Enhanced Efficacy of a Novel Controlled Release Paclitaxel Formulation (PACLIMER Delivery System) for Local-Regional Therapy of Lung Cancer Tumor Nodules in Mice


Lung Cancer Program and Division of Pulmonary/Allergy/Critical Care Medicine, University of Alabama at Birmingham, Birmingham, Alabama 35294-0007 [E. H., R. I. G.], and Guilford Pharmaceuticals, Inc., [W. D., R. G. L.], Baltimore, Maryland 21224

ABSTRACT

The efficacy of systemic chemotherapy for non-small cell lung cancer (NSCLC) has improved with newer agents. However, the response rates and prolonged survival times achieved by chemotherapy remain modest, and these small gains are obtained at the cost of significant toxicity. In this study, the efficacy of a controlled release formulation of paclitaxel was compared with conventional paclitaxel in animals with human lung cancer xenografts. Paclitaxel (10%) was encapsulated in a proprietary polymer in the form of microspheres (PACLIMER Delivery System). Tumor nodules comprised of two different cell lines (A549 and H1299) were treated by a single i.p. or intratumoral administration of conventionally formulated paclitaxel or a single intratumoral injection of the PACLIMER Delivery System. In vitro testing demonstrated that paclitaxel was released slowly from the microspheres with >80% released after 90 days. Direct comparison of the highest dose for all formulations (24 mg/kg) showed that for nodules comprised of either NSCLC cell line, growth of the PACLIMER Delivery System-treated nodules were inhibited significantly more than the groups treated with conventional paclitaxel or the vehicle controls. Tumor volume doubling times for A549 and H1299 nodules treated with PACLIMER Delivery System were 60 and 35 days, respectively, compared with 10 and 11 days, respectively, in the nodules treated with the conventional paclitaxel by intratumoral administration. We conclude that intratumoral administration of the PACLIMER Delivery System may substantially increase the efficacy of paclitaxel for the therapy of local-regional NSCLC.

INTRODUCTION

NSCLC is one of the leading causes of cancer-related mortality and morbidity in the developed world. Importantly, large numbers of NSCLC patients have disease that is limited to the thorax, where more effective local therapies could impact significantly on mortality and morbidity. For example, in a large autopsy study reported by Matthews (1), 48% of individuals dying with the squamous cell variant of NSCLC had disease limited to the thorax. In the same study, 18% of adenocarcinoma NSCLC patients had disease limited to the thorax (1). In this context, the bronchioloalveolar cell variant of NSCLC has also been found to metastasize less commonly than typical adenocarcinoma beyond the thorax (2, 3). Furthermore, among the large fraction of surgically treated patients that experience recurrences, two large studies have demonstrated that 34–41% of NSCLC recurrences are within the thorax (4, 5).

In recognition of the importance of extensive or metastatic intrathoracic NSCLC that is not amenable to resection, conventional local-regional therapies have long been a mainstay of therapy. The most widely used form of local-regional therapy for intrathoracic NSCLC is external beam radiotherapy. In the years 1990–1995, based on the National Cancer Institute database analysis, 45% of NSCLC patients were treated with external beam radiotherapy (6). The well-recognized limitations of external beam radiotherapy include normal lung parenchyma damage that is poorly tolerated by the significant fraction of NSCLC patients with advanced chronic obstructive pulmonary disease.

Paclitaxel is one of the newer chemotherapy agents that has been used for extensive local-regional NSCLC. Paclitaxel acts in part by reversibly binding microtubules (7, 8) at the NH2-terminal region of the β-tubulin subunit (9, 10). The binding of the drug to the β-tubulin subunit interferes with microtubule depolymerization, although the precise changes in relative mass of polymerized versus depolymerized tubulin subunits is concentration dependent (11–13). Importantly, the activity of paclitaxel appears to be independent of the p53 status of the cells (14–17). The observation that paclitaxel was more cytotoxic toward actively dividing cells (18, 19) and that cells arrested at the G2-M phase (20, 21) initially suggested a straightforward mechanism of toxicity. Additional studies have shown clearly that the consequences of paclitaxel-mediated microtubule stabilization extend beyond the direct arrest of mitosis to include the modulation of apoptosis-regulating proteins. Several investiga-
tors have shown that paclitaxel leads to the phosphorylation of bcl-2 (22–24) and that the phosphorylated bcl-2 is inactivated (25).

Several mechanisms of paclitaxel resistance have been described. Because a primary target of the drug is the β-tubulin molecule, investigators have directed studies toward variations in the tubulin molecules, finding that many resistant cells have distinct changes in the relative expression of β-tubulin isoforms (26–28). In addition, mutant β-tubulin molecules have been identified in resistant cells (29). Paclitaxel resistance has also been found to be associated with overexpression of HER2/neu (30) and increased levels of the membrane scaffolding protein, caveolin-1 (31). The role of drug transport molecules in paclitaxel resistance is less clear at this time. Knockout mice without mdr-1 have impaired clearance of paclitaxel from the gut (32, 33), but the clinical relevance is unclear (34).

Although systemically administered paclitaxel has been shown to have efficacy for NSCLC, the effect is modest, as evidenced by a review that found a mean partial plus complete response rate of 26% in nine separate studies of NSCLC (35). We hypothesized that a controlled release formulation of paclitaxel (PACLIMER Delivery System) delivered into NSCLC tumors would be more effective than the same dose of conventionally formulated paclitaxel. Animal testing with engrafted tumor nodules showed that PACLIMER Delivery System was superior to both systemic and intratumoral conventionally formulated paclitaxel.

**MATERIALS AND METHODS**

**Cell Lines.** Human NSCLC cell lines A549, H1299, H838, H1650, H358, and A427 were obtained directly from the American Type Culture Collection (Rockville, MD). All cell lines were grown in DMEM/F-12 medium (Mediatech, Herndon, VA) supplemented with 10% fetal bovine serum (growth medium) under antibiotic-free conditions at 37°C in a 5% CO2 atmosphere.

**In Vitro Sensitivity Assays.** The growth-inhibitory effects of paclitaxel on each of the human NSCLC cell lines were quantified by a colorimetric assay. Cells were seeded at 5 × 103 cells/well in 96-well microtiter plates and allowed to attach overnight. Paclitaxel (Sigma-Aldrich, St. Louis, MO) was dissolved in DMSO at a stock concentration of 5 mg/ml. Serial dilutions were made with growth medium and added to the wells to achieve concentrations of 0–1000 nM. Two days later, cell growth was quantified using the reagents and instructions of the CellTiter 96 Aqueous Non-Radioactive Cell Proliferation assay (Promega Corp., Madison, WI).

**Paclitaxel Formulations.** The PACLIMER Delivery System was made by incorporating paclitaxel in a biodegradable polyphosphoester polymer, p(DAPG-EOP), in the form of microspheres at 10% (w/w) loading through a solvent evaporation process. Briefly, paclitaxel and p(DAPG-EOP) were dissolved in ethyl acetate and pumped through an in-line homogenizer with 0.5% PVA solution to a container with an overhead stirrer. After the microspheres hardened, they were filtered and lyophilized.

**Clinical-grade paclitaxel (Hausser, Boulder, CO) was conventionally formulated for the tumor treatments by dissolving it in ethanol/Cremophor-EL (1:1), and then diluting it to the proper concentrations with 0.9% NaCl so that the injection volume was comparable for all groups.

**In Vitro Characterization of PACLIMER.** The size of the PACLIMER Delivery System microspheres was determined by a single particle optical sensing system (Model 770; Particle Sizing Systems, Langhome, PA). Paclitaxel release from the PACLIMER Delivery System microspheres was quantified over time by an *in vitro* release assay. Approximately 10–20 mg of PACLIMER Delivery System microspheres were incubated in 60 ml of PBS (pH 7.4) at 37°C. To maintain a sink condition, 2

**Table 1 In vitro growth inhibition of NSCLC cell lines by paclitaxel**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>GI50 (nM)</th>
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<tbody>
<tr>
<td>A549</td>
<td>40.5</td>
</tr>
<tr>
<td>H1299</td>
<td>58</td>
</tr>
<tr>
<td>H838</td>
<td>19.5</td>
</tr>
<tr>
<td>H358</td>
<td>29.3</td>
</tr>
<tr>
<td>H1650</td>
<td>26.5</td>
</tr>
<tr>
<td>A427</td>
<td>22.5</td>
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</tbody>
</table>

**Fig. 1 PACLIMER Delivery System composition.** Shown is a scanning electron micrograph and a schematic diagram of the PACLIMER Delivery System. *Legend* to the right identifies the two components illustrated as solid and gray circles within the schematic cross-section of the microspheres.

**Fig. 2 In vitro release of paclitaxel from the PACLIMER Delivery System.** Shown are the amounts of paclitaxel released from the PACLIMER Delivery System as a function of time. *Abscissa,* time in days; *ordinate,* cumulative percentage of paclitaxel released. *Bars,* SE.
ml of octanol were placed on top of the PBS layer to continuously extract the released paclitaxel from the PBS. At specific time points, octanol was removed, and a fresh 5 ml of octanol were layered on top of the PBS. Paclitaxel presented in the octanol was assayed by high-performance liquid chromatography analysis (mobile phase: 60% acetonitrile in water for 10 min, then hold for 0.5 min, followed by 40% acetonitrile for 1.5 min) after a 1:3 dilution with acetonitrile.

Fig. 3  Time-dependent changes in tumor nodule sizes after different treatments. All data points shown here are the mean of two experiments; bars, SE. All of the groups shown in the three graphs for each cell line were performed simultaneously in each experiment but are presented on three different graphs for each cell line for clarity. A, A549 nodules treated with PACLIMER Delivery System (PLM) 24 mg/kg compared with the i.p. conventional paclitaxel vehicle (IP PTX vehicle), i.t. conventional paclitaxel vehicle (IT PTX vehicle), and i.t. PACLIMER Delivery System vehicle (IT PLM vehicle). B, same as A with H1299 tumor nodules. C, A549 nodules treated with PACLIMER Delivery System (PLM) 24 mg/kg compared with PACLIMER Delivery System 12.5 mg/kg or 4 mg/kg doses. D, same as C with H1299 tumor nodules. E, A549 nodules treated with paclitaxel 24 mg/kg by conventional formulation via i.p. administration (IP PTX) or i.t. administration (IT PTX), or PACLIMER Delivery System via i.t. administration (IT PLM). F, same as E with H1299 tumor nodules.
Animal Model and Testing. Athymic nude Balb/c mice engrafted with human NSCLC cell lines A549 and H1299 were used to quantify the relative efficacy of the different paclitaxel formulations and routes of administration. All of the animal experiments were performed in full compliance with a protocol approved by the University of Alabama at Birmingham Animal Use Committee. Engraftment methods were exactly as we have described previously (36). Tumor nodules were allowed to grow to an estimated volume of 200–300 mm$^3$ prior to treatment; tumor volumes were estimated using the formula of length $\times$ width $\times$ height directly measured with calipers. Each animal was weighed at the time of treatment so that dosages could be adjusted to achieve the mg/kg amounts reported. For i.p. injections, animals received a total injection volume of $\sim 0.1$ ml. For i.t. injections, animals received a single injection volume of $\sim 0.1$ ml with a 21-gauge needle placed in the center of the tumor. The i.t. injections were infused over 10–15 s, and the needle was allowed to remain in place for an additional 15–20 s before removal. After the treatments, all mice were tagged, and tumors were measured three times weekly with calipers. Animals were weighed once a week.

RESULTS

The PACLIMER Delivery System is a novel, controlled release formulation of paclitaxel (Fig. 1). In this form, paclitaxel was incorporated into the biodegradable polymer p(DAPG-EOP) as a solid solution in the form of microspheres, i.e., the drug molecules were homogeneously dispersed among the polymer molecules. Paclitaxel was released from the PACLIMER Delivery System primarily by degradation of the polymer. The PACLIMER Delivery System microspheres manufactured for the experiments reported here were first characterized for size and release characteristics in vitro. The microspheres were found to range in size from 20 to 200 $\mu$m in diameter, with a median diameter of 53 $\mu$m (see “Materials and Methods”). An in vitro release assay showed that the encapsulated paclitaxel was continuously released over a 90-day period of time at a rate of $\sim 1$–2% per day (Fig. 2).

As a first step toward testing the efficacy of the PACLIMER Delivery System on NSCLC cell lines, the paclitaxel sensitivities of several NSCLC cell lines were determined by in vitro testing (Table 1). The growth of all six cell lines was comparably inhibited by paclitaxel with concentrations causing a 50% reduction in growth (GI$_{50}$) ranging from 19.5 to 40.5 $\mu$m. On the basis of these findings, the A549 and H1299 cell lines were chosen for further testing in the animal model experiments.

Tumor nodules formed by s.c. engraftment of the two NSCLC cell lines were treated with various formulations of paclitaxel (Fig. 3). Three vehicle control groups were used (i.e., conventional formulation vehicle, i.e. conventional formulation vehicle, and i.t. PACLIMER Delivery System vehicle consisting of microspheres without drug), and all of three vehicle controls had comparable rates of steady growth throughout the 30-day posttreatment period in both cell lines (Fig. 3, A and B). For both the A549 and H1299 tumor nodules, the PACLIMER Delivery System dose of 24 mg/kg produced the greatest reduction in tumor growth compared with the PACLIMER Delivery System at doses of 12.5 and 4 mg/kg (Fig. 3, C and D). The i.p. and i.t. administration of conventionally formulated paclitaxel at 24 mg/kg produced a minimal reduction in growth rate that was not significantly different from the corresponding vehicle controls. In striking contrast, the PACLIMER Delivery System 24 mg/kg group had a marked reduction in growth rate compared with both of the conventional paclitaxel groups of 24 mg/kg (Fig. 3, E and F). For the A549 tumor nodules, the difference between the PACLIMER Delivery System and i.t. paclitaxel was highly significant ($P < 0.006$), as was the difference between the PACLIMER Delivery System and i.p. paclitaxel ($P < 0.0008$) as determined by the two-tailed $t$ test. For the H1299 tumor nodules, the difference between the PACLIMER Delivery System and i.t. paclitaxel was also significant ($P < 0.04$), as was the difference between the PACLIMER Delivery System and i.p. paclitaxel ($P < 0.04$). Animal weights increased in all of the groups over time without significant differences between groups (not shown), and none of the treatment groups were associated with any overt toxicity.

The tumor volume doubling time was determined from the tumor measurements in all treatment groups (Fig. 4). In the A549 cells, the doubling time of the PACLIMER Delivery System 24 mg/kg group was estimated at 60 ± 9.4 days, compared with 11.5 ± 2.3 and 10.2 ± 4.7 days for the paclitaxel 24 mg/kg given via the i.p. and i.t. routes, respectively. The H1299 cell doubling time in the PACLIMER Delivery System 24 mg/kg group was estimated to be 35 ± 8 days, compared with 12 ± 1.9 and 11.2 ± 1.9 days for the paclitaxel 24 mg/kg given via the i.p. and i.t. routes, respectively.

DISCUSSION

In the experiments reported here, conventionally formulated paclitaxel was compared with a novel, proprietary, controlled release formulation of paclitaxel that is designated as the PACLIMER Delivery System. The PACLIMER Delivery System in this study was in the form of injectable microspheres that were intended for direct, i.t. injection. The experiments compared a single i.t. administration of PACLIMER Delivery System to equivalent dosing with conventionally formulated paclitaxel that was given locally as an i.t. injection or systemically by i.p. injection. In both NSCLC cell lines, the PACLIMER Delivery System caused a sustained, significantly greater reduction in tumor nodule growth compared with the conventional paclitaxel.

The PACLIMER Delivery System dosing was based on the total amount of paclitaxel within the microspheres, not on the amount of drug actually released into the tumor masses. Although paclitaxel release over time within the tumor nodules was not measured in these experiments, the in vitro release data (see Fig. 2) suggest that the peak paclitaxel levels in the PACLIMER Delivery System-treated animals were lower than in the groups receiving the conventionally formulated paclitaxel at 24 mg/kg. The lower doses of the PACLIMER Delivery System, i.e., 12.5 and 4 mg/kg, did not appear to have any efficacy. In the context of the numbers of animals in each group ($n = 6$), it is possible that lesser amounts of growth inhibition at the lower doses may not have been detectable by this experimental design. Higher doses of the PACLIMER Delivery System were not investigated, in large part because of anticipated
difficulties in administering the larger volume required into the tumor nodule sizes studied here. These findings are consistent with previous reports that the sustained exposure of tumor cells to low concentrations of paclitaxel can be more efficacious than shorter exposure to higher concentrations (37, 38). It seems reasonable to speculate that the local release achieved with the PACLIMER Delivery System would be less likely to cause systemic toxicity than continuous systemic administration of conventionally formulated paclitaxel over similar periods of time.

Others have described previously alternative sustained release formulations of paclitaxel with different release characteristics. Dordunoo et al. (39) encapsulated paclitaxel in poly(ep-silon-caprolactone) microspheres, finding that only 25% of the drug was released in 6 weeks in in vitro assays. Wang et al. (40) used poly(lactic-co-glycolic acid) microspheres for the encapsulation of paclitaxel and found a relatively constant release rate over 3 weeks in vitro but did not evaluate their formulation in vivo. Park et al. (41) encapsulated paclitaxel in polyanhydride discs but described the release rate as too slow for clinical utility. In contrast, Fung et al. (42) described the encapsulation of paclitaxel in a polyanhydride pellet that was implanted in normal mouse brains, where high levels of paclitaxel were detected within 3 mm of the pellet with lower doses detectable for up to 5 cm from the pellet. It is important to realize that all of these formulations described previously differed significantly from that used in this report on the basis of formulation compositions and release characteristics. Furthermore, none of these prior studies have reported the relative efficacy of their specific formulations for i.t. use in comparison with conventionally formulated paclitaxel.

Systemic chemotherapy, even with the taxanes, has had...
limited success in the treatment of NSCLC. The results of the preclinical animal experiments presented here suggest a new paradigm for local-regional chemotherapy of NSCLC that capitalizes on the local, controlled delivery of paclitaxel via the PACLIMER Delivery System. The PACLIMER Delivery System could be administered via percutaneous needles directed into parenchymal masses by fluoroscopic or computed tomographic guidance. Alternatively, the PACLIMER Delivery System could be administered via the endobronchial route using a bronchoscopically directed needle. We conclude that these results support the development of human studies for the assessment of safety and efficacy of PACLIMER Delivery System therapy of local-regional NSCLC.

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