Pharmacokinetics of paclitaxel administered by hyperthermic retrograde isolated lung perfusion techniques

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Objective: Although paclitaxel is widely used as a systemic agent for the treatment of solid tumors, limited information is available concerning administration of this taxane by regional techniques. The present study was undertaken to evaluate the pharmacokinetics and acute toxicity of paclitaxel administered by hyperthermic retrograde isolated lung perfusion techniques to ascertain its potential for the regional therapy of unresectable pulmonary neoplasms.

Methods: Adult sheep underwent 90 minutes of retrograde isolated lung perfusion with escalating doses of paclitaxel and moderate hyperthermia using a protein-free, oxygenated extracorporeal circuit and a steady perfusion pressure of 14 to 16 mm Hg. An additional animal received paclitaxel by means of 1-hour central venous infusion. Paclitaxel concentrations in lung tissues, perfusates, and systemic circulation were determined by high-performance liquid chromatography techniques. Cytotoxicity of paclitaxel in cancer cells and in normal human bronchial epithelial cells was evaluated in vitro using 4, 5-dimethylthiazo-2-yl-2,5-dipagnyl tetrazolium bromide assays. Lung tissues were examined by hematoxylin-and-eosin techniques.

Results: Paclitaxel concentrations (maximum concentration and area under the plasma concentration time curve) in perfused tissues increased with escalating perfusate doses. Uptake of drug into lung parenchyma appeared saturable at high paclitaxel exposure; a substantial pharmacokinetic advantage was observed. Paclitaxel concentrations in systemic circulation were undetectable or exceedingly low after perfusion. Histopathologic examination of lung tissues harvested 3 hours after completion of isolated lung perfusion revealed no immediate toxicity, even at a paclitaxel exposure 20-fold higher than that achievable after 1 hour of intravenous administration at the maximum tolerable dose in human subjects. Moderate hyperthermia enhanced paclitaxel-mediated cytotoxicity 5- to 100-fold in cultured cancer lines. No paclitaxel toxicity was observed in cultured normal human bronchial epithelial cells after exposure to paclitaxel under normothermic or hyperthermic conditions.

Conclusions: These data support further evaluation of paclitaxel administered by hyperthermic retrograde isolated lung perfusion techniques for the treatment of unresectable malignant pulmonary tumors.

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Nearly one third of all patients with extrathoracic malignant tumors have pulmonary metastases. Many patients, particularly those with soft tissue sarcomas, die of metastatic disease confined to the lungs. Treatment of these individuals remains controversial.¹

Nearly 60 years ago, Alexander and Haight² reported prolongation of survival in 12 patients undergoing pulmonary metastasectomy; the algorithms that they used to select patients for resection remain valid today. Current studies have confirmed the role of surgical intervention in patients with pulmonary metastases and have demonstrated that survival correlates with completeness of resection, histology, and number of pulmonary nodules.³ In general, patients with metastatic melanomas do poorly, despite apparently complete resections (5-year survival of <25%), whereas those with germ-cell cancers tend to do quite well after pulmonary metastasectomy (5-year survival of approximately 60%). Patients with metastases from epithelial malignant tumors have intermediate survivals. Collectively, these data indicate that some patients can be salvaged by metastasectomy alone; however, the majority of individuals either present with or subsequently develop inoperable disease.

Whereas the role of systemic chemotherapy is debatable,⁴ it is conceivable that high doses of cytotoxic agents administered by isolated lung perfusion techniques may prove efficacious for the treatment of unresectable pulmonary malignancies. Doxorubicin, cisplatin, and tumor necrosis factor have been administered by antegrade isolated lung perfusion; however, their efficacies and toxicities in this context have not been fully defined.⁵⁻⁸ In the present study we used a sheep model to evaluate the feasibility of hyperthermic retrograde isolated lung perfusion, and the pharmacokinetics of paclitaxel administered by these techniques. Herein we report a profound pharmacokinetic advantage for paclitaxel administered in this manner, and demonstrate significant enhancement of paclitaxel-mediated cytotoxicity by moderate hyperthermia in cultured cancer lines but not in normal bronchial epithelial cells. These data support the evaluation of paclitaxel administered by means of isolated lung perfusion for the treatment of inoperable pulmonary malignancies.

Materials and Methods

Administration of Paclitaxel by Means of Isolated Lung Perfusion or Intravenous Infusion

All animal experiments were approved by the National Cancer Institute (NCI) Animal Care and Use Committee and were in strict compliance with the "Guide for the Care and Use of Laboratory Animals." The NCI is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

Adult sheep received 1 mg of atropine sulfate intramuscularly 1 hour before sedation, with 10 mg of diazepam administered intravenously. The left external jugular vein was cannulated for venous access, and anesthesia was induced with approximately 20 mg/kg pentobarbital administered intravenously (given to effect). The animals were intubated with a single-lumen endotracheal tube and maintained under general anesthesia with an average of 2.5% isoflurane and 1 L/min oxygen flow. Dexamethasone (20 mg) and diphenhydramine (50 mg) were administered intravenously. The left chest was prepared and entered through a lateral thoracotomy through the fifth intercostal space. The descending aorta was cannulated for arterial line placement. The inferior pulmonary ligament was incised, and the left mainstem bronchus was dissected free of adjacent nodal tissues and encircled with an umbilical tape. Bronchial arteries were ligated at the tracheobronchial angle. The pericardium was incised, and the ligamentum arteriosum was divided; the left main pulmonary artery and the left superior and inferior pulmonary veins were isolated and encircled with vascular loops. Heparin (300 U/kg) was administered intravenously, and vascular clamps were placed on the proximal left pulmonary artery and across the left atrium to isolate the left lung. A DLP retrograde cardioplegia cannula (DLP Medtronic, Grand Rapids, Mich) was inserted into the isolated left atrium, and a 20F angled DLP venous cannula was placed into the left main pulmonary artery; the cannulas were connected to a closed, oxygenated extracorporeal circuit, silicone bonded to minimize leaching of di-2-ethylhexylphthalate during the perfusion. After partial inflation of the left lung, the left bronchus was clamped to provide continuous positive airway pressure of 5 to 10 mm Hg during the perfusion. The previously ligated bronchial arteries were transected to allow back bleeding from the left lung. One liter of Ringer's lactate containing 250 μ g of prostaglandin E₁ (Prostin VR; Pharmacia, Kalamazoo, Mich) was infused over 5 minutes in a retrograde manner through a single pass to flush the lung and dilate the pulmonary vasculature; effluent was discarded. A 3-L protein-free perfusate consisting of Ringer's lactate containing allogeneic packed red blood cells (final hematocrit, 10 g/dL) and the appropriate dose of paclitaxel (Bristol Myers Squibb, Evansville, Ind) was infused continuously in a retrograde manner under moderate hyperthermia (perfusate temperature, 39.5°C). Effluent from transected bronchial vessels was returned to the circuit for reinfusion. Flow rates were adjusted to maintain a perfusion pressure within the pulmonary veins of 14 to 16 mm Hg, as measured with a pressure transducer connected to the cardioplegia cannula. Perfusion continued in this manner for 90 minutes, during which the total volume of perfusate typically circulated through the lung at least 9 times. After the perfusion, the lung was flushed with 1 L of Ringer's lactate, cannulas were removed, and vessels were repaired. Ventilation and perfusion were reestablished in the perfused lung, and the animal was maintained under general anesthesia for an additional 3 hours to enable collection of samples for pharmacokinetic and histopathologic analysis. Animals were then put to death under anesthesia by NCI veterinary staff.

One sheep received paclitaxel intravenously by means of central venous infusion over 1 hour after left thoracotomy. The sheep was maintained for 7 hours under general anesthesia for collection of lung and peripheral blood samples and subsequently put to death as described above.

Analysis of Paclitaxel Levels in Perfusates, Plasma, and Lung Tissues

Perfusate samples were collected before addition of paclitaxel to the pump, immediately before perfusion, and at 5, 10, 15, 20, 30, 45, 60, and 90 minutes after initiation of perfusion. Peripheral blood samples were collected at 0, 5, 30, 60, 90, 120, 150, and 270 minutes relative to initiation of perfusion, or at 0, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270, 300, 360, and 420 minutes relative to initiation of intravenous infusion. Perfusate and blood samples were collected in heparinized tubes, placed on ice until completion of the procedure, and then centrifuged; plasma was removed and stored at -70°C . Lung tissue samples were collected at 0, 5, 30, 60, 90, 100, 120, 150, and 270 minutes relative to initiation of perfusion or at 0, 30, 45, 60, 90, 120, 180, 240, and 270 minutes relative to initiation of intravenous infusion. Tissue samples were immediately snap-frozen in liquid nitrogen, and stored at -70°C until prepared for analysis as previously described,⁹ with modification. Briefly, after addition of 1 mL of 0.9% NaCl, thawed lung tissue samples were disrupted with a homogenizer. Paclitaxel was recovered from tissue homogenates, as well as perfusate and plasma samples by means of acetonitrile extraction, and analyzed with reverse-phase high-performance liquid chromatography,¹⁰ with a lower limit of detection of 20 ng/mL. Standard curves were constructed by adding appropriate amounts of paclitaxel (100-1000 ng) to 300 mg of nonperfused lung tissue, followed by extraction and analysis as described above. Standard curves for perfusate and plasma samples were constructed by using analogous techniques.

Pharmacokinetic Analysis

Pharmacokinetic parameters were calculated by using noncompartmental analysis. The terminal elimination rate constant (K_e) was determined from linear regression analysis. The plasma elimination half-life ($t_{1/2}$) was calculated as follows:

$$t_{1/2} = 0.693/K_e.$$

The area under the plasma concentration time curve (AUC) for each animal was calculated by using the linear trapezoidal method from time 0 to the last concentration time point obtained (AUC_{0-t}), and extrapolated to infinity by dividing the last concentration by the terminal elimination rate constant. The maximum concentration ($C_{\max}$) for perfusate was obtained at time 0. $C_{\max}$ values for lung tissue and plasma were obtained from observed experimental values.

Histologic Analysis of Lung Tissues

Three hours after completion of the lung perfusion, tissue samples were obtained from the perfused and the contralateral, unperfused lung. Tissue samples were formalin fixed, paraffin embedded, sectioned, and stained with hematoxylin-eosin. The slides were evaluated in a blinded manner by a senior pathologist (P.H.D.) for evidence of acute lung injury.

Cell Lines and In Vitro Proliferation Assays

The non-small cell lung cancer lines H1299, H460, and H358; the melanoma lines SK-mel-5 and 586 mel; and the fibrosarcoma cell line HT1080 were available in tissue-culture banks at the NCI. Normal human bronchial epithelial cells were purchased from

Clonetics (Bio Whittaker, Inc, Walkersville, Md). Cell lines were maintained in RPMI medium supplemented with 10% fetal calf serum and antibiotics. Paclitaxel was purchased from Bristol Myers Squibb. 4, 5-Dimethylthiazol-2-yl-2,5-diphenyl tetrazolium bromide was obtained from Sigma (St Louis, Mo). For in vitro proliferation assays, cells were seeded in flat-bottomed 96-well microtiter plates at densities optimized for each cell line. After overnight incubation, cells were exposed to varying doses of paclitaxel at 37°C or 39.5°C for 90 minutes. After 90-minute drug treatment, paclitaxel was removed, media was replenished, and cells were incubated for 3 days at 37°C . Cell viability was quantitated by 4, 5-dimethylthiazol-2-yl-2,5-diphenyl tetrazolium bromide colorimetric assays.¹¹ Dose-response curves for cells treated with paclitaxel were plotted as a fraction of viable cells relative to cells grown in normal media. Paclitaxel 50% inhibitory concentration values for cells treated under normothermia or hyperthermia were derived from the respective dose-response curves. Reduction of paclitaxel 50% inhibitory concentration values in cells treated with the drug at 39.5°C indicated an increase in the cellular responsiveness to paclitaxel under hyperthermic conditions.

Statistical Analysis

Data from proliferation assays were expressed as means $\pm$ SD. Two-tailed Student *t* tests were performed with a Prism 2.0 software package from Graph Pad Software (San Diego, Calif) to determine the significance of treatment effects in cultured cells under normothermic or hyperthermic conditions.

Results

Retrograde isolated lung perfusion was successfully completed in 10 sheep. The first animal received perfusate without paclitaxel; as assessed by fluorescein techniques, perfusion appeared quite uniform in the isolated lung (data not shown). On the basis of these initial observations, an additional 9 sheep underwent isolated lung perfusion with paclitaxel. Depending on the size of the animals, flow rates typically ranged between 300 and 500 mL/min. Two heat exchangers were used to maintain hyperthermia in the lung (average temperature, 38.5°C - 39.5°C , as measured with temperature probes in the upper and lower lobes). During the procedure, perfusate emanated across the visceral pleura, and retrograde bronchial arterial flow was clearly evident. Although not precisely quantitated, this effluent, which was continually returned to the pump, was at least several hundred milliliters.

Concentration-time profiles and pharmacokinetic parameters for paclitaxel perfusates are summarized in Figure 1 and Table 1, respectively. The $C_{\max}$ of paclitaxel in perfusates increased with dose; however, the $C_{\max}$ at a dose of 2 mg (lowest dose) was lower than expected (0.23 vs 0.6 $\mu\text{g/mL}$), possibly due to nonspecific binding of paclitaxel to the extracorporeal circuit. At higher doses, the $C_{\max}$ was consistent with doses administered. The elimination rate constant decreased, and elimination half-life increased with escalating drug dose. Paclitaxel concentrations in perfusates

Figure 1. Paclitaxel concentration-time profile in perfusates. Doses are 2 mg (squares), 20 mg (diamonds), 40 mg (inverted triangles), 200 mg (triangles), and 800 mg (circles).

TABLE 1. Pharmacokinetic parameters of paclitaxel in perfusates

Dose (mg)	C _{max} (µg/mL)	Ke (h ⁻¹)	T1/2 (h)	AUC(0-1.5) (µg · h ⁻¹ · mL ⁻¹)
2	0.23	1.11	0.62	0.09
2	0.20	1.56	0.44	0.02
20	4.26	0.69	1.01	1.95
40	12.36	0.45	1.53	7.07
40	11.53	0.41	1.71	6.99
200	65.87	0.19	3.63	70.00
200	72.15	0.12	5.68	73.70
800	307.00	0.09	7.57	348.00
800	272.59	0.10	6.93	356.87

decreased in a biexponential manner. At the high doses, the slope of the elimination phase was diminished, suggesting that the uptake of paclitaxel into pulmonary tissues was saturable.

Concentration-time profiles and pharmacokinetic parameters for paclitaxel in lung tissues are represented in Figure 2 and Table 2, respectively. Paclitaxel concentrations in lung tissues increased nonlinearly with escalating dose and

Figure 2. Paclitaxel concentration-time profiles in lung tissues after perfusion and 1-hour intravenous paclitaxel infusion. Paclitaxel was administered by isolated lung perfusion at doses of 200 mg (filled squares) and 800 mg (filled circles) and intravenously at a dose of 200 mg (open squares).

TABLE 2. C_{max} and AUC of paclitaxel in lung tissues after perfusion or intravenous infusion

	Dose (mg)	C _{max} (ng/mg)	AUC(0-4.5)
			(µg · h ⁻¹ · mg ⁻¹)
Perfusion	2	1.96	7.04
	2	2.04	5.02
	20	11.9	26.05
	40	14.5	32.9
	40	15.6	26.24
	200	60.4	96.7
	200	41.4	60.8
	800	85.8	165.2
Intravenous infusion	800	94.3	202.4
	200	25.4	73

peaked between 60 and 90 minutes; tissue levels rapidly declined during washout. Lung tissue C_{max} and AUC increased disproportionately with dose, gradually approaching a plateau. The average C_{max} (50 ng/mg) in lung tissues obtained when 200 mg (78 µmol/L) of paclitaxel was used in the perfusion was approximately 2-fold higher than that observed after systemic infusion of the same dose of paclitaxel over 1 hour; tissue AUCs under these conditions were relatively comparable (Table 2). The plasma C_{max} (15.1 µg/mL) and AUC (26 to 30 µg · h⁻¹ · mg⁻¹) observed after a 1-hour infusion of 200 mg of paclitaxel (approximately 150 mg/m²) in sheep were comparable with those reported by Maier-Lenz and colleagues¹² after a 1-hour infusion of 225 mg/m² (the maximum tolerated dose) in cancer patients. When the dose of paclitaxel in the perfusate was increased to 800 mg (approximately 320 µmol/L), the C_{max} of paclitaxel in perfused tissues was approximately 86

Figure 3. Representative sections of perfused and contralateral, nonperfused lung parenchyma (A and B, respectively) from a sheep receiving 800 mg of paclitaxel by means of isolated lung perfusion.

TABLE 3. Plasma paclitaxel concentrations ($\mu\text{g/mL}$) during and after perfusion or intravenous infusion

Time (h)	Perfusion					Intravenous infusion	
	40 mg	200 mg	200 mg	800 mg	800 mg	Time (h)	200 mg
0.08	BLQ	BLQ	BLQ	BLQ	BLQ	0.5	6.49
0.5	BLQ	BLQ	BLQ	BLQ	BLQ	0.75	8.23
1	BLQ	BLQ	BLQ	BLQ	BLQ	1	15.11
1.5	BLQ	BLQ	BLQ	BLQ	BLQ	1.5	7.57
1.66	BLQ	0.127	0.089	ND	0.038	2	4.94
1.83	BLQ	ND	0.052	0.19	ND	2.5	4.09
2	BLQ	ND	ND	0.12	ND	3	3.05
2.5	BLQ	0.023	0.027	0.06	0.18	3.5	2.9
4.5	BLQ	BLQ	BLQ	0.016	0.06	4	2.37
						4.5	1.78
						5	1.78
						6	1.31
						7	1.05

BLQ, Below limits of quantitation; ND, not determined.

ng/mL, and the AUC was approximately $165 \text{ ng} \cdot \text{h}^{-1} \cdot \text{mg}^{-1}$ (Figure 2 and Table 2).

Paclitaxel concentrations in the systemic circulation were undetectable at all perfusate doses during the isolated lung perfusion (Table 3). After restoration of circulation to the perfused lung, systemic levels were either undetectable or extremely low; no drug was detected in any systemic blood sample after perfusion with 2, 20, or 40 mg of paclitaxel. After 200- and 800-mg perfusions, paclitaxel could be detected after the washout period but quickly disappeared. C_{max} in plasma after these perfusions (approximately 0.10 and 0.19 $\mu\text{g/mL}$, respectively) was significantly lower than that observed after a 200-mg intravenous infusion (15 $\mu\text{g/mL}$). These data indicate that most of the paclitaxel was removed by the postperfusion flush, and that retained drug was not rapidly released from the lung into the systemic circulation (Figure 2 and Table 3).

No severe immediate clinical toxicity was evident in these animals; oxygen saturations were approximately 93%

to 95% after perfusion, and only mild, transient bronchorrhea was observed in animals perfused with high doses of paclitaxel once ventilation resumed in the perfused lung. Histopathologic examination of lung tissues obtained 3 hours after completion of the isolated lung perfusion revealed no evidence of pulmonary hemorrhage, alveolar edema, or interstitial thickening, even at a paclitaxel exposure 20-fold higher (320 $\mu\text{mol/L}$) than that achievable after a 1-hour administration of paclitaxel at the maximum tolerable dose of 225 mg/m^2 in patients with cancer (15 $\mu\text{mol/L}$, Figure 3).¹²

As defined by Collins¹³ and Dedrick,¹⁴ the pharmacokinetic advantage for regional delivery (Rd) of chemotherapeutic agents can be calculated with the following formula:

$$\text{Rd} = (\text{AUC}_L/\text{AUC}_P)_1/(\text{AUC}_L/\text{AUC}_P)_2,$$

where L_1 and P_1 refer to lung and plasma values observed during regional drug delivery (in this case isolated lung

Figure 4. Paclitaxel 50% inhibitory concentration (IC_{50}) in cultured cancer lines under normothermic or hyperthermic conditions.

perfusion), and L_2 and P_2 refer to values obtained after intravenous administration. Using the data from the 200-mg perfusion and 200-mg intravenous infusion experiments (Tables 1, 2, and 3), and assuming that the AUC was less than 0.1 for the plasma during the perfusion (no detectable levels of paclitaxel were recorded),

$$Rd = (0.079/0.1)/(0.073/23.06^*) = 250.$$

Collectively, these data indicate that paclitaxel can be administered by means of hyperthermic retrograde isolated lung perfusion in large animals without obvious immediate toxicity, and that paclitaxel concentrations in pulmonary tissues after isolated lung perfusion greatly exceed those potentially achievable by systemic administration in clinical settings.

Because moderate hyperthermia is known to enhance the tumoricidal activity of cytotoxic agents such as melphalan and cisplatin,^{15,16} additional experiments were performed to evaluate the effects of hyperthermia on paclitaxel-mediated toxicity in cultured cells. Brief hyperthermia (analogous to what was achieved during isolated lung perfusion) significantly enhanced paclitaxel-mediated toxicity in all cancer cells. The effects were most dramatic in lines established from melanomas and sarcomas, which typically are refractory to paclitaxel,^{17,18} and no toxicity was observed after paclitaxel exposure under normothermic or hyperthermic conditions in normal human bronchial epithelial cells (Figures 4 and 5).

Discussion

Delivery of chemotherapeutic agents via selective lung perfusion is an attractive means to control unresectable neoplasms or prevent recurrence after pulmonary metastasectomy. Techniques for isolated lung perfusion were described by Jacobs and colleagues¹⁹ and Pierpont and

*AUC 0-4.5 hours.

Figure 5. Effects of paclitaxel in cultured normal human bronchial epithelial cells under normothermic or hyperthermic conditions.

Blades²⁰ nearly 40 years ago. However, the role of isolated lung perfusion in cancer therapeutics has not been established, primarily because of the invasiveness of the procedure, and the lack of specificity regarding uptake and cytotoxicity of the chemotherapeutic agents used to date.⁵ Furthermore, antegrade perfusion techniques may not be optimal for drug delivery to pulmonary neoplasms which frequently derive their blood supply from the bronchial circulation^{21,22}; indeed, chemotherapeutic agents administered by means of selective bronchial artery infusion can mediate regression of pulmonary neoplasms.²³ Conceivably, retrograde perfusion techniques that exploit collaterals between pulmonary and bronchial venous systems may enhance drug delivery to metastatic lesions in the lungs. Furthermore, use of novel agents that exhibit preferential cytotoxicity in cancer cells may enhance the efficacy of

isolated lung perfusion, thereby justifying the invasiveness of this procedure.

In the present study, we sought to determine the potential of paclitaxel for regional lung delivery. This prototypic taxane was chosen for study because it induces dose-dependent apoptosis preferentially in cancer cells by a variety of mechanisms not solely related to microtubule stabilization.²⁴⁻²⁶ In addition, paclitaxel exhibits a broad spectrum of activity with minimal pulmonary toxicity, even when administered systemically at extremely high doses (775-825 mg/m² over 24 hours) in patients with cancer.^{17,27} A protein-free perfusate was used to enhance availability of paclitaxel, which is highly protein bound. Our experiments indicated a disproportional increase of $C_{\max}$ and AUC of paclitaxel in lung parenchyma with escalating perfusate dose, indicating nonlinear, potentially saturable uptake into lung tissues. The fact that the $C_{\max}$ and AUC continued to increase without reaching a plateau indicates that the transport of paclitaxel into lung tissues was not fully saturated, even with a perfusate dose of 800 mg (320 μ mol/L). The calculated regional delivery (Rd) value suggests that the pulmonary exposure of paclitaxel administration after hyperthermic retrograde isolated lung perfusion is 250-fold more efficient than that achievable by systemic infusion, thus confirming a profound pharmacokinetic advantage for paclitaxel administered by means of isolated lung perfusion techniques. The fact that paclitaxel levels in the systemic circulation were either undetectable or extremely low after isolated lung perfusion indicates that some paclitaxel is retained in the lungs, and that systemic toxicity should be minimal, despite paclitaxel exposure far in excess of what is achievable with systemic infusion.

The rationale for considering paclitaxel for administration by means of isolated lung perfusion is supported by recent studies indicating that 1-hour paclitaxel infusions can mediate tumor regressions comparable with those of more traditional dose regimens but with less toxicity in patients with solid tumors.²⁸ Maier-Lenz and colleagues¹² evaluated the toxicity and pharmacokinetics of intravenous paclitaxel administered over 1 hour in 34 patients with incurable malignancies. Paclitaxel dose was escalated from 150 mg/m² to 250 mg/m²; dose-limiting neurotoxicity was observed in 2 of 3 patients treated with 250 mg/m². No pulmonary toxicity was observed at any dose level. At the maximum tolerable dose of 225 mg/m², plasma $C_{\max}$ was 14.5 μ g/mL, and AUC was 26 μ g h/mL; comparable values were obtained in sheep after a 1-hour infusion of 150 mg/m² paclitaxel (total dose, 200 mg; Table 3). Much higher values were readily achieved with hyperthermic retrograde isolated lung perfusion in these animals.

Potential limitations of this study include the small number of sheep used for the perfusion experiments and the absence of intermediate or long-term toxicity data pertain-

ing to these animals. However, our primary objectives were to determine the feasibility of hyperthermic retrograde isolated lung perfusion and to evaluate the pharmacokinetics of paclitaxel administered by means of these techniques; as such, the study was not designed to examine acute or subacute toxicities related to paclitaxel exposure. Despite the fact that relatively few animals were used in this study, pharmacokinetic parameters were quite reproducible within dose cohorts. Furthermore, no histologic evidence of lung injury was evident 3 hours after completion of the lung perfusion at any dose, suggesting that paclitaxel mediates no immediate toxicities under these exposure conditions. Clearly, this analysis was insufficient to fully define the toxicity profile of paclitaxel administered by means of isolated lung perfusion techniques. An institutional review board-approved, phase I, dose-escalation study is currently underway to comprehensively evaluate the toxicities and efficacy of paclitaxel administered by means of hyperthermic retrograde isolated lung perfusion in patients with unresectable malignant pulmonary tumors.

The clinical formulation of paclitaxel contains 50% cremophor EL, a polyoxyethylated castor oil diluent, and 50% ethanol, both of which may significantly influence the uptake and distribution of paclitaxel in pulmonary tissues during isolated lung perfusion. Cremophor is known to cause nonlinear pharmacokinetics of paclitaxel during intravenous infusion; these effects vary with paclitaxel dose and infusion duration.²⁹⁻³¹ Entrapment of paclitaxel in cremophor micelles can significantly reduce drug availability.³⁰ In experiments relevant to our own studies, Knemeyer and colleagues³² analyzed the effects of cremophor-EL on urine, bladder tissue, and plasma pharmacokinetics of intravesical paclitaxel. The threshold cremophor concentration for micelle formation was 0.008%. Ethanol concentrations up to 1% and cremophor at concentrations below 0.01% did not alter the free fraction of paclitaxel. Higher concentrations of these diluents, particularly cremophor-EL, significantly diminished paclitaxel partitioning across the urothelium, and decreased bladder paclitaxel concentrations, but did not alter the urine or plasma pharmacokinetics of this taxane. Interestingly, whereas the intravesical preparation of paclitaxel facilitated micelle formation with diminished drug availability, the concentration of cremophor was insufficient to influence distribution of paclitaxel once it had penetrated into bladder tissue.

The analytic techniques used in our lung perfusion experiments could not discriminate between free and micellar forms of paclitaxel. In all probability, free paclitaxel markedly diminished as the paclitaxel dose was escalated in the perfusate. Hence micelle formation may have contributed to the nonlinear, potentially saturable uptake of paclitaxel into lung tissues during the perfusion experiments. Nevertheless, the fact that the $C_{\max}$ and AUC of paclitaxel in pulmonary

parenchyma increased in a dose-dependent manner despite cremophor concentrations greatly exceeding critical micellar concentration in perfusates suggests that a dynamic equilibrium between tissue and free drug and micellar forms of paclitaxel in the perfusate facilitated drug uptake into the lung. Using an ex vivo model system, Creel and colleagues³³ observed that paclitaxel concentration and permeation distance in arterial tissues increased with perfusate concentration and exposure duration. Paclitaxel concentrations in the arterial wall greatly exceeded perfusate concentrations. Thus interactions with hydrophobic elements within the blood vessel wall conceivably could enhance uptake of paclitaxel from lung perfusates and influence the rate at which paclitaxel moves into extravascular tissues during isolated lung perfusion. Collectively, these observations suggest that the pharmacokinetics of paclitaxel administered by hyperthermic isolated lung perfusion techniques may be highly complex. The animal model did not allow us to evaluate the antitumor effects of paclitaxel administered in this manner.

Although not formally defined, the mechanisms by which hyperthermia potentiates the cytotoxicity of chemotherapeutic agents have been attributed to selective dilation of tumor neovasculature, as well as enhanced drug uptake caused by increased membrane permeability; these latter effects appear to be more pronounced in chemoresistant cells.^{34,35} Rietbroek and colleagues³⁶ observed no thermal enhancement of paclitaxel cytotoxicity in R-1 rhabdomyosarcoma or SW-1573 lung carcinoma cells in vitro. Leal and coworkers³⁷ reported that high temperatures (43°C) protected MCF-7 breast cancer cells from paclitaxel cytotoxicity, despite increased intracellular drug concentrations. On the other hand, Cividalli and associates³⁸ recently observed that hyperthermia significantly enhanced paclitaxel cytotoxicity in mouse mammary adenocarcinoma cells in vivo. Experiments presented in this article clearly indicate that moderate hyperthermia enhances paclitaxel-mediated cytotoxicity in epithelial cancer cells, as well as in melanoma and sarcoma cells; this phenomenon does not occur in normal human bronchial epithelial cells after paclitaxel exposure. These observations may be clinically relevant. Melanomas and sarcomas typically exhibit resistance to taxanes.^{17,18,40} Conceivably, hyperthermia may significantly enhance the sensitivity of these malignancies to paclitaxel, especially when administered by means of regional perfusion techniques at doses exceeding those achievable by means of systemic administration. Furthermore, because paclitaxel is a potent inhibitor of angiogenesis,^{41,42} the antitumor effects of this taxane administered under hyperthermic conditions in vivo may be more pronounced than already suggested by our in vitro studies. The dramatic pharmacokinetic advantage and potential therapeutic index of paclitaxel administered by means of hyperthermic retrograde isolated lung perfusion support

further evaluation of this agent for the regional therapy of inoperable pulmonary malignancies.

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