treatment, while others received intratumoral bolus injections (5 mg) of carboplatin, doxorubicin, or 5-FU. Treatments into the tumor center were made as a single injection (150 μl) of either sustained release microspheres or bolus injections containing a total of 5 mg of carboplatin, doxorubicin, or 5-FU. Five mg of total drug was

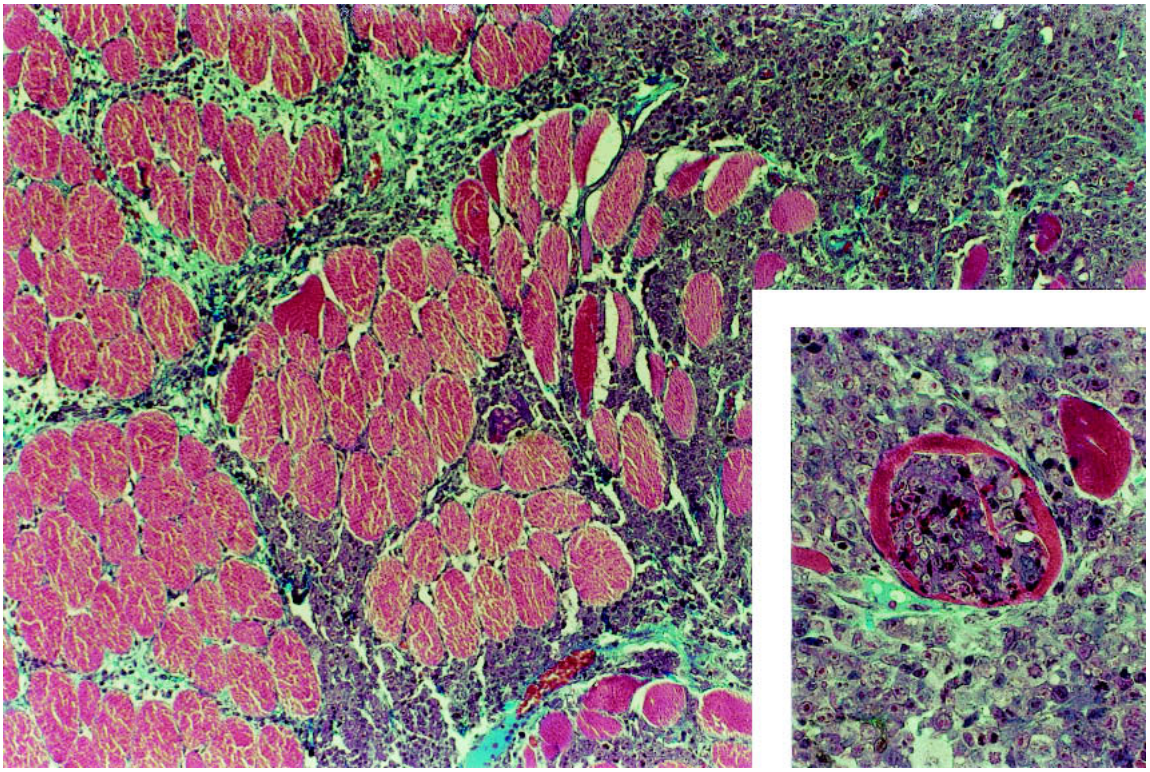

Fig. 1. Representative photomicrograph of a 10-day-old MATB-III tumor implanted into the biceps femoralis muscle. Note the dense infiltration of tumor cells (purple) into the myocytes comprising the muscle fibers (red) and through the collagen (blue) of the surrounding tissue. The insert illustrates the invasion of tumor cells into an individual muscle fiber. Note that the tumor cells have invaded the muscle fiber and appear to be destroying it from the inside out. Original magnification = 100×, insert = 200×.

chosen here because dose escalation beyond 5 mg did not produce any additional efficacy in the above studies.

Parallel studies examined the benefits of multiple, temporally-spaced microsphere treatments as well as the simultaneous delivery of doxorubicin and 5-FU (i.e., drugs that are combined clinically to treat solid tumors). Microspheres were injected along the tumor perimeter. Animals received blank microspheres, doxorubicin microspheres alone (5 mg total drug), 5-FU microspheres alone (5 mg total drug), or a combination of doxorubicin and 5-FU microspheres (2.5 mg of each for a total of 5 mg drug). For combination chemotherapy, the doxorubicin- and 5-FU-loaded microspheres were blended together and injected as a single suspension. Animals received 5 separate treatments beginning 7 days following tumor implantation and then again on days 21, 35, 49, and 63. Tumor sizes were recorded every 2 to 3 days and any animal with a tumor volume greater than 12,000 mm^3 was euthanized using CO_2 asphyxiation.

Intramuscular Tumors

Ten days following tumor implantation, microspheres were injected into a single site in the tumor center (5 mg drug/150 μl) or in a circular pattern along the tumor/muscle perimeter. For injections along the tumor perimeter, 6 separate injections (25 μl/site) were made equidistantly from one another and contained a total of 5.0 mg of carboplatin, doxorubicin, or 5-FU. Unlike the s.c. tumors, which retained a well-defined border, the i.m. tumors grew deep within the leg muscle, making a clear delineation between the tumor and muscle difficult. Accordingly, pilot studies were used to estimate the location of the outer growing edge of the tumor. This was accomplished by injecting methylene blue dye containing microspheres into the tumor at varying distances from the tumor center. After injection, the tumor was dissected free to measure the distance of the injection site from the outer edge. Using this information, the individual injection chemotherapeutic microsphere injections were made approximately 4 mm from the center of the tumor to place each injection approximately 2 mm from the perimeter of the tumor. Separate animals served as controls and received blank microspheres or bolus injections of carboplatin, doxorubicin, or 5-FU (5 mg) into either the tumor center or along the tumor/muscle perimeter. Repeated measures, using palpation, of the i.m. tumors were not possible because the tumors grow deep within the leg muscle. Accordingly, we used "time to disability" as a surrogate measure for tumor growth. Pilot work demonstrated that once disability occurred, the size of the tumor was consistently large, infiltrative into the muscle and required sacrifice for humane reasons. Twice daily animals were placed on a bench top and observed for 2 m by an individual blinded to the animals' experimental condition for any impairment in use of the previously-implanted leg (i.e., dragging the leg, inabilty to place weight on the leg, notable limp, inability to flex the joint of the leg, etc.). This allowed the use of a Kaplan-Meier plot to depict between group differences over time and also significantly reduced the numbers of animals needed as separate treatment groups did not have to be killed at different time points to assess tumor growth. In

all studies, animals were killed at the first indication of disability and the tumor was excised and measured.

Separate studies determined the efficacy of multiple, temporally-spaced treatments with doxorubicin and 5-FU microspheres either alone or in combination. Animals received 5 separate treatments with blank microspheres, doxorubicin microspheres (5 mg/treatment), 5-FU microspheres (5 mg/treatment), or a combination of doxorubicin and 5-FU microspheres (2.5 mg of each/treatment). Treatments were made 10, 24, 38, 52, and 66 days following implantation.

A final set of animals received intravenous (i.v.) infusions of carboplatin, doxorubicin, or 5-FU as previously described (19). Immediately following placement of a polyethylene cannula into the jugular vein, the animals were placed in polystyrene buckets for i.v. infusions using a syringe pump interfaced with a swivel linked infusion line (Instech; Plymouth Meeting, Pennsylvania). Animals received 5 mg of total drug either once on day 10, or over 4 separate treatments beginning on day 10 and repeated every 3 to 4 days. The 4 individual treatments were fractionated to provide 1.25 mg per treatment for a total of 5 mg over the same approximate duration of delivery provided by the chemotherapeutic microspheres. Pilot studies in normal, non tumor-bearing animals determined that the doses used for systemic delivery approached the maximally tolerated doses. In each case, increasing the dose by merely 50% resulted in significant weight loss (16–24%) and mortality (13–38%); we thus concluded that we had achieved the maximum tolerated therapeutic dose. Animals were monitored daily and were euthanized at the first sign of morbidity. The tumors were excised and measured.

Statistics

The effects of sustained release chemotherapy on s.c. tumor growth was analyzed using a repeated measures analysis of variance (JMP, SAS Institute Inc., Cary, North Carolina). Time to disability was analyzed using non-parametric Kruskal-Wallis statistics to determine overall treatment effects. The non-parametric modification of the Neuman-Keuls test was used for subsequent pair-wise comparisons. Minimal statistical significance in all cases was defined as $P < 0.05$.

RESULTS

In Vitro Release of Chemotherapeutic Drugs from Microspheres

The loading density of the chemotherapeutic agents in the microspheres was 10% for carboplatin, 9.2% for doxorubicin, and 9.8% for 5-FU. The release of 5-FU was more rapid than either carboplatin or doxorubicin. After 3 days, the cumulative release of 5-FU was 58.3%, while carboplatin and doxorubicin release was 27.1% and 36.4%, respectively. By day 10, 80% of doxorubicin and 5-FU had been released, while 80% of the carboplatin was not released until 14 days. Approximately 90% of each drug was released within 14 days.

Comparison of Sustained Release Chemotherapeutic Microspheres *vs.* Bolus Injections on Subcutaneous Tumor Growth

The s.c. tumors normally grew rapidly and all non-treated animals were sacrificed within 18–19 days due to tumor growth**.** These tumors were characterized by densely packed neoplastic cells, variegated with intense vasculature and enclosed in a thick fibrous capsule.

Injections of chemotherapeutic microspheres along the outer perimeter of the tumor produced a significant, dose-related delay in tumor growth, relative to animals receiving either no treatment or injections of blank microspheres (Figs. 2–4). Delayed tumor growth was observed following treatment with each chemotherapeutic agent, although the minimally effective dose of sustained release carboplatin (Fig. 2) was higher than that for doxorubicin (Fig. 3) or 5-FU (Fig. 4). While total doses of 0.1 mg doxorubicin and 5-FU significantly delayed tumor growth ($P < 0.01$), carboplatin did not impact tumor growth until the dose was elevated to 1.0 mg. For each chemotherapeutic agent, 5 mg of total drug was maximally effective and no further benefits were obtained by increasing the dose to 10 mg. In contrast to the benefits of microsphere injections along the outer perimeter of the tumor, a single injection of sustained release microspheres into the tumor center (containing 5 mg of total drug) did not impact tumor growth. Bolus injections into either the center or the outer perimeter of the tumor, did not impact tumor growth (Figs. 2–4).

Five separate treatments with doxorubicin- or 5-FU-loaded microspheres, each separated by 14 days, produced an even more robust effect, completely eradicating tumors in all animals within 61 and 75 days following doxorubicin and 5-FU microspheres, respectively (Fig. 5). Combining the doxorubicin and 5-FU microspheres produced even more rapid tumor regression (day 48) than achieved by twice the dose of either drug alone.

Comparison of Sustained Release Chemotherapeutic Microspheres *vs.* Bolus and Systemic Chemotherapy on Intramuscular Tumor Growth

Intramuscular tumors grew rapidly in the control groups (no treatment or blank microspheres) and all animals were euthanized within 18 to 19 days due to the disability of the tumor-bearing leg (Figs. 2 and 6). These tumors differed from the more traditional s.c. tumor in that they were not encapsulated but invaded the surrounding muscle bundles, manifested by large areas of interspersed myocytes and neoplastic cells (Fig. 1). As with the s.c. tumors, the i.m. tumors were highly vascularized and exhibited no areas of necrosis.

Injections of chemotherapeutic microspheres along the tumor/muscle perimeter significantly delayed the onset of disability (Table I, Fig. 6). The benefits of sustained release chemotherapy were greatest with doxorubicin (27% or 4/15 of the animals never demonstrated any disability over the duration of the experiment; $P < 0.001$), followed by 5-FU (7% or 1/15 of the animals never demonstrated any disability over the duration of the experiment, $P < 0.01$), and finally carboplatin (all animals euthanized by day 26, $P < 0.05$).

Intratumoral bolus injections and systemic chemotherapy did not impact disability (Table I). Dissection of the tumor-bearing leg revealed that the i.m. tumors were eradicated in animals surviving the duration of the experiment without disability. In contrast, all disabled animals had large end-stage tumors that were comparable to those in control animals. The growth rate of i.m. tumors was not impacted by intratumoral bolus injections or systemic chemotherapy. End-

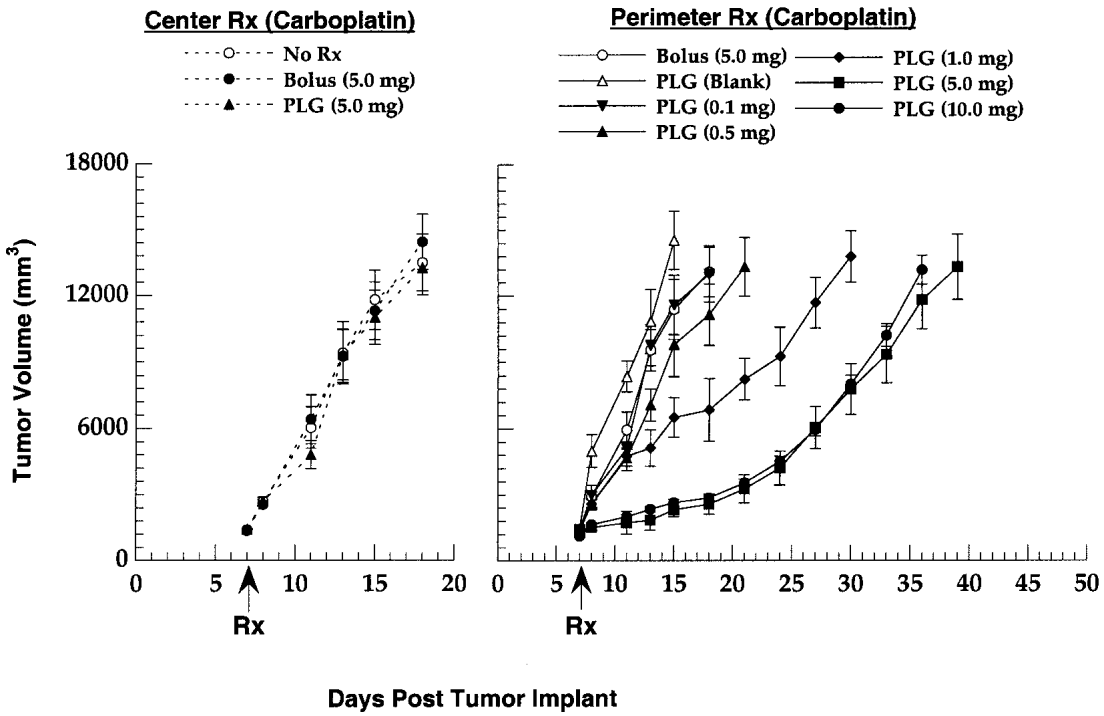

Fig. 2. Growth of s.c. tumors following a bolus injection of carboplatin (5 mg) or injection of sustained release carboplatin microspheres (0.1–10.0 mg total drug) into either the center of the tumor or into 6 sites along the perimeter of the tumor. Sustained release of carboplatin produced a dose-related suppression in tumor growth when the microspheres were injected along the tumor perimeter (right panel). In contrast, tumor growth was not impacted by injections of carboplatin-loaded microspheres (5 mg total drug) into the center of the growing tumor mass (left panel), nor intratumoral bolus chemotherapy (5 mg) into the center of the tumor (left panel) or along the tumor perimeter (right panel). Sample size is 15/group. Data are presented as mean ± SEM tumor volume.

stage tumor volumes across these studies were 6979 to 7739 mm^3.

Multiple, temporally-spaced injections of chemotherapeutic microspheres along the perimeter of the i.m. tumors were more effective than single treatments (Fig. 6B). Again, tumors grew rapidly in control animals and all animals were sacrificed within 20 days, due to disability of the implanted leg. In contrast, no tumor-associated disability was seen in 40% (6/15) of the 5-FU-treated ($P < 0.001$) and 53% (8/15) of the doxorubicin-treated animals ($P < 0.001$). Simultaneous delivery of doxorubicin and 5-FU, achieved by blending and injecting individual doxorubicin- and 5-FU-loaded microspheres, was significantly more effective than either drug alone, with 80% (12/15) of the animals never exhibiting any disability of the implanted leg ($P < 0.01$ vs doxorubicin or 5-FU). The tumors were eradicated in all animals surviving the duration of the experiment.

DISCUSSION

The results presented here detail several new findings regarding the use of sustained release chemotherapeutic microspheres to treat solid peripheral tumors. They established that: 1) microspheres can be easily injected into, or around, solid tumors to provide sustained, local delivery of chemotherapeutic drugs, 2) injections of sustained release microspheres into the outer perimeter of solid tumors were effective, while identical injections into the center of the tumor were ineffective, 3) sustained local delivery of chemotherapy around the tumor was superior to both equimolar intratumoral bolus injections as well as the maximum tolerated systemic dose, 4) multiple, temporally spaced treatments with chemotherapeutic microspheres were more effective than a single treatment, and 5) simultaneous delivery of multiple sustained release chemotherapeutic agents was superior to single agent therapy; thus, chemotherapeutic microspheres provide the opportunity for convenient polypharmacy.

Conventional routes of administering chemotherapeutic drugs, including systemic (1–4) administration and intratumoral bolus injections (5–7) do not achieve adequate delivery of the drugs to solid tumors. In the present studies, intratumoral bolus injections and systemic administration of the maximum tolerated dose of chemotherapy produced negligible effects in two different tumor models (implanted s.c. and i.m.). In contrast, the growth of both tumor types was markedly suppressed by injections of sustained release microspheres. While demonstrating that injections of sustained release microspheres can significantly impact tumor growth, these studies also revealed that the precise pattern and/or location of the injections dictates whether efficacy occurs. When dispersed over 6 injection sites along the outer perimeter of both s.c. and i.m. tumors, sustained release microspheres produced a significant, dose-related suppression of tumor growth using 3 different chemotherapeutic agents (carboplatin, doxorubicin, and 5-FU). In contrast, microsphere injections into the tumor center were completely ineffective even though the center of the tumor was not necrotic and contained apparently viable cells. These results are similar to

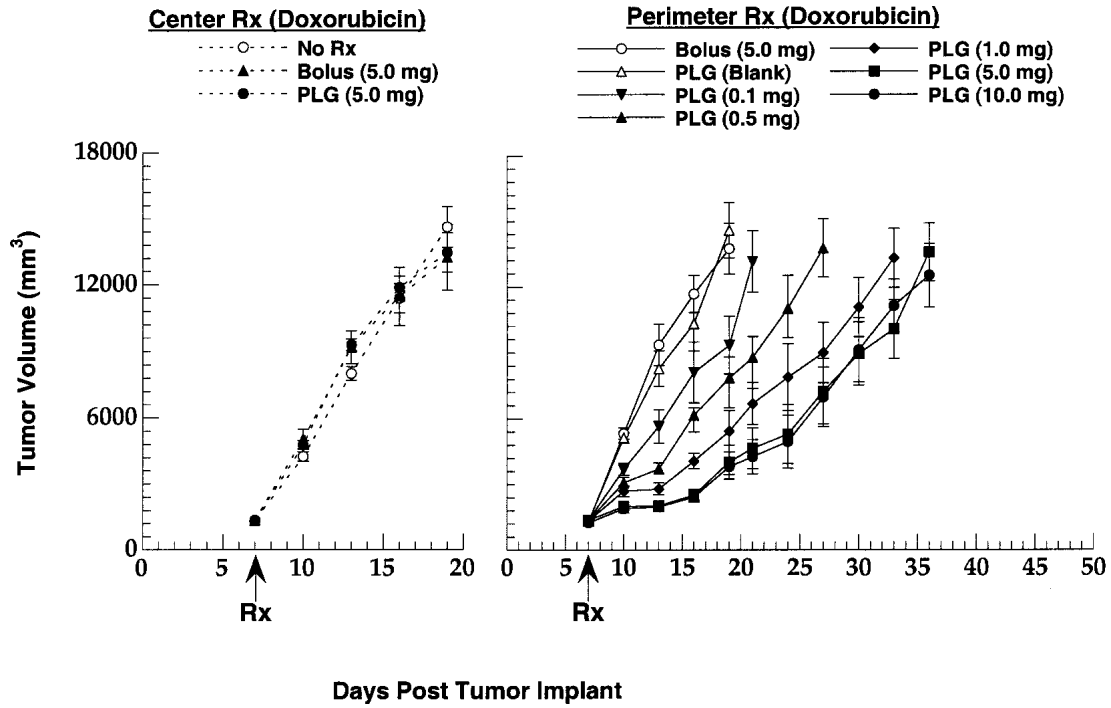

Fig. 3. Growth of s.c. tumors following a bolus injection of doxorubicin (5 mg) or injection of sustained release doxorubicin microspheres (0.1–10.0 mg total drug) into either the center of the tumor or into 6 sites along the perimeter of the tumor. In contrast, sustained release of doxorubicin produced a dose-related suppression in tumor growth when the microspheres were injected along the tumor perimeter (right panel). In contrast, tumor growth was not impacted by injections of doxorubicin-loaded microspheres (5 mg total drug) into the center of the growing tumor mass (left panel) nor intratumoral bolus chemotherapy (5 mg) into the center of the tumor (left panel) or along the tumor perimeter (right panel). Sample size is 15/group. Data are presented as mean ± SEM tumor volume.

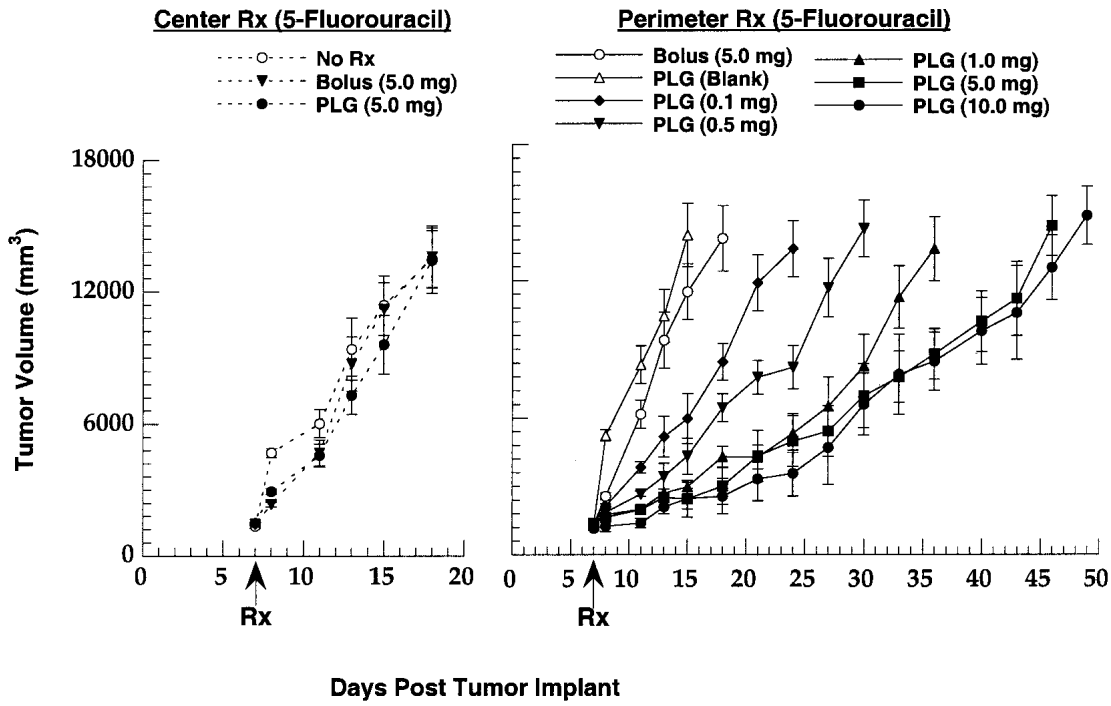

Fig. 4. Growth of s.c. tumors following a bolus injection of 5-FU (5 mg) or injection of sustained release 5-FU microspheres (0.1–10.0 mg total drug) into either the center of the tumor or into 6 sites along the perimeter of the tumor. Similar to the data in Figs. 1 and 2, sustained release of 5-FU produced a dose-related suppression in tumor growth when the microspheres were injected along the tumor perimeter (right panel). In contrast, tumor growth was not impacted by injections of 5-FU-loaded microspheres (5 mg total drug) into the center of the growing tumor mass (left panel) nor intratumoral bolus chemotherapy (5 mg) into the center of the tumor (left panel) or along the tumor perimeter (right panel). Sample size is 15/group. Data are presented as mean ± SEM tumor volume.

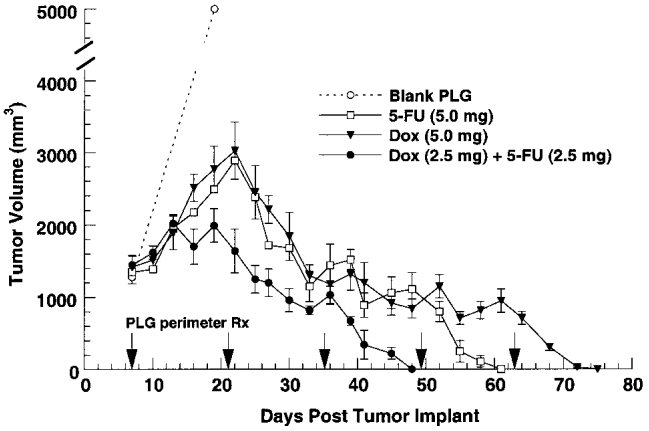

Fig. 5. Growth of s.c. tumors after 5 separate treatments with microspheres containing 5-FU or doxorubicin alone (5 mg total drug per injection) or a combination of 5-FU and doxorubicin (2.5 mg of each for 5 mg of total drug). In contrast to the results obtained using a single treatment (Figs. 3–5), 5 sequential treatments (each separated by 14 days) resulted in complete regression of the tumors in all groups. The most rapid effects occurred when microspheres containing either 5-FU or doxorubicin were blended together and injected as a simple suspension. Sample size is 15/group. Data are presented as mean ± SEM tumor volume.

those recently reported in a rodent model of glioma where injections of microspheres along the outer border of a glioma enhanced survival while injections directly into the tumor mass had a minimal impact on survival (12,13). Together with the previous results in rodent glioma models, the present data in solid tumors indicate that the location of microsphere injections (i.e., inside vs. around the border of a tumor) plays a critical role in the effectiveness of local, sustained release chemotherapy.

If the efficacy of local sustained release chemotherapy is confirmed clinically, control of solid tumors using surgery or radiation could be greatly enhanced. Direct or local treatment of many types of solid tumors (including head, neck, liver, prostate, pancreatic, and glioma) is often limited by the lack of accessibility to parts of the tumor or because of the inexpendable nature of its adjacent tissue (24–26). These tumors can, however, be imaged using either ultrasound or computerized tomography, making it possible to inject drugs directly into or around the tumor. In these cases, local injections of chemotherapeutic microspheres could be made to increase the likelihood of achieving cytotoxic drug concentrations to the tumor.

Because injections of microspheres can be accomplished using a standard hypodermic needle with minimal invasiveness to the injected tissue, repeated, temporally spaced treatments are possible. The present studies examined the benefits produced by either a single treatment with chemotherapeutic microspheres or 5 separate treatments, each separated by 14 days. A single treatment with chemotherapeutic microspheres significantly slowed the growth of both the s.c. and i.m. tumors. However, 5 separate and temporally-spaced treatments eradicated 100% of the s.c. tumors and 40–53% of the i.m. tumors. These impressive effects were obtained even though no attempt was made to selectively target specific areas of tumor growth and under conditions where it was difficult, if not impossible, to precisely define the perimeter of the i.m. tumors. In clinical practice, standard imaging techniques make it possible to define the anatomic boundaries of tumors (27,28) and then target them with chemotherapeutic micro-

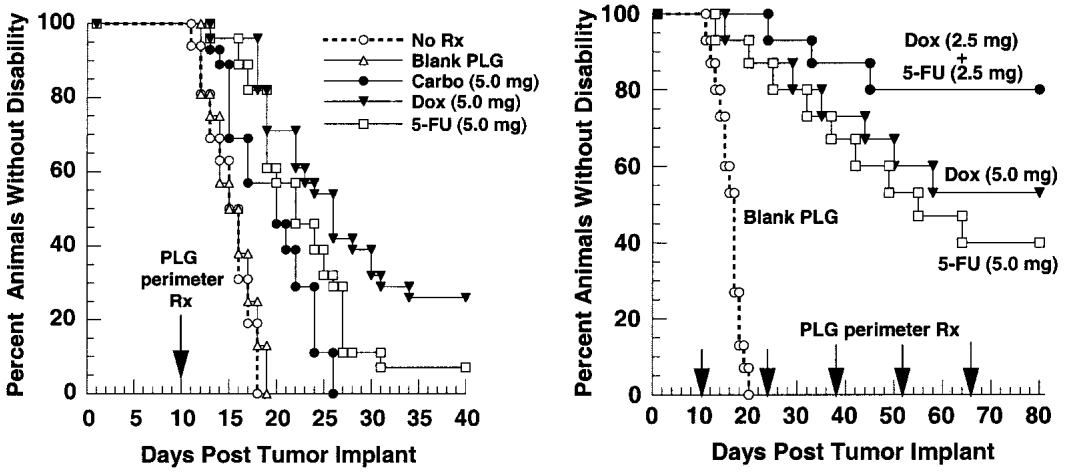

Fig. 6. Effects of sustained release carboplatin, 5-FU, and doxorubicin (5 mg total drug) on growth of i.m. tumors as determined by time to onset of impaired use of the tumor-bearing leg (see text for details regarding blinded assessment). Left panel: Animals received a single treatment with microspheres 10 days following tumor implant. Relative to animals receiving either no treatment or blank microsphere injections, sustained release carboplatin, doxorubicin, and 5-FU all significantly delayed the onset of disability. Note also that 20% of the doxorubicin and 7% of the 5-FU treated animals did not exhibit any impairment due to growth of the tumor. Right panel: Animals received 5 sequential treatments with microspheres containing 5-FU or doxorubicin alone (5 mg total drug per injection) or a combination of 5-FU and doxorubicin (2.5 mg of each for 5 mg of total drug) beginning 10 days after tumor implantation. As was shown in the s.c. tumor model (Fig. 4), 5 separate treatments, each separated by 14 days, was significantly more effective than a single treatment. Note that 60% of the doxorubicin, 47% of the 5-FU, and 80% of the doxorubicin with 5-FU treated animals did not develop any disability due to tumor growth. Sample size is 15/group.

Table I. Effects of Chemotherapeutic Agents Delivered by Sustained Release Microspheres, Bolus Injections, or Systemic Infusions

Treatment	(N)	Days to 50% disability	Days to 100% disability
No Treatment (vehicle)	(18)	15	18
	(15)	15	19
Carboplatin (5 mg):			
S.R. microspheres - center	(18)	17	22
S.R. microspheres - periphery	(15)	20	26*
Bolus - center	(18)	17	22
Bolus - periphery	(18)	19	23
Intravenous - single Rx	(15)	15	18
Intravenous - fractionated Rx	(15)	18	22
Doxorubicin (5 mg):			
S.R. microspheres - center	(18)	17	20
S.R. microspheres - periphery	(15)	26	>40***
Bolus - center	(18)	17	20
Bolus - periphery	(18)	17	22
Intravenous - single Rx	(15)	14	16
Intravenous - fractionated Rx	(15)	19	23
5-FU (5 mg):			
S.R. microspheres- center	(18)	17	20
S.R. microspheres-periphery	(15)	22	>40**
Bolus - center	(18)	17	20
Bolus - periphery	(18)	15	21
Intravenous - single Rx	(15)	14	17
Intravenous - fractionated Rx	(15)	18	21

Note: Using disability as an objective and functional index of growth of the deep intramuscular tumor, comparisons of different treatment modalities demonstrated that only local, sustained release (S.R.) chemotherapy delivery around the tumor periphery produced reliable treatment effects. See text for details. *P, 0.05, **$P < 0.01$, ***$P < 0.001$. See text for details.

sphere injections, perhaps providing even greater opportunity for improved outcome.

Microspheres also provide the opportunity for simple and convenient polypharmacy, potentially producing greater effects than can be achieved with single drugs. Here we blended and injected individual preparations of doxorubicin- and 5-FU-loaded microspheres and evaluated the resulting combination chemotherapy on tumor growth. These chemotherapeutic agents are commonly combined in clinical practice and have different mechanisms of cell killing. Doxorubicin produces cellular DNA damage by inhibiting topoisomerase and generating semiquinone free radicals, while 5-FU interferes with DNA replication by inhibiting thymidine production (29). While doxorubicin- and 5-FU loaded microspheres suppressed the growth of both the s.c. and i.m. tumors, the greatest effects were obtained when microspheres releasing doxorubicin and 5-FU were mixed together. This enhanced effect was achieved even though the amount of each chemotherapeutic drug (doxorubicin and 5-FU) delivered was only one half the high dose of each drug when tested alone. It should be mentioned that the doses of both doxorubicin and 5-FU used here were equivalent, while conventional clinical systemic dosing paradigms typically employ doses of 5-FU that are 10-fold greater than doxorubicin (30). Future studies using sustained release microspheres to locally deliver multiple chemotherapeutic agents should continue to optimize drug combinations based on considerations of cell cycle specificity, different modes of action of the drugs and varied toxicities. As drugs with novel anti-tumor actions emerge, including radiation sensitizers, hormones and monoclonal antibodies, greater opportunities for developing synergistic polypharmacy using local sustained delivery will occur.

In summary, the results of these studies suggest that sustained release chemotherapeutic microspheres can suppress the growth of previously established solid peripheral tumors and that repeated, temporally-spaced treatments can completely eradicate solid tumors in animal models. The benefits of sustained release microsphere treatments were demonstrated in two different models, using tumors grown both subcutaneously and intramuscularly. If confirmed clinically, this approach may enhance the efficacy of chemotherapeutic agents in situations where focal therapy is useful, without significantly increasing the toxic liability of the agents.

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