Translymphatic Chemotherapy by Intrapleural Placement of Gelatin Sponge Containing Biodegradable Paclitaxel Colloids Controls Lymphatic Metastasis in Lung Cancer

Jiang Liu, Dale Meisner, Elizabeth Kwong, Xiao Y. Wu, and Michael R. Johnston

Abstract

As a means of treating lymphatic metastasis from lung cancer, the pharmacokinetics and therapeutic effects of an intrapleural (ipl) implantable drug delivery system consisting of a gelatin sponge impregnated with polylactide-co-glycolide paclitaxel (PLGA-PTX) microspheres were studied. PLGA-PTX with 7% (w/w) drug loading were incorporated into gelatin matrix. The pharmacokinetics were studied in rats with one of the following regimens: (a) Taxol 8 mg/kg by i.v. injection; (b) Taxol 8 mg/kg ipl; (c) PLGA-PTX (100 mg/kg) ipl; (d) sponge containing PLGA-PTX (100 mg/kg) ipl. PTX concentrations in lymph node and plasma were determined by liquid chromatography mass spectrometry, and the area under the curve (AUC) was calculated. Therapeutic efficacy was assessed in an orthotopic lung cancer model with tumor resection 14 days following tumor implantation. Animals were randomized to ipl placement of PLGA-PTX sponge, placebo sponge, or no treatment. Lymph node metastases were examined at 32 d. The results show that the mediastinal lymph node AUC was significantly higher with ipl placement of PLGA-PTX sponge compared with i.v. and ipl administration of Taxol. This represents 100- to 400-fold increase of lymphatic drug exposure compared with i.v. dosing. Peak plasma concentration was significantly reduced in the PLGA-PTX sponge group compared with i.v. dosing. PLGA-PTX particles were microscopically identified in lymphatic tissue and resulted in an 80% reduction of lymphatic metastasis compared with controls. Translymphatic-targeted drug delivery significantly decreases lymphatic metastasis in an orthotopic lung cancer model. This effect may be attributable to the improved distribution of PTX to the lymphatic system. [Cancer Res 2009;69(3):1174–81]

Introduction

Lung cancer is a devastating disease in both men and women. It kills more people than breast cancer, colon cancer, and prostate cancer combined. Despite a rapid development of various existing and novel anticancer agents, chemotherapy given by the conventional i.v. route has only achieved modest responses and survival benefits but at the cost of significant toxicity (1). Lymph node involvement is the single strongest prognostic factor for lung cancer in the absence of systemic metastasis (2), and eradication of metastatic tumor cells from the regional lymphatic system, including lymphatic vessels and lymph nodes, poses a significant challenge in the treatment of lung cancer. Even patients with early disease who have undergone potentially curative surgery still have a significant incidence of recurrence and subsequent death (3). Approximately 50% of patients with pathologic Stage I or Stage II non–small cell lung cancer (NSCLC) develop recurrent disease, in part, attributable to lymphatic micrometastasis (4).

To control lymphatic metastasis, anticancer agents must be delivered in tumoridal concentrations from the site of application to the site of action (tumor involved lymph nodes or the lymphatics where tumor cells potentially dwell). Conventional chemotherapy may not effectively enter into the lymphatic system because of dose-limiting toxicities and failure to access lymphatics from the i.v. route, presumably because of a “blood-lymph barrier”. The blood-lymph barrier was first described by Fischer-Brugge and colleagues (5) in 1950. Subsequently, Grotte (6, 7) examined the molecular sieving property of the blood-lymph barrier using dextrans. The transport of dextran from plasma into lymph was highly selective, depending on the size of the molecule. However, the significance of a blood-lymph barrier has been largely ignored in cancer research and treatment. Lymphatic drug delivery becomes even more compromised after extensive cancer surgery due to a disruption of the blood supply to the lymphatic tissue.

The highly effective physiologic function of the lymphatic system in clearing foreign particulate matter provides a solid rationale for using colloidal particulates as drug transport vehicles to target the lymphatic system. Colloidal drug delivery systems may thus improve the therapeutic effect of both existing and novel anticancer agents.

We have explored the intrapleural (ipl) administration of colloidal particulates as an alternate route to target thoracic lymphatics (8), and developed a biodegradable polymeric micro-particulate lymphatic targeting system (9). The system is composed of a proprietary formulation of gelatin sponge containing polylactide-co-glycolide paclitaxel (PTX) microspheres (PLGA-PTX). This system showed sustained drug release properties in vitro and exhibited lymphatic targeting capability in rat models (9). Its proposed initial clinical application involves intraoperative placement of a PLGA-PTX sponge into the surgical field in close proximity to mediastinal lymphatics after resection of the primary lung cancer. PLGA-PTX microparticles are released as the sponge matrix disintegrates. Theses particles are then selectively taken up into lymphatic channels and delivered to regional lymph nodes where they slowly release PTX to treat potential occult metastatic disease.
PTX has gained widespread use in the treatment of a variety of carcinomas and has become a first line chemotherapeutic agent for NSCLC. However, there is a lack of pharmacokinetic data with regard to its lymphatic distribution especially through ipl administration. The only pharmacokinetic study of intrapleurally administered PTX was its use in patients with malignant pleural effusions (10).

The goal of the present study was to investigate the lymphatic and systemic distribution of PTX given intrapleurally to rats using the PLGA-PTX sponge system compared with that of the conventional formulation of PTX (Taxol) given i.v. and ipl. The therapeutic efficacy of the system was then assessed in an orthotopic lung cancer model.

Materials and Methods

Chemicals and Reagents

Injectable Taxol was obtained from Bristol-Myers Squibb. PLGA-PTX microspheres, and gelatin sponges containing PLGA-PTX microspheres (PLGA-PTX sponge) were prepared according to the method described previously (9). The mean size of the microspheres was 3.5 ± 2.1 μm, which is considered to be suitable for targeting regional lymph nodes via ipl administration (11, 12). The formulated PLGA-PTX microspheres, with a size of less than 5 μm, were then incorporated into a noncross-linked gelatin matrix to form an implantable sponge device. High performance liquid chromatography (HPLC) grade acetonitrile, trifluoroacetic acid (TFA), and all other chemicals were obtained from Sigma Aldrich Chemical.

Animals

Female Sprague-Dawley rats weighing ~250 grams and 4-wk-old male Rowett nude rats were obtained from Charles River Laboratories, Inc. Animals were maintained under specific pathogen-free conditions in microisolation cages under controlled light, temperature, and humidity. All animals were fed autoclaved food and water ad libitum. Animals were euthanized by CO2/O2 asphyxiation after the experiments. Before the animal study, institutional animal care approval was obtained from University Health Network.

Drug Administration

Three types of paclitaxel dosage forms were used in the study: injectable Taxol (30 mg/5 mL), PLGA-PTX microspheres, and PLGA-PTX microspheres imbedded in the gelatin sponge. The drug administration includes i.v. and ipl administration of Taxol; ipl administration of PLGA-PTX suspension; ipl implantation of PLGA-PTX sponge.

For i.v. dosing, Taxol was diluted with an equal volume of saline for tail vein injection. The drug was slowly administered over 8 min per injection. For ipl administration, Taxol and PLGA-PTX suspension were given into the pleural cavity under isoflurane inhalation anesthesia by means of a transthoracic procedure described previously (8). Taxol was administered without further dilution, whereas PLGA-PTX microspheres were dispersed in injectable water with 0.1% Tween 80. The volume of ipl injection was limited to 1.5 mL.

For ipl sponge implantation, the PLGA-PTX sponge was placed into the pleural space through a left thoracotomy as described in our previous study (9). In brief, a 1.5-cm incision was made over the fifth intercostal space into the pleural cavity. The gelatin sponge containing PLGA-PTX was placed along the mediastinal pleura. The rib cage was reapproximated with 4-0 silk suture and the skin incision closed with wound clips.

Experimental Design of Pharmacokinetic Study

Eighty four rats were randomized into four experimental groups:

Group I: Taxol given i.v.
Group II: Taxol given ipl.
Group III: PLGA-PTX given ipl.
Group IV: PLGA-PTX sponge given ipl.

The dose of Taxol chosen for group I and group II was 8 mg/kg, which approximates the LD50 of Taxol on quick i.v. bolus in Sprague-Dawley rats (13). The dose of PLGA-PTX chosen for group III and group IV was 100 mg/kg (7 mg/kg PTX). Each group was further divided according to the sampling times after chemotherapy administration. The sampling times for group I and group II were 1, 3, 8, 12, 24, 48 h, and 7 d. Based on our in vitro drug release profile (9), we anticipated that drug release would last for at least several weeks. Thus, the sampling times for group III and group IV were 12, 24 h, 3 d, 1, 2, 3, and 4 wk. At each time point (n = 3), drug concentrations were determined in the left mediastinal lymph nodes (LLN), right mediastinal lymph nodes (RLN), and plasma. PTX quantitation was performed using liquid chromatography mass spectrometry (LC-MS/MS; Table 1).

Pharmacokinetic Analysis

Sample processing. Peripheral blood was collected into a heparinized vacutainer tube (BD). The blood was centrifuged at 1,500 g for 20 min to obtain plasma. The samples were stored at −20°C until analyzed. One hundred microliters of a plasma sample were mixed with 50 μL of internal standard (IS) docetaxel (5 μg/mL) then treated with 2.0 mL of acetonitrile and vortexed. After centrifugation at 3,200 g for 20 min, the organic layer was transferred to another polypropylene tube and evaporated. The residue was reconstituted in 200 μL of a 30% acetonitrile solution with 0.1% TFA. Both ipsilateral (LLN) and contralateral (RLN) mediastinal lymph nodes were collected when the animals were sacrificed. The samples were weighed and stored at −80°C. After adding 50 μL IS, the lymph nodes were homogenized (Polytron; PCU-11) in 5.0 mL of acetonitrile. The probe was washed with 1 mL of acetonitrile to recover any remaining drug after homogenization. The homogenate was centrifuged and the acetonitrile extract was removed and evaporated. The residue was redissolved in 400 μL of 30% acetonitrile with 0.1% TFA.

Table 1. Experimental design of pharmacokinetic study

<table>
<thead>
<tr>
<th>Study arms</th>
<th>Number of animals</th>
<th>Dose (PTX)</th>
<th>Sampling time</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Taxol i.v.)</td>
<td>n = 3 each time point</td>
<td>8 mg/kg (LD50)</td>
<td>1, 3, 8, 12, 24, 48 h, 7 d</td>
<td>Plasma, LLN, RLN</td>
</tr>
<tr>
<td>Group II (Taxol ipl)</td>
<td>n = 3 each time point</td>
<td>8 mg/kg (LD50)</td>
<td>1, 3, 8, 12, 24, 48 h, 7 d</td>
<td>Plasma, LLN, RLN</td>
</tr>
<tr>
<td>Group III (PLGA-PTX ipl)</td>
<td>n = 3 each time point</td>
<td>7 mg/kg (PLGA-PTX 100 mg/kg, 7% drug loading)</td>
<td>12, 24 h, 3 d, 1 wk, 2, 3, 4 wk</td>
<td>Plasma, LLN, RLN</td>
</tr>
<tr>
<td>Group IV (PLGA-PTX Sponge ipl)</td>
<td>n = 3 each time point</td>
<td>7 mg/kg (PLGA-PTX 100 mg/kg, 7% drug loading)</td>
<td>12, 24 h, 3 d, 1, 2, 3, 4 wk</td>
<td>Plasma, LLN, RLN</td>
</tr>
</tbody>
</table>

Abbreviation: LD50, median lethal dose.
LC-MS/MS. LC-MS/MS consists of an HPLC (Agilent) and the Applied Biosystems/Sciex QTrap LC-MS/MS mass spectrometer. Nitrogen gas was used as nebulizing gas, curtain gas, and collision gas. The spray voltage was set to 5,500 V, and the orifice voltage was set to 20 V. The mass spectrometer was operated in positive ion MRM mode with collision energy set to 35 V. Precursor ion selection was done with Q1 and product ions were selected by Q3. The analytes were monitored with a dwell time of 250 ms. The precursor/product ion pairs were 854 to 509.20 Da for paclitaxel, and 808.30 to 182 Da for the IS. Isocratic and C18 column were used for HPLC separation. The HPLC mobile phase was 50% acetonitrile with 1% acetic acid. The flow rate was set at 500 μL/min.

Preparation of stock solutions, calibration standards. Standard stock solutions of PTX were prepared in 50% acetonitrile (1 mg/mL) and stored at 4°C. Working solutions were prepared by diluting standard stock solutions to concentrations of PTX ranging from 20 ng/mL to 40 μg/mL. IS, docetaxel, was prepared at 5 μg/mL in 50% acetonitrile and stored at 4°C.

Calibration curves were prepared by spiking 100 μL blank donor plasma or tissue homogenate with appropriate amounts of PTX to give final concentrations of 5 ng/mL to 10 μg/mL (n = 10). Calibration curves were obtained by least-square linear regression, weighted by the reciprocal of the concentration, using the peak height ratio of drug to IS.

Extraction efficiency. Aliquots of the prepared stock solutions of PTX were added separately to 100 μL of plasma or lymph node tissue homogenate to yield concentrations of 0.025, 2.5, and 7.5 μg/mL. The samples were subjected to extraction as described above. Peak area ratios of the extracted and nonextracted samples were compared. Extraction recovery was determined as the mean ± SD of three samples.

H460 Orthotopic and Pneumonectomy Lung Cancer Models
An orthotopic lung cancer model previously reported with endobronchial implantation of NCI-H460 tumor cells was used in this study. The implanted cells produced a lung tumor with consistent mediastinal lymph node metastases but limited systemic tumor spread (14). Within 3 wk, all animals developed a primary lung tumor and mediastinal lymph node metastasis. For the present study, this model was expanded to include surgical resection of the primary lung tumor so that adjuvant therapies could be studied. Fourteen days after tumor cell implantation, the animals underwent left pneumonectomy to completely remove the primary tumor. At this time point, regional lymph node metastases were not grossly detectable. The animal was anesthetized by inhalation of 5% isoflurane then orally intubated with a 16-gauge angiocatheter (BD; Utah 84070) and placed in a right lateral decubitus position. The catheter was connected to a rodent ventilator delivering a tidal volume of 10 mL/kg at a rate of 80 to 100 breaths per minute, maintaining 5% isoflurane. The left side of the chest wall was cleaned, shaved, and sterilized with Betadine. A 1.5-cm anterolateral skin incision was made over the fifth intercostal space and was extended into the pleural cavity. The left hilum was then ligated with a 4-0 silk suture. The left lung was excised just distal to the ligature. The resection margin was cauterized. In some groups, a gelatin sponge was placed on the medial side of the mediastinum after pneumonectomy. The ribs were reapproximated with 4-0 silk suture and the incision was closed with wound clips. Animals were prophylactically treated with Augmentin (Beecham Labs) in drinking water at 0.35 mg/mL for 1 wk.

Therapeutic Efficacy Study
After successful left pneumonectomy, the animals were assigned to three experimental groups:

Group I: no treatment control (n = 10);
Group II: ipl implantation of placebo gelatin sponge as sham control (n = 5);
Group III: ipl implantation of gelatin sponge containing PLGA-PTX (100 mg/kg with 7% w/w PTX loading) (n = 10).

The mean animal body weight was 180 ± 20 grams at the time of surgery. Therefore, each treated animal received a sponge containing 18 mg of PLGA-PTX. Figure 1 illustrates the ipl sponge implantation after left pneumonectomy. The animals were sacrificed 32 d after pneumonectomy, at which time most control animals manifested overt evidence of tumor progression.

Assessment of lymph node metastasis. The presence of lymphatic metastasis and the number of all the mediastinal lymph nodes recovered were documented. The pattern of PLGA-PTX particle distribution and disintegration of the sponge were grossly assessed and photographed. Mediatinal lymph nodes and lymphatic tissue were removed, fixed in 10% buffered formalin, and embedded in paraffin. All tissues were serially sectioned and stained with H&E for microscopic examination to confirm the presence or absence of tumor. Any macroscopic or microscopic tumor deposit discovered was considered a metastasis. The primary end points were the incidence of lymph node metastasis and the volume of tumor contained within the lymph nodes, which was reflected by the lymph node weight.

Scanning and transmission electron microscopic examination. Block samples of parietal pleura were taken from the costal regions of PLGA-PTX sponge–treated animals. They were immersed in modified Karnovsky fixative for 2 h and were postfixed in cacodylate-buffered (pH 7.4) 1% osmium tetroxide for 1 h and dehydrated in a graded series of alcohol, dried by the t-butyl alcohol drying method, sputter coated, and viewed under an S-3400 SEM (Hitachi Ltd). For transmission electron microscopic examination (TEM), the pleura was cut into small pieces, immersed in the Karnovsky fixative for 2 h, and postfixed in cacodylate buffered (pH 7.4) 2% osmium tetroxide/1% potassium ferrocyanide for 2 h. They were then dehydrated in a graded series of ethanol and embedded in epoxy resin. Thin sections were cut, stained with methanolic uranyl acetate and aqueous lead citrate, and viewed under H-7000 TEM equipped with an AMT digital camera (Hitachi Ltd).

Figure 1. Illustration of ipl implantation of gelatin sponge containing PLGA-PTX microspheres after surgical resection (left pneumonectomy) of an orthotopic human H460 lung cancer in a nude rat. A, view of pleural cavity after left pneumonectomy; B, resected lung cancer; C, gelatin sponges containing PLGA-PTX microspheres; D, ipl implantation of the sponge.
Statistical Analysis

All data were presented as mean ± SE unless specified otherwise. Area under the concentration versus time curve (AUC) was determined using Prism version 4 (GraphPad Software). All pharmacokinetic data were compared between groups at each time point with the Mann-Whitney test (two tailed). For all statistical procedures, \( P \) values of <0.05 was considered significant. Comparison of lymph node weight was performed using ANOVA or unpaired Student’s \( t \) test. \( \chi^2 \) test was used to compare the incidence of metastasis between treatment and control arms.

Results

Assay validation characteristics. Spiked drug concentrations were linearly related to the peak area ratios of the drug versus IS over the concentration range from 5 ng/mL to 10 \( \mu \)g/mL. A typical correlation coefficient was >0.998. The average extraction recovery of paclitaxel from plasma and lymph node homogenate was 102.6% ± 4.6% and 59.4% ± 5.8%, respectively.

Paclitaxel distribution in the lymph node tissue. In the given experimental time, the AUC\(_{0-28d}\) for LLN was significantly higher with ipl administration of PLGA-PTX and gelatin sponge containing PLGA-PTX (6,904.3 ± 141.7 and 6,470.3 ± 211.5 \( \mu \)g-d/g, respectively) compared with that of i.v. and ipl administration of Taxol (15.95 ± 2.4 and 155.5 ± 10.9 \( \mu \)g-d/g, respectively; \( P < 0.01 \)). This represents a >400-fold increase in drug exposure to lymphatic tissue compared with i.v. administration of Taxol (Fig. 2A and B). Similarly, ipl administration of PLGA-PTX and sponge containing PLGA-PTX resulted in a significantly higher AUC\(_{0-28d}\) for RLN (1,028.3 ± 61.5 and 933.4 ± 85.9 \( \mu \)g-d/g, respectively; \( P < 0.01 \)). This represents approximately a 100-fold increase in drug exposure between PLGA-PTX and PLGA-PTX sponge treatment. However, the time to reach the peak concentration, \( T_{\text{max}} \), differed, with a peak concentration in ipsilateral and contralateral lymph nodes at 24 hours for PLGA-PTX and at 3 days for the PLGA-PTX sponge treatment.

Plasma profile of paclitaxel. Plasma pharmacokinetic data for rats treated with four different regimens are shown in Fig. 3A. The ipl dosing of PLGA-PTX and Taxol was well-tolerated. A few animals manifested acute respiratory distress after receiving slow i.v. injection of 8 mg/kg Taxol. But all animals survived during the predetermined time of the experiment. After i.v. and ipl dosing of 8 mg/kg Taxol, plasma paclitaxel concentration reached its peak at 1 and 3 hours, respectively, followed by an exponential decrease over 48 hours. By day 7, the PTX level in the plasma was undetectable. The C\(_{\text{max}}\) for i.v. and ipl dosing was 14.950 ± 1.610 \( \mu \)g/mL and 0.665 ± 0.085 \( \mu \)g/mL, respectively. In distinction, animals that received PLGA-PTX and the PLGA-PTX sponge had a slow increase in the plasma concentration and reached a C\(_{\text{max}}\) of

![Figure 2](https://example.com/figure2.png)

**Figure 2.** A, LLN paclitaxel concentration versus time profiles in various experimental arms (n = 3 for each time point); B, comparison of LLN AUC values (mean ± SE) with various experimental arms; C, RLN paclitaxel concentration versus time profiles in various experimental arms (n = 3 for each time point); D, comparison of RLN AUC values (mean ± SE) with various experimental arms. *, \( P < 0.01 \) (Mann-Whitney test).
Thirty-two of a total of 40 lymph nodes (80%) obtained (Fig. 4B) was significantly lower compared with the nontreatment controls between the two control groups. Figure 4C There was no difference in the pattern of lymph node metastasis involvement, all of which were in the contralateral mediastinum. 26 lymph nodes (15.4%) from the treatment group showed tumor metastases. Four of from control animals (28 from nontreatment controls and 12 from sham controls) were found to contain tumor metastases. Four of nontreatment controls (10 of 10) and sham controls (5 of 5; < 0.01; Fig. 4F). The incidence of lymphatic metastasis was significantly lower in the treatment group (2 of 10) compared with that of the sham controls (5 of 5; < 0.01; Fig. 4F). Efficacy of translymphatic chemotherapy in the H460 lung cancer model. Intrapleural implantation of a PLGA-PTX sponge as an adjuvant chemotherapy significantly reduced lymphatic tumor metastasis. The incidence of lymphatic metastasis was significantly lower in the treatment group (2 of 10) compared with that of the nontreatment controls (10 of 10) and sham controls (5 of 5; P < 0.01; Fig. 4A). The tumor burden of the cancerous lymph nodes was significantly lower compared with the nontreatment controls (Fig. 4B). Thirty-two of a total of 40 lymph nodes (80%) obtained from control animals (28 from nontreatment controls and 12 from sham controls) were found to contain tumor metastases. Four of 26 lymph nodes (15.4%) from the treatment group showed tumor involvement, all of which were in the contralateral mediastinum. There was no difference in the pattern of lymph node metastasis between the two control groups. Figure 4C and D show photographs of autopsies performed on control and treated animals, respectively.

Lymphatic absorption of PLGA-PTX microspheres. PLGA-PTX microspheres were evident microscopically in mediastinal lymph nodes retrieved from animals implanted with the PLGA-PTX sponge (white beads indicated by arrows in Fig. 5A). Scanning electron microscopic examination (SEM) confirmed that PLGA-PTX microspheres released from the sponge matrix into the pleural cavity were taken up into the lymphatic stomata of the parietal pleura (Fig. 5B) and penetrated into the subpleural space, which is rich in lymphatic lacunae (Fig. 5C). TEM revealed that PLGA-PTX microspheres passed the wall of the lymphatic lacuna to enter their lumen (Fig. 5D).

Discussion
In the present study, the pharmacokinetics of a proprietary controlled release formulation of paclitaxel, designated as the PLGA-PTX sponge system, was investigated in comparison with conventionally formulated Taxol, and the therapeutic efficacy of the system was further examined in an orthotopic lung cancer rat model. At a single relatively low dose of PTX (7 mg/kg), both PLGA-PTX microspheres and PLGA-PTX sponge placed within the pleural cavity resulted in a substantially greater lymphatic drug exposure compared with conventional Taxol (8 mg/kg) administered i.v. or ipl. The favorable lymphatic biodistribution of PTX is probably due to the selective lymphatic uptake of PLGA-PTX microspheres. This is supported by the histologic finding of PLGA-PTX microspheres in mediastinal lymphatic tissue up to 4 weeks after the sponge placement. Because ipl administration of PLGA-PTX and PLGA-PTX sponge result in similar pharmacokinetic profiles, it can be inferred that the noncross-linked gelatin sponge has limited effect on the microsphere release, except for an initial mild delay in T_max. The longer time to reach peak lymph node concentration with the sponge treatment probably reflects an additional process of particle release from the sponge matrix.

The dosing of PLGA-PTX and PLGA-PTX sponge system was based on the total amount of PTX within the microspheres, not on the amount of drug actually released into the lymphatic tissue. Accordingly, the total PTX recovered from the lymphatic tissue includes both released PTX and nonreleased PTX. Although PTX release over time was not measured in these experiments, the amount in 50 days with an average daily release of PTX was released from the polymer slowly to ~37% of the total amount in 50 days with an average daily release of ~0.3% to 0.8% of total drug loading (data not shown). Theoretically, it may be feasible to measure the released drug if an appropriate solvent can be chosen to extract PTX from tissue without disruption of PLGA. The tissue homogenates may require microfiltration to separate PLGA-PTX from released PTX. Technically, however, it would be very difficult given the small lymph node volume available from

![Figure 3](image-url)

**Figure 3.** A, plasma paclitaxel concentration versus time profiles with various experimental arms (n = 3 for each time point); B, comparison of plasma AUC values (mean ± SE) with various experimental arms.
each experimental rat. Current aggregate measurement of drug distribution does not confound interpretation of total PTX delivery to the mediastinal lymphatic tissue.

PTX delivered via PLGA microspheres resulted in dramatically reduced peak plasma concentration ($C_{\text{max}}$), the value that correlates best with systemic toxicities. Therefore, PLGA-PTX and the PLGA-PTX sponge system would probably be less likely to cause systemic toxicity than systemic administration of Taxol. Several colloidal PTX systems have been developed as alternatives to systemic drug delivery of PTX (15). In general, the free drug may readily extravasate to normal tissues, whereas the size of the drug-loaded particulate may limit the systemic distribution. In the present study, PTX slowly released from the PLGA-PTX system generated a sizable plasma AUC. Because the plasma concentration-time curves of PLGA-PTX and PLGA-PTX sponge have shown no regression by the end of the 4-week experiment, the actual AUC is undoubtedly higher. This may have potential therapeutic benefits in inhibiting tumor metastasis in the systemic circulation as sustained exposure of tumor cells to low concentrations of PTX can be more efficacious than shorter exposure to higher concentrations (16, 17). PTX can be very effective in producing one log cell kill of A549 cells with a concentration of 10 nmol/L as long as the exposure time is longer than 24 hours (18). Yamori and colleagues (19) reported that 50% growth-inhibitory concentrations (GL50) for 7 lung cancer cell lines ranged from 4 to 24 nmol/L (mean, 9.9 nmol/L) in vitro. Under the present experimental conditions, the administration of PLGA-PTX by ipl administration was associated with plasma levels, which are required to induce relevant cytotoxic effects in vitro. However, the overall plasma concentrations were significantly lower than a threshold concentration of 0.1 μmol/L, which may elicit hematologic side effect in clinical patients (20, 21).

Two types of controlled drug release can be achieved, temporal and distribution control (22). In temporal control, drug delivery systems aim to deliver the drug over an extended duration or at a specific time during treatment. In distribution control, drug delivery systems aim to target the release of the drug to the precise site of action within the body. Systems using either of these two mechanisms have been developed for clinical therapy. However, integration of both mechanisms in a single device to improve cancer chemotherapy is rarely seen. Despite the similar pharmacokinetic profile with the two PLGA-PTX formulations found in the study, our observational findings revealed that the sponge system resulted in more concentrated PLGA-PTX in the

![Figure 4](https://www.aacrjournals.org/doi/figure/10.1158/0008-5472.CAN-08-1753)
mediastinal area, reflecting a better distribution control release. We favor the sponge system over the free PLGA-PTX because of its intended clinical use as an immediate adjuvant during cancer surgery. The sponge would facilitate appropriate placement in the surgical field, such as in continuity with the mediastinal pleura, and shield the microspheres from the drainage tubes used in many cancer surgical procedures.

I.p. administration of polylactide nanoparticle encapsulated with PTX in ovarian cancer xenografts was studied by Lu and colleagues (23). In their study, the nanoparticulate PTX significantly inhibited local tumor progression and peritoneal ascites. The PTX concentration of pelvic lymph nodes (\(1\mu g/g\)) was 20-fold higher than that of free PTX treated animals at 48 h after i.p. administration. However, this concentration is 50- to 350-fold lower than the lymph node treated with PLGA-PTX–based system in this study. The most likely explanation for this magnitude of difference is that particles of \(<200\text{ nm}\) size cannot be sufficiently retained in regional lymph nodes.

A biodegradable polymer PTX microsphere, Paclimer, was developed and examined in various cancer xenografts and patients with ovarian cancer (24, 25). Paclimer contains 10% PTX (w/w) with microspheres ranging in size between 20 to 200 \(\mu m\) in diameter (median diameter of 53 \(\mu m\)). The system has been studied for intratumoral and i.p. administration. Although the micro–particulate PTX can be delivered locally to control tumor growth and ascites, particles in this size range tend to remain within the administration site, such as peritoneal cavity (26).

The present study shows that i.p. implantation of a gelatin sponge containing PLGA-PTX exhibits substantial therapeutic efficacy in inhibiting occult lymphatic metastasis or micrometastasis when used as an adjuvant treatment in an orthotopic lung cancer model. This effect is attributable to a unique formulation and mode of administration resulting in an improved distribution of PTX to the targeted lymphatic system. The mechanism of how PLGA-PTX microspheres are selectively taken up by the regional lymphatics and lymph node has not been completely elucidated. But very likely, they enter the rich pleural lymphatic network through direct openings on the surface of the pleural membrane (27, 28). The accumulation of PLGA-PTX particles in the vicinity of lymphatic vessels suggests that these lymphatics are also potential therapeutic targets. Our immunohistochemistry assay using lymphatic vessel endothelial receptor 1 (LYVE-1) antibody has also shown that LYVE-1-positive structures were primarily found in the paranodal and mediastinal lymphoid tissue (data not shown).

The clinical potential and possibilities of translymphatic drug delivery are numerous. The system may effectively avoid the first-pass metabolism of PTX as the drug is released on the site of targeted lymphatics and lymph node before it is exposed to alteration by liver enzymes. Therefore, it may greatly increase the bioavailability of PTX. Because PTX shows cytotoxicity at nanomolar concentrations (29, 30), and also shows a radiosensitizing effect to arrest cells at the G2–M transition of the cell cycle (31), it may have potential to improve the efficacy of adjuvant radiotherapy.

The system provides a formulation alternative to deliver pharmacologically effective amounts of PTX to the targeted lymphatic tissue. It avoids the use of Cremophor EL, an excipient present in Taxol for i.v. administration, which may cause severe anaphylaxis. Imbedded in the gelatin sponge matrix, the system can be inserted during a surgical procedure as an adjuvant or neoadjuvant therapeutic modality to control lymphatic metastasis.
Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References


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Translymphatic Chemotherapy by Intrapleural Placement of Gelatin Sponge Containing Biodegradable Paclitaxel Colloids Controls Lymphatic Metastasis in Lung Cancer

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