Paclitaxel Liposome Aerosol Treatment Induces Inhibition of Pulmonary Metastases in Murine Renal Carcinoma Model

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ABSTRACT

The present studies were undertaken to evaluate the pulmonary pharmacokinetics and therapeutic efficacy of paclitaxel (PTX) administered by aerosol. PTX was encapsulated into dilaurylphosphatidylcholine liposomal formulations (PTX-DLPC). The deposition and clearance of PTX-DLPC in the lungs administered by aerosol or i.v. at comparative doses was performed, and PTX was quantitatively determined in tissue extracts by high-performance liquid chromatography analysis. The murine renal carcinoma (Renca) pulmonary metastases model was used to determine the therapeutic effect of drug formulation administered by aerosol. PTX-DLPC aerosols were generated with the Aero-Mist jet nebulizer (cis-USA). The most effective schedule of treatment was when mice inhaled the drug for 30 min 3 days per week. There was a significant reduction of the lung weights and reduced number of visible tumor foci on the lung surfaces of mice treated with PTX aerosol ($P < 0.004$ and $P < 0.01$, respectively) compared with control groups. Inhalation of PTX-DLPC also led to prolonged survival in mice inoculated with Renca cells. The results of the present studies demonstrate the therapeutic potential of aerosol technology for lung cancer treatment.

INTRODUCTION

The lungs are the common site of both metastases and primary neoplasia. The average lung cancer mortality is 90% and is the leading cause of cancer-related deaths in both men and women. One reason for the poor survival is that traditional methods of treatment, such as surgical resection, radiation, and chemotherapy have failed to eradicate lung cancer. Systemic chemotherapy has been used with little success because most current drugs delivered in this way are quickly destroyed or inactivated in blood and liver. Subsequently, not enough drug reaches the tumor at the therapeutic dose. Furthermore, drugs administered systemically may cause life-threatening systemic toxicity. An ideal chemotherapy would involve the administration of high concentrations of active agents directly and continuously to the target tissue to allow the maximum effect at the site of interest without adversely affecting other organs. For pulmonary diseases, aerosol technology has been developed to achieve this objective. This localized delivery method has a number of potential advantages over systemic delivery. Lungs provide a large absorptive surface for aerosol deposition and allow the drug to avoid the first-pass metabolic degradation (1–3).

Our research group has developed experimental liposome aerosol formulations for pulmonary delivery of various active compounds, including the new potent anticancer lipophilic derivative of camptothecin 9-nitrocamptothecin (9NC). The aerosolized liposome 9NC formulation has proved effective against human cancer xenografts and experimental pulmonary metastases in mice at doses significantly lower than used by other routes of administration (4, 5). The human trials based on these results are under way.

PTX is the antineoplastic drug that demonstrated a therapeutic potential in lung cancer patients (6–9). PTX possesses a unique mechanism of action that differs from other anticancer drugs. It stabilizes microtubules by suppressing dynamic changes, affecting both growing and shortening, and leading to mitotic arrest (10, 11). In addition to the effects on mitosis, the broad antitumor activity of PTX may be the consequence of effects on the regulation of the cell cycle progression (12, 13). There may also be initiation of apoptosis (14, 15), alterations in the expression of gene products critical for tumor angiogenesis (16), metastases (17), or the host immune response (18). In the clinic, PTX is administered by continuous i.v. infusion. However, PTX possesses a very low solubility in conventional aqueous vehicles, and the preparation approved for clinical use solubilizes PTX in mixture of polyethoxylated castor oil and ethanol. This vehicle may cause severe hypersensitivity reactions in humans (19, 20). Liposomes were found to be a viable alternative for the therapeutic use of PTX because of its improved toxicological and pharmacological characteristics (21, 22).

In the present report, we compared the effectiveness of PTX delivered to the lungs by liposome aerosol with i.v. administration of the same preparation. We also studied a thera-

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3 The abbreviation used are: PTX, paclitaxel; DLPC, dilaurylphosphatidylcholine; Renca, renal carcinoma cell line; HPLC, high-performance liquid chromatography.
peutic efficiency of PTX aerosol treatment on the growth of pulmonary metastases in the murine Renca model. We chose this model because renal cell carcinoma is characterized by a lack of early disease symptoms, which leads to a distant metastatic formation, including the lungs, in a majority of the patients at the time of diagnosis.

**MATERIALS AND METHODS**

**Chemicals.** PTX was obtained from SuperGen, Inc. (San Ramon, CA). DLPC was purchased from Avanti Polar Lipids (Alabaster, AL). Organic solvents (HPLC grade) were obtained from Fisher Scientific. Sterile water for irrigation was purchased from Baxter Healthcare Corporation (Deerfield, IL).

**Animals.** Female ICR and BALB/c mice (7–8 weeks old) were obtained from Harlan Sprague Dawley (Indianapolis, IN) and housed in standard cages with food and water provided ad libitum. Experiments were performed with the approval of the Institutional Animal Care and Use Committee.

**Cell Culture and Animal Model.** The mouse Renca cell line was kindly provided by Dr. Robert Wiltrout, National Cancer Institute (Frederick, MD). The cells were maintained in vivo by serial renal passages according to the protocol provided by Dr. Wiltrout. Before in vivo implantation, Renca cells were cultured in vitro for two passages as described previously (23). To induce pulmonary metastases, 100,000 cells were injected i.v. in 0.2 ml of saline via tail vein in syngeneic BALB/c mice.

**Preparation of PTX-DLPC.** Liposomal formulation of PTX was prepared as described previously (24). Briefly, stock solutions of DLPC and PTX were prepared in t-butanol. Aliquots of PTX and DLPC (1:10, w/w) were mixed and then frozen at −70°C and lyophilized overnight to dryness. The formulations were stored sealed at 2°C and 70% RH. The distribution half-life of PTX in suspension before nebulization was 10 mg/ml.

**Aerosol Drug Delivery and Dosage Calculation.** The treatment with aerosol of mice that bore pulmonary tumors was performed as previously described (4). Briefly, an AERO-MIST jet nebulizer (cis-USA, Bedford, MA) was used to generate aerosol particles at the air flow rate of 10 liters/min. The aerosol particles were measured by Andersen Cascade Impactor and had a mass median diameter of 2.2 ± 0.2 μm (24). Aerosol particles with this size will deposit predominantly in the lung periphery. Mice were placed in sealed plastic cages and exposed to aerosol for 30 min. The aerosol was generated with 5% CO₂-enriched air obtained by mixing normal air and CO₂ with a blender (Bird 3M, Palm Springs, CA) and the CO₂ concentrations were calibrated with a Fluid Fyrite (Bacharach Inc., Pittsburgh, PA). The use of carbon dioxide increased pulmonary deposition of PTX 3- to 4-fold (24), and the total deposited dose during a 30-min inhalation of PTX-DLPC was 5 mg/kg.

**Pharmacokinetic Studies.** ICR tumor-free mice were used for these studies. One group received 5 mg/kg PTX i.v. via the tail vein. The other group received the same dosage of PTX during a 30-min inhalation. At each time point, three mice were killed by exposure to Isoflurane, USP (Abbott Laboratories, Chicago, IL) and exsanguinated. The lungs were rapidly excised, weighed, frozen and stored at −70°C until analyzed.

The extraction procedure for PTX has been described previously (24). Briefly, the sample was homogenized in 3 ml of ethylacetate in a mini-beadbeater. Homogenates were transferred to 10-ml glass tubes and centrifuged at 1000 × g for 10 min. The supernatant fraction was separated, and organic solvent was evaporated with air. The residue was reconstituted in 0.2 ml of methanol:acetoritile (2:1, v/v), sonicated in a water-bath sonicator, and centrifuged at 1000 × g for 10 min. Supernatant fractions were analyzed by HPLC.

PTX was quantified by reverse-phase HPLC with monitoring on a Waters 486 UV absorbance detector at 227 nm (Waters, Milford, MA). All of the measurements were made at room temperature on Waters Nova-Pak C18 column (3.9 × 150 mm). The mobile phase was composed of 49% acetonitrile and 51% water.

**In Vivo Antitumor Activity.** BALB/c mice were inoculated with tumor cells on day 0. On day 1, they were randomly divided into groups of 10 mice. One group of mice was left untreated, the second group of mice received 5 mg of PTX/kg by aerosol, and the third group inhaled blank liposomes (DLPC) at the dose equivalent to that in the same dose of PTX formulation. Mice were treated for 2 weeks. After that, they were killed by exposure to Isoflurane, USP, and exsanguination. Lungs were resected and weighed. After that, lungs were fixed in Bovin's fixative for tumor enumeration and sizing. In survival studies, mice were getting treatment until they died.

**Statistical Methods.** The statistical significance of differences between groups was calculated by Student’s *t* test. Evaluation of survival data was performed using Gehan’s *t* test (Primer of Biostatistics 4.0 software). *P* < 0.05 were considered to be statistically significant.

**RESULTS**

**Pulmonary Pharmacokinetics of PTX-DLPC Administered by Aerosol or i.v.** Fig. 1 shows the recovery of PTX from the lungs after single doses of 5 mg/kg PTX-DLPC given by aerosol or i.v. Observations of aerosol concentrations were started 5 min after the start of 30-min aerosol exposure. Measurements after i.v. treatment were begun 1 min after bolus injection of drug. Comparison of the area under the curve (AUC) revealed that in the lungs, AUC in the aerosol group was 26-fold higher than that after the i.v. injection (33.4 and 1.3 mg-h/g, respectively; *P* = 0.001, two-tailed *t* test). In the lungs at 1 min after i.v. treatment, there were 11.7 μg/g of PTX, but no other values exceeded 0.8 μg/g. The distribution half-life (*t*½) for PTX administered by aerosol or i.v. was 0.71 h and
PTX Liposome Aerosol for Cancer

0.02 h, respectively, and lung concentrations after aerosol treat-
ment during the 3-h period of observation ranged from 5.5 to
23.1 μg/g of tissue.

Treatment of Renca Pulmonary Tumors by PTX-DLPC

Inhalation. To study the in vivo activity of the liposomal
preparation of PTX administered by aerosol, studies were per-
formed in BALB/c mice with Renca pulmonary metastases.
Mice received the first treatment at the dose of ~5 mg/kg by
inhalation for 30 min 24 h after tumor inoculation. Additional
treatments were given three times weekly at the same dosage
and continued for 2 weeks. At that time, diffusely distributed,
numerous, small tumor lesions were visible on lung surfaces.
The antitumor effect of treatment was evaluated by the follow-

Table 1 Effect of PTX liposome aerosol treatment on murine
Renca metastases

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean lung weight (mg/±SD)</th>
<th>Mean tumor size, mm [range]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated (n = 10)</td>
<td>242 ± 32</td>
<td>478 ± 102</td>
</tr>
<tr>
<td>DLPC (n = 10)</td>
<td>213 ± 27</td>
<td>489 ± 135</td>
</tr>
<tr>
<td>PTX-DLPC (n = 10)</td>
<td>179 ± 16</td>
<td>332 ± 143</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated (n = 10)</td>
<td>417 ± 65</td>
<td>374 ± 70</td>
</tr>
<tr>
<td>PTX-DLPC (n = 10)</td>
<td>292 ± 39</td>
<td>382 ± 52</td>
</tr>
</tbody>
</table>

DISCUSSION

In our previous studies, we have shown that the aerosol
method of drug delivery, using 9NC, can be effectively used for
experimental lung cancer therapy (5). To increase the deposition
efficiency of the drugs via aerosol, we used a modification by
the addition of 5% CO2 to the breathing air. CO2-enriched air
significantly increased the pulmonary concentration of anticanc-

er drugs by about 3-fold compared with that achieved with
ambient air (24). In the present studies with this methodology,
we found higher concentrations of PTX in the lungs than the
concentration after i.v. injection. The clearance of inhaled PTX
from the lungs was also slower.

In clinical trials, PTX is administered by continuous infu-
sion because it provides prolonged exposure of the neoplastic
cells to the drug. Liebmann et al. (25) had shown in vitro that
the cytotoxicity of the drug was more dependent on increasing
the duration of exposure than on increasing PTX concentrations.
Aerosol delivery provides a continuous and direct exposure of
the lungs to the drug. In our experiments, mice bearing Renca
pulmonary metastases received ~5-mg/kg doses of PTX by
inhalation three times weekly during 2 weeks. This dose was
substantially lower than the most frequently used doses for i.v.
or i.p. administration. ≥20 mg/kg per injection (26–28). Sur-

Fig. 1 Pulmonary pharmacokinetics of PTX-DLPC administered by
aerosol (○) or i.v. (●). Mice inhaled the drug for 30 min; starting time,
0 (total deposited dose, 5 mg of PTX/kg). Bolus i.v. injection with 5 mg
of PTX/kg was given into tail vein at time 0.
vival studies demonstrated prolonged mean survival of treated mice compared with untreated (up to 25%). The dose-dependence studies revealed that aerosol treatment three times weekly was more effective than twice per week. However, when we increased the frequency of treatments up to five times per week, we noticed an increased aggressiveness in mice behavior after 10–14 days of treatment, which might be explained in part by PTX-induced neurological toxicity (data not shown).

Although numerous preclinical studies have demonstrated the effectiveness of PTX in different tumor models, they used only invasive techniques, and the majority of the experiments were done with solid tumor xenografts. To our knowledge, this is the first study demonstrating the effectiveness of PTX aerosol treatment for local therapy of lung tumors in mice. However, although we did not achieve complete tumor growth arrest in this animal model, we believe that the effectiveness of this treatment may be further improved with combination therapy, using agents that have different mechanisms of action on cancer cells.

REFERENCES


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