The Suppression of Human Prostate Tumor Growth in Mice by the Intratumoral Injection of a Slow-Release Polymeric Paste Formulation of Paclitaxel

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ABSTRACT

Most patients that present in the clinic with prostate cancer have either localized or recurrent postradiotherapy therapy tumors that may be amenable to injectable treatments using slow-release cytotoxic drugs. The objective of this preclinical study was to design an injectable polymeric paste formulation of paclitaxel for intratumoral injection into nonmetastatic human prostate tumors grown s.c. in mice. Paclitaxel was dissolved (10% w/w) in a blend of a biodegradable triblock copolymer of random copolymer of \( \text{DL-lactide} \) and \( \epsilon \)-caprolactone (PLC) with poly(ethylene glycol) [PEG; PLC-PEG-PLC] blended with methoxypoly(ethylene glycol) in a 40:60 ratio. Human prostate LNCaP tumors grown s.c. in castrated athymic male mice were injected with 100 \( \mu \)l of this paste at room temperature. Changes in tumor progression were assessed using both serum prostate-specific antigen (PSA) levels and tumor size. Paclitaxel inhibited LNCaP cell growth in vitro in a concentration-dependent fashion with an IC\(_{50}\) of 1 nM. Apoptosis was documented using DNA fragmentation analysis. The paste formulation solidified over a period of 1 h both in vitro and in aqueous media at 37°C as the methoxypoly(ethylene glycol) component partitioned out of the insoluble PLC-PEG-PLC/paclitaxel matrix. The semisolid implant released drug at a rate of about 100 \( \mu \)g/day in vitro. In control mice treated with paste without paclitaxel, serum PSA levels increased from 2–8 ng/ml (mean, 4.3 ± 2 ng/ml) to 60–292 ng/ml (mean, 181 ± 88 ng/ml), and tumor volume increased from 30 to 1000 mm\(^3\). In mice treated with a single 100-\( \mu \)l injection 3 weeks after castration (early-phase treatment group), tumors decreased in volume from a mean of 43 ± 19 mm\(^3\) to nonpalpable, and PSA levels decreased from a mean of 22 ± 8 to 2 ± 1 ng/ml by 8 weeks after castration. In mice treated 5 weeks after castration (androgen-independent tumors; late-phase treatment group), tumors decreased in volume from a mean of 233 ± 136 mm\(^3\) to nonpalpable, and serum PSA decreased from 24 ± 8 to 9 ± 4 ng/ml. Observed side effects of the treatment were limited to minor ulceration at the needle injection site in paclitaxel-treated mice only. The controlled-release formulation can be injected via 22-gauge needles and is effective in inhibiting LNCaP tumor growth and PSA levels in mice bearing multiple nonmetastatic tumors. Paclitaxel may be an effective therapy for patients with localized tumors recurring after radiotherapy and for some patients with localized tumors who are not candidates for radical treatment.

INTRODUCTION

Prostate cancer is the second highest cause of cancer death in men (1). The incidence of prostate cancer continues to rise each year due to an increased awareness of the disease and an aging population (2, 3). Over the past decade, substantial improvements in diagnosis and staging of the disease have been made with the combined use of digital rectal examination, measurement of serum PSA levels, and transrectal ultrasound. Earlier diagnosis of prostate cancer has resulted in a stage migration, with an increased proportion of men diagnosed with clinically localized disease and a 50% decrease in the incidence of stage D2 disease over the last 2 years (4, 5). Hence, most men diagnosed with prostate cancer during the 1990s were found to have localized disease. However, the potential benefits of detection of earlier phase, localized disease must be balanced by the risk of treatment-related morbidity and associated increased costs.

The natural history of localized prostate cancer is variable but is generally one of slow local progression with later development of regional and distant metastasis. More than two-thirds of patients will suffer local or systemic progression, and more than half of patients with prostate cancer will die from their disease within 10 years (6). Significant morbidity can result from local tumor progression, including bladder outlet and ureteral obstruction, pain, and bleeding. Although radical prostatectomy and radiation therapy are both effective forms of treatment, both have low but significant morbidity, and not all patients are suitable candidates. In addition, serum PSA measurements allow for the detection of biochemical and local recurrences after radiotherapy and identify men destined to experience disease recurrence, who may benefit from early and additional adjuvant therapy. Androgen withdrawal therapy remains the only efficacious treatment for advanced or recurrent prostate cancer. However, both surgical castration and LHRH agonist treatments are associated with significant short-term and long-term side effects. Unfortunately, LHRH agonist treatment is also expensive. Furthermore, over time, the tumor becomes hormone resistant. Therefore, patients with recurrent localized tumors after radiotherapy and some patients with localized tumors who are not candidates for aggressive therapy would benefit from additional well tolerated, minimally invasive, local treatment modalities.

The anatomical location of the prostate gland is amenable to direct local injection with agents capable of inducing apoptosis. Brachytherapy for localized prostate cancer is now performed by transperineal placement of radioactive seed implants under transrectal ultrasound guidance. It is conceivable that the prostate gland could also be infiltrated with chemotherapeutic agents using similar techniques. Paclitaxel has been described previously to have cytotoxic activity against a number of prostate cancer cell lines in vitro (7–9). The nonmetastatic cell line JCA-1 established from primary human prostate tissue was shown to be sensitive to paclitaxel with an IC\(_{50}\) value of 20 nM (9). Similarly, the LNCaP cell line was shown to undergo apoptosis when subjected to paclitaxel concentrations in the low micromolar concentration range, although the IC\(_{50}\) was not measured in that study (8). Paclitaxel has also been shown to have a potent inhibitory effect on angiogenesis (10, 11), a process that has been proposed as a target for the chemotherapeutic treatment of prostate cancer (12). Unfortunately, the low water solubility of paclitaxel necessitates the use of ethanol/cremophor formulations for i.v. admin-

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3 The abbreviations used are: PSA, prostate-specific antigen; PEG, poly(ethylene glycol); McPEG, methoxypoly(ethylene glycol); PLC, copolymer of \( \text{DL-lactide} \) and \( \epsilon \)-caprolactone.

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istration with significant toxicity including hypersensitivity reactions to the cremophor (13, 14).

An alternative approach to the administration of this drug for the treatment of localized prostate tumors might be via a controlled-release implant that could deliver pharmacologically effective doses of paclitaxel to the tumor site. Such a site-directed paclitaxel delivery system might avoid the systemic toxicities associated with the repeated use of the cremophor/paclitaxel formulation. In our laboratory, we have developed a variety of biodegradable injectable polymeric paste formulations based on the low melting point polymers poly(e-caprolactone) and a triblock copolymer of poly(D,L-lactide) and PEG (15, 16). In this work, a biodegradable triblock copolymer of a random PLC with PEG was used to form PLC-PEG-PLC. The injectable polymer paste was formulated by blending PLC-PEG-PLC with MePEG in a 40:60 ratio.

We have previously described the use of the LNCaP tumor model as a valid model for the in vivo study of the effects of androgen ablation or chemotherapy in prostate cancer (17, 18). Serum PSA levels are directly proportional to tumor volume in noncastrated mice, and both androgens and tumor volume are important codeterminants of circulating PSA levels. Immediately after castration, serum PSA levels decrease rapidly by approximately 80% and increase up to 20-fold after androgen supplementation, without detectable castration-induced tumor cell death or concomitant changes in tumor volume. These changes in PSA production in vivo reflect changes in androgen-regulated PSA gene expression. Beginning approximately 4 weeks after castration, PSA production rises to precastration levels, and tumor volume increases in the absence of testicular androgens, reflecting the onset of androgen-independent regulation of the PSA gene and androgen-independent tumor growth (17, 18). The LNCaP prostate tumor model permits study of the efficacy of castration in combination with treatment with other apoptotic enhancers.

The objective of this study was to determine the effectiveness of intratumoral delivery of paclitaxel in an injectable, biodegradable polymeric paste formulation against s.c. grown LNCaP tumors in mice. This system does not directly model tumors localized in the prostate gland or tumors that have been previously exposed to radiation that might be treated with site-directed controlled-release anticancer formulations in humans. However, the model allows for the determination of the efficacy of the paste formulation against localized human prostate tumors. The formulation was used as early adjunct therapy to castration in androgen-dependent LNCaP tumors and as salvage therapy in recurrent androgen-independent tumors.

MATERIALS AND METHODS

Paclitaxel Paste Formulation. The controlled-release formulation consisted of a triblock copolymer blended with the water-soluble polymer MePEG in a 40:60 ratio containing 10% paclitaxel (w/w). This formulation is a viscous liquid or paste at room temperature and was designed to be injected intratumorally in mice containing multiple LNCaP tumors, where it sets to a solid implant within 1 h at the tumor site. The manufacture of 2-g batches of 10% paclitaxel (w/w) loaded paste was achieved as follows: 200 mg of paclitaxel (Hauser Chemical Co., Boulder, CO) were blended into 1080 mg of MePEG (M_w 350; Union Carbide, Danbury, CT) at 5 min at 40°C, followed by 720 mg of a PLC-PEG-PLC triblock copolymer (Angiotech Pharmaceuticals, Inc., Vancouver, British Columbia, Canada). This mixture was stirred for 15 min at 30°C so that all of the paclitaxel was dissolved in the liquid polymer blend, and then the polymer-drug solution was drawn up into 1-ml Luer lock syringes (BD Labware, Bedford, MA), capped, and stored at 4°C until use. Control paste (without the drug) was manufactured by blending PLC-PEG-PLC with MePEG in a 40:60 ratio (w/w) at 50°C for 5 min, loaded into syringes, and stored at 4°C until use.

In Vitro Drug Release Studies. Fifteen mg of the 10% drug-loaded paste were weighed into 20-ml glass scintillation vials (Fisher Scientific). The vials were then warmed to 50°C on a 60 degree angle until the fluid paste collected at the base of the vial, and then the vial was cooled quickly to 4°C so that the paste solidified as a defined mass. Four ml of cold PBS (pH 7.4) containing human serum albumin at 0.2% (Fraction 4; Boehringer Mannheim, Mannheim, Germany) were added to the vial, followed by 4 ml of 1-octanol (Fisher Scientific). The vials were then capped and placed in a 37°C oven. As paclitaxel was released from the paste into the PBS, a concentration gradient-dependent diffusion of drug was established to the 1-octanol phase, in which the paclitaxel is highly soluble. Samples of the 1-octanol phase were removed, and the paclitaxel concentration in the samples was determined spectrophotometrically using absorbance at 232 nm. Samples were analyzed every day for the first week and then analyzed at appropriate time points afterward. The sample was then gently placed back in the remaining 1-octanol in the vial. Paclitaxel concentrations in the 1-octanol were also determined on days 10, 20, 30, 40, 50, and 60 by performance liquid chromatography methods. Briefly, 50 μl of the 1-octanol sample were diluted into 450 μl of methanol for analysis by high-performance liquid chromatography (Waters 600 series) using a mobile phase of 58% acetonitrile:37% water:5% methanol (flow rate = 1 ml/min) with a 20-μl injection volume onto a reverse-phase C18 column (Waters, Nova-Pak) and detection at 232 nm.

Animals and Cell Lines. Male 6–8-week-old athymic nude mice (BALB/c strain) were purchased from Charles River Laboratory (Montreal, Quebec, Canada). LNCaP cells (passage number 40–48) were maintained in RPMI 1640 (Terry Fox Laboratory, Vancouver, British Columbia, Canada) with 5% fetal bovine serum (Life Technologies, Inc., Burlington, Ontario, Canada) as described previously (17).

In Vitro Mitogenic Assays. To measure the effect of paclitaxel on LNCaP cell growth in vitro, we used a 96-well assay based on the uptake and elution of crystal violet dye by the cells in each well (17). Three thousand LNCaP cells were plated per well in 96-well plates (Falcon) in RPMI 1640 with 5% fetal bovine serum, and the cell media were changed to various concentrations of paclitaxel 24 h later. The medium was changed every 2 days; 7–10 days later, the cells were fixed in 1% glutaraldehyde (Sigma) and stained with 0.5% crystal violet (Sigma). Plates were washed and air-dried, and the dye was eluted with 100 μl of Sorenson’s solution (9 mg of trisodium citrate in 305 ml of distilled water, 195 ml of 0.1 n hydrochloric acid, and 500 ml of 90% ethanol). Absorbance of each well was measured by a Titertek Multiskan TCC/340 (Flow Laboratories, McLean, VA) at 560 nm. Control experiments demonstrated that absorbance was directly proportional to the number of cells in each well.

DNA Fragmentation Analysis. DNA fragmentation was analyzed by agarose gel electrophoresis as described previously (19). Briefly, fragmented genomic DNA was extracted by incubation of cells in Tris extraction buffer [10 mM Tris-HCl (pH 7.4), 10 mM EDTA, and 0.5% Triton X-100] at 4°C for 5 min. The cell suspension was centrifuged for 10 min at 4°C. The resultant supernatant was incubated for 1 h at 37°C in the presence of proteinase K (400 μg/ml) before extraction with phenol/chloroform. The fragmented DNA was precipitated with salt and isopropyl alcohol and then analyzed by agarose gel electrophoresis.

Inoculation of LNCaP Cells. All animals were anesthetized with methoxyfluorane before injection of LNCaP cells. To establish s.c. tumors, 2 × 10^6 LNCaP cells were suspended in 75 μl of RPMI 1640 plus 5% FBS and 75 μl of Matrigel (Collaborative Biomedical Laboratories, Bedford, MA) and injected via 27-gauge needle into the s.c. space of the flank region. Tumors were measured twice weekly using calipers, and their volumes were calculated by the following formula: length × width × height × 0.5236 (20).

Determination of Serum PSA Levels. Blood samples were obtained by tail vein incision of mice at specified times as described previously (17). Serum PSA levels were determined by an enzymatic immunoassay kit with a lower limit of sensitivity of 0.2 ng/ml (Abbott IMX, Montreal, Quebec, Canada) according to the manufacturer’s protocol. Fifteen μl of mouse serum were diluted with 135 μl of diluent to perform the assay; the lower limit of sensitivity in this murine model was therefore 2 ng/ml.

Treatment Protocols. When LNCaP tumor volume reached between 100–200 mm^3 and serum PSA levels were approximately 10 ng/ml, mice were anesthetized using methoxyfluorane and castrated via abdominal approach (18). The castration of all treatment groups helped to avoid any possible paclitaxel-related fluctuations in androgen-regulated serum PSA production. Mice were then randomly divided into three groups: (a) a control group treated...
with control paste; (b) an early-phase treatment group treated 3 weeks after castration, when serum PSA levels were still at nadir levels; and (c) a late-phase treatment (androgen-independent) group treated >6 weeks after castration, when serum PSA had increased to levels above the precastration levels. Mice in control and the early- and late-phase treatment groups were treated with a single intratumoral injection of 100 µl of control paste or 10% paclitaxel-loaded paste (containing approximately 10 mg of drug). Tumor volume and serum PSA levels were measured once weekly. Data points for both sets of experiments were expressed as average tumor volumes ± SEs of the mean based on at least five determinations.

To determine the effects of systemic uptake of paclitaxel on tumor volume and serum PSA, a set of mice was treated by injection of the paste at a remote location away from the tumor. Five mice, each bearing one tumor, were castrated and treated with 100 µl of 10% paclitaxel-loaded paste by injection (i.e., using the same treatment protocol used for other mice). However, these mice were treated by injection at a site more than 1 cm from the tumor masses.

Apoptosis Immunolabeling. Mice were treated under the early-phase treatment regimen with paclitaxel paste or with control paste. After 3 days, the mice were sacrificed, and the tumors were excised from the s.c. sites. The tumors were fixed in 10% neutral buffered formalin and embedded in paraffin. Fixed sections (5 µm) were cut from the specimens and stained for apoptosis (ApoTag kit; Oncor, Gaithersburg, MD) using a methylene blue counterstain. Apoptotic cells stain brown using this method.

RESULTS

Effect of Paclitaxel on LNCaP Cells in Vitro. Paclitaxel suppressed LNCaP cell growth in vitro as shown in Fig. 1. Concentrations as low as 0.1 nM inhibited LNCaP cell growth with an IC₅₀ below 1 nM. Cell detachment began after 48 h and was associated with DNA laddering on agarose gel electrophoresis, consistent with apoptosis (Fig. 1, inset).

Release of Paclitaxel from the Polymeric Paste. In vitro studies showed that paclitaxel released slowly from the paste (Fig. 2). After an initial burst of paclitaxel release over the first few days (approximately 5% of the encapsulated drug was released), the release rate decreased to 0.5% per day for the next 2 weeks. After 2 weeks, the paste pellet began to disintegrate, and release rates increased to approximately 1.2% of the drug released per day.

Effect of Intratumoral Paste Injection on Serum PSA Levels. Initial pilot studies were performed to determine the biocompatibility of the polymeric paste and to determine whether the intratumoral injection of the control paste alone had any effect on tumor growth rates. These experiments showed no apparent local or systemic adverse reactions to the injection of control paste when injected either intratumorally or in other s.c. areas.

Changes in LNCaP tumor growth and serum PSA levels between control and early versus late phase paclitaxel paste-treated mice are compared in Fig. 3. A—C, respectively. Mean pretreatment PSA levels were 24.1 ± 7, 21.9 ± 7.6, and 24.4 ± 7.6 ng/ml for all control, early-phase, and late-phase treatment groups, respectively. After castration, serum PSA decreased by 60–70% in all three groups and reached nadir levels by 2 weeks after castration. In the control mice (n = 7), serum PSA increased beginning at 3–4 weeks after castration, and by week 10, serum PSA levels had risen to a mean of 180 ± 88 ng/ml (range, 60–292 ng/ml). In contrast, serum PSA levels decreased in mice treated with a single intratumoral injection of paclitaxel paste. In the early-phase treatment group injected 3 weeks after castration (n = 7), serum PSA levels decreased in all mice from a mean preinjection level of 7.26 ± 3.8 ng/ml at 3 weeks after castration to 1.83 ± 1.01 ng/ml by 7 weeks after castration (Fig. 3B). By week 11, one mouse had a slightly elevated level of 13 ng/ml, but all other serum PSA levels remained below or near detectable levels (2.2 ± 0.25 ng/ml). In the late-phase treatment group (n = 5), serum PSA levels decreased from 24.4 ± 7.6 to 8.72 ± 3.7 ng/ml (Fig. 3C). By week 5, four mice had serum PSA levels that were higher than the precastration levels, and these mice were treated with 10% paclitaxel-loaded paste. The one remaining mouse was treated at week 8, when the serum PSA level had risen to 52 ng/ml. After treatment, serum PSA levels decreased in all mice to a mean of 4.6 ± 2.0 ng/ml by 4 weeks after injection. It should be noted that the data in Fig. 3C show the mean values of all of the serum PSA values at each time point, including the data from the mouse treated at week 8, although this mouse was treated 2 weeks later than the other mice.

Effect of Intratumoral Injection of Paste on LNCaP Tumor Growth in Vivo. Changes in LNCaP tumor volume paralleled changes in serum PSA as shown in Fig. 4. Mean pretreatment LNCaP tumor volumes were 57 ± 12, 68 ± 24, and 82 ± 40 mm³ for the control, early-phase treatment, and late-phase treatment groups, respectively. After castration, a minimal decrease in tumor volume was

![Fig. 2. Cumulative release of paclitaxel from 10% (w/w) paclitaxel-loaded paste in vitro. Drug-loaded paste (15-mg samples) was placed in 4 ml of PBS. Released drug was partitioned into 4 ml of 1-octanol (layered on top of the PBS) and quantitated by UV/VIS absorbance at 232 nm.](image-url)
observed. In the control mice ($n = 7$), tumor volume continued to increase after injection to a mean of $398 \pm 37 \text{ mm}^3$ by week 8, as shown in Fig. 4A. These tumors grew rapidly to over $1000 \text{ mm}^3$. Accurate LNCaP tumor volume measurements were difficult after paste injection because of persisting paste in the tumor bed. In the early-phase treatment group, in which animals were treated 3 weeks after castration ($n = 7$; Fig. 4B), tumor decreased in volume from a preinjection mean of $43 \pm 19 \text{ mm}^3$ three weeks after castration to nonpalpable by 7 weeks after castration. By week 11, there was still no evidence of tumor growth. In the late-phase treatment group ($n = 5$; Fig. 4C), tumor decreased in volume from $233 \pm 136 \text{ mm}^3$ to nonpalpable within 2 weeks of treatment with paclitaxel paste. These tumors remained nonpalpable at week 16. On exposure of the tissue area around the implants after this time (at the time of sacrifice), there was no visible evidence of residual tumor tissue. Although histological analysis of the tissue from this area might be performed to confirm the full extinction of tumor growth, this was not possible in these mice. There was no clear boundary between the tissue and the implant; therefore, collection of all of the appropriate tissue was not possible. The nonpalpability of the tumors some 11 weeks after treatment may be considered strong evidence of the extinction of the tumors. This is further supported by nondetectable serum PSA levels at the later stages of the experiments.

It was considered possible that the drug might be released from the polymer into the systemic circulation and that systemic drug (not locally released drug) might be responsible for the sustained decrease in serum PSA levels. After treatment of mice by injection of drug-loaded paste at a remote location away from the tumor, serum PSA levels and tumor volumes continued to rise steadily. Five weeks after this remote injection treatment, serum PSA had increased by 98% (mean value), and tumor volumes had increased by 113%. These data clearly demonstrated that any paclitaxel released into the systemic circulation was not responsible for the efficacy of the intratumoral paclitaxel formulation observed in the early- and late-treatment groups.
Radical prostatectomy or radiotherapy remain the only curative treatment options for clinically localized prostate cancer, whereas androgen ablation therapy is the only effective form of systemic therapy for men with advanced disease. Measurement of PSA levels in the early detection of prostate cancer has produced a stage migration and a 50% decrease in the incidence of stage D2 disease over the last 2 years (21). Almost 90% of men diagnosed with prostate cancer in North America present with localized disease (4, 5). Although refinements in the treatment of localized disease have significantly reduced the morbidity of therapy, alternative, less invasive forms of therapy are more appropriate for men with life expectancies of less than 10 years or those with low-volume, low-grade disease, which is more likely to have an indolent natural history. Furthermore, use of serum PSA levels to detect biochemical recurrences after radiotherapy identifies men destined to experience disease recurrence who may benefit from adjuvant systemic or local therapy. Salvage prostatectomy in radiation failures is technically difficult and is associated with high rates of urinary incontinence and rectal injury (22). Similarly, addition of cryotherapy in radiation failures is associated with low success rates and a 30% risk of urinary incontinence (23). Hence, new treatment strategies using less invasive local treatments are required for patients with localized prostate cancer who are not candidates for radical therapy or for patients who develop a local recurrence after radical therapy to reduce the risk of local and/or metastatic progression of the disease.

Despite several hundred clinical studies of both experimental and approved single agents, systemic chemotherapy has limited antitumor activity in hormone-refractory prostate cancer (24). The poor response to systemic chemotherapy is seen in part because patients are generally old with poor performance status, which compromises their chemotherapeutic schedules. Estramustine and vinblastine, which inhibit microtubule function, have shown some efficacy against prostate cancer both in vitro (7, 9, 24) and in vivo (25–27). Paclitaxel, an anticancer agent isolated from the bark of Taxus brevifolia, has a broad range of antineoplastic activity and a unique mechanism of action involving stabilization of microtubules and angiogenesis inhibition (28, 29). Paclitaxel has shown efficacy against advanced breast, ovarian, and non-small cell lung cancer (25). Recently, paclitaxel has been reported to inhibit human prostate cancer cell growth in vitro (7–9). In this study, we have shown that paclitaxel induced apoptosis in the human prostate cancer cell line LNCaP with an IC50 of about 0.1 nM, confirming a previous report that described susceptibility of this cell line to paclitaxel at 10 μM (8). The reduced tolerance to systemic chemotherapy in elderly patients may be circumvented by developing methods to deliver high and sustained concentrations of cytotoxic agents within the local tumor environment.

Walter et al. (30) described the effectiveness of a paclitaxel-loaded polymeric implant placed besides brain tumors or within tumor resection sites in rats after invasive surgery. We have previously described the effective use of biocompatible, biodegradable polymeric pastes for the site-directed delivery of antineoplastic agents such as paclitaxel (15) or bis(maltolato)oxovanadium (31). These surgical pastes were originally designed as an adjunct to tumor resection therapy whereby a residual slow-release formulation of the drug would be applied to the resection site to prevent tumor regrowth. Such pastes were composed of PCL blended with MePEG and applied as a viscous molten paste at 56°C setting to a solid drug-polymer implant at body temperature. Pilot studies using this paclitaxel-loaded polycaprolactone paste applied intratumorally in the s.c. LNCaP tumors in mice were successful in reducing both serum PSA levels and tumor volume.6 However, the paste was very difficult to inject because of the viscosity of the polymer, and some large tumors failed to respond fully to the drug implant, probably due to the very slow release of the formulation (15).

The polymeric paste formulation used in this study is a viscous liquid or paste at room temperature that may be injected through a small-gauge needle and that solidifies within 1 h in vivo. This forms a controlled-release implant that releases the drug locally at the site of action. In vitro studies showed that the formulation released paclitaxel at a rate of approximately 1% of the total encapsulated drug per day (Fig. 2). This novel polymeric injection vehicle takes advantage of the hydrophobic nature of paclitaxel, which facilitates dissolution of the drug in the hydrophobic PLC-PEG-PLC/McPEG blend. On injection into an aqueous compartment, the MePEG partitions out of the blend, and the residual PLC-PEG-PLC/paclitaxel components solidify. This solidification arises from two factors, the increasing melting point of the blend (due to the decreasing concentration of the low melting

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point McPEG component) and the partial precipitation of the paclitaxel in the blend as the concentration of the drug increases. The high affinity of the drug for the hydrophobic matrix and the low solubility of the drug in the aqueous tissue compartment lead to the controlled release of the drug from the semisolid implant. In vitro studies in aqueous buffer, 10% paclitaxel-loaded paste showed evidence of mass loss and of diminished structural integrity after 2 weeks. The paste completely degraded in 3–4 months. After s.c. injection/implantation in tumors, there was evidence of residual paste at week 8 in both the early- and late-phase treatment groups, indicating that degradation was incomplete in vivo at that time.

These studies were designed to assess the efficacy of an injectable slow-release depot formulation of paclitaxel against human tumors grown in mice. This system does not directly model the clinical situation for human prostate cancer patients because the tumor is not growing in the prostate gland, and the tumor has not been subjected to radiation therapy. However, this animal model may reflect a potential treatment method for patients with recurrent prostate cancer, using androgen ablation with an adjuvant cytotoxic agent. Serum PSA levels in this s.c. LNCaP model have been shown to be directly related to tumor volume (17) and therefore provide an additional surrogate marker to monitor tumor progression and treatment efficacy. Serum PSA levels and tumor volume in the early- and late-phase (androgen-independent)-treatment groups decreased after paclitaxel paste injection but continued to increase in control mice. The therapeutic efficacy of paclitaxel paste resulted from the induction of apoptosis in the LNCaP tumors, which was documented both in vitro and in vivo. Although paclitaxel has been previously reported to inhibit endothelial cell growth and to have antiangiogenic effects (10, 11), the apoptotic effects of paclitaxel on LNCaP tumor cells probably represent the major inhibitory effect of the drug on tumor growth described in this study. Future studies may investigate the effect of paclitaxel on tumor-associated angiogenesis in mice. Local ulceration was present in some mice, probably due to irritation and scratching of the skin followed by paclitaxel inhibition of wound healing, as has been reported previously for this drug (32, 33).

In summary, we have provided preclinical efficacy data and proof of principle for a site-directed, injectable, controlled-release formulation of paclitaxel as an effective treatment for localized prostate tumors. The paste formulation released paclitaxel into local tumor tissues and induced apoptotic regression of androgen-independent LNCaP prostate tumors. Although the study was performed in mice, we believe that the paclitaxel paste may offer a potentially effective therapy for patients with localized tumors recurrting after radiotherapy and for some patients with localized tumors who are not candidates for radical treatment. Future studies may investigate combination therapy of the paste with radiation in LNCaP tumors grown in mice and issues of toxicity.

REFERENCES

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