Regional Drug Delivery for Inoperable Pulmonary Malignancies

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Abstract Unresectable primary lung cancers and inoperable pulmonary metastases contribute significantly to cancer mortality throughout the world. Isolated lung perfusion and inhalation techniques are potential strategies to enhance delivery of chemotherapeutic as well as biologic agents to the lungs while minimizing systemic toxicities. This chapter reviews current efforts pertaining to regional drug delivery for inoperable pulmonary malignancies.

Keywords Pulmonary metastases • Isolated lung perfusion • Inhalational drug delivery

1 Introduction

Primary and metastatic tumors involving the lungs cause considerable morbidity and mortality in cancer patients. In 2010, approximately 222,500 Americans developed lung cancer [1]. Many of these individuals presented with tumors that were confined to the chest, yet unresectable due to anatomic or physiologic limitations. Currently, median survival of patients with limited-stage small-cell or stage IIIA/B non-small-cell lung cancers treated with chemotherapy and/or radiation approximates 14 months [2, 3]. Whereas most of these individuals die from extrathoracic metastatic disease, a significant number develop life-threatening complications due to uncontrolled growth of their primary tumors. Recalcitrant local disease following definitive induction therapy often precedes the development of systemic metastases in lung cancer patients.

Nearly one third of all patients dying from malignancies of non-thoracic origin suffer from pulmonary metastases [4]. Many patients, particularly those with sarcomas, succumb to uncontrolled pulmonary metastases in the absence of other systemic disease; treatment of these individuals remains controversial. Pulmonary metastasectomy may be beneficial in selected patients. Analysis of more than 5,000 cases entered onto the International Registry of Pulmonary Metastases indicated that survival following pulmonary metastasectomy is contingent on the histology, disease-free interval following resection of the primary malignancy, number of pulmonary nodules, and completeness of resection [5]. Overall, patients with metastatic melanomas do poorly despite complete resections (5-year survival <25 %); in contrast, individuals with germ cell cancers fare much better following pulmonary metastasectomy,

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M.A. Rudek et al. (eds.), *Handbook of Anticancer Pharmacokinetics and Pharmacodynamics*, Cancer Drug Discovery and Development, DOI 10.1007/978-1-4614-9135-4_25,

with 5-year survivals of approximately 60 %. Patients with metastases from epithelial cancers have intermediate survivals. These findings have been confirmed and extended by numerous single-institution studies demonstrating potential efficacy of pulmonary metastasectomy for a variety of tumor histologies in properly selected patients [6–9]. Whereas these data indicate that some individuals with pulmonary metastases can be salvaged by resection alone, the majority of patients either present with or eventually develop multiple metastases that are inoperable. Recurrent disease following complete pulmonary metastasectomy is often attributable to outgrowth of chemo-resistant micrometastases present at the time of initial diagnosis.

Although efficacious for eradication of pulmonary metastases related to lymphoid or germ cell tumors, systemic chemotherapy has not proven to be uniformly beneficial for the treatment of pulmonary metastases secondary to epithelial or sarcomatous malignancies [9–11]. Frequently, systemic toxicities limit optimal dosing of chemotherapeutic agents in patients with these tumors. Conceivably, administration of cytotoxic agents by regional techniques may reduce tumor burden within the lungs while minimizing systemic toxicities in patients with pulmonary metastases. This chapter reviews recent experience pertaining to regional therapy of inoperable pulmonary malignancies.

2 Anatomy of the Pulmonary System

The high frequency of primary and metastatic cancers involving the lungs is attributable to the large surface area of respiratory epithelia at risk for malignant degeneration following carcinogen exposure and the extensive capillary system that entraps circulating cancer cells within the pulmonary interstitium. The lungs are perfused by two circulatory systems [12]. The pulmonary artery (PA) normally delivers all of the output from the right ventricle; although deoxygenated, blood within the PA is sufficient to maintain viability of normal lung parenchyma. The bronchial arterial circulation, emanating from several branches of the descending aorta, provides additional nutrient support to the airway mucosa [13]. Primary lung cancers, as well as metastatic lesions, frequently derive significant, and at times preferential, nutrient support from the bronchial circulation [14–16].

Inhalation- or perfusion-related pulmonary injuries are manifested by desquamation of airway epithelia, alveolar protein accumulation, and edema with or without fibrosis within the interstitial space [17–20]. Depending on the severity of the insult, life-threatening, irreversible interstitial fibrosis may ensue, manifested either as acute respiratory failure or more insidious, restrictive lung disease [21]. The fragility of the pulmonary interstitium, and its limited potential for recovery following severe insults, must be considered when contemplating regional delivery of cytotoxic agents for the management of inoperable pulmonary malignancies.

3 Isolated Lung Perfusion and Other Regional Delivery Techniques

3.1 Nitrogen Mustard and Melphalan Preclinical Studies

Administration of cytotoxic agents by selective lung perfusion was first reported shortly after techniques for cardiopulmonary bypass were established. In 1960, Pierpont and Blades [22] utilized a closed extracorporeal circuit to administer nitrogen mustard to dogs via antegrade [PA, inflow; pulmonary vein (PV), outflow] isolated lung perfusion techniques. Ten of 23 dogs receiving 0.4 mg/kg of nitrogen mustard via 15-min isolated lung perfusion (ILuP) survived the procedure. No washout was used following the perfusion, and three of these ten animals exhibited neutropenia. Histologic changes consistent with acute pneumonitis were evident in the perfused lungs. No dogs survived

Table 1 Concentration of melphalan in perfusate and lung tissue

Drug	Animals	Route	Dose or initial conc.	Lung (μg/g)	References
Melphalan	Rat	ILuP	2 mg	62.2 (34.3)	[25]
		ILuP	1 mg	3.3 (0.09)	
		IV	1 mg	6.9 (1.9)	
Melphalan	Rat	ILuP	0.5 mg	40.9 (3.8)	[30]
		ILuP	1 mg	50.5 (2.6)	
		IV	0.5 mg	0.8 (0.5)	
		IV	1 mg	1.7 (0.2)	

Numbered in parenthesis are standard deviations. *ILuP* isolated lung perfusion, *IV* intravenous infusion

perfusion at higher doses. Subsequently, Jacobs et al. [23] administered escalating doses of nitrogen mustard to dogs via ILuP techniques. In contrast to what was observed by Pierpont and Blades [22], Jacobs et al. [23] noted that doses of nitrogen mustard up to 1.6 mg/kg were tolerated when this agent was administered by 30-min perfusion at flow rates that maintained normal physiologic pressures within the pulmonary arterial system. Creech et al. [24] described techniques for simultaneous bilateral lung perfusion in animals and reported the results of bilateral ILuP in one lung cancer patient as part of a large study involving regional perfusion of a variety of malignancies involving the limbs, pelvis, abdominal viscera, and lungs.

Although additional studies of ILuP with nitrogen mustard were not pursued, a number of preclinical studies have been performed to determine the potential efficacy of the mustard derivative melphalan, for regional treatment of pulmonary metastases. Nawata et al. [25] evaluated the pharmacokinetics and antitumor activity of melphalan administered by ILuP techniques in a rodent sarcoma model. Rats received MCA-induced sarcoma cells via intrajugular vein injection and 7 days later were randomized to receive 1 or 2 mg of melphalan intravenously, 2 mg of melphalan via ILuP (approximately 7–8 mg/kg) administered over 20 min at a rate of 0.5 mL/min, or buffered hetastarch. Seven days following treatment, cohorts of animals were euthanized and pulmonary nodules enumerated. Melphalan concentrations in pulmonary tissues following ILuP were considerably higher than those observed following systemic administration of melphalan (62.2 ± 34.3 μg/g vs. 6.9 ± 1.9 μg/g or 3.3 ± 0.9, respectively) (Table 1). A tenfold reduction in the number of pulmonary nodules was observed in melphalan-perfused lungs relative to lungs from animals receiving intravenous melphalan or hetastarch perfusions. Sixty-seven percent of animals receiving melphalan lung perfusions tolerated contralateral pneumonectomy, compared to 80 % of animals receiving perfusions with hetastarch. No animals survived intravenous administration of melphalan.

Hendriks et al. [26] evaluated the efficacy of melphalan administered by ILuP in a rodent model of adenocarcinoma pulmonary metastases. Median survival of rats receiving unilateral melphalan (2 mg) lung perfusions was 81 ± 12 days compared to untreated animals with bilateral pulmonary metastases (18 ± 1 days) or unilateral metastases (28 ± 3 days) or animals treated with 0.5 mg of melphalan intravenously (37 ± 6 days).

Ueda et al. [27] evaluated long-term pulmonary toxicity of melphalan in a rodent perfusion model. Rats underwent 20-min ILuP with 1 mg of melphalan and were randomly euthanized at monthly intervals for 6 months. In melphalan-treated lungs, perivascular as well as peribronchial edema with septal thickening and interstitial inflammation were observed 30 days following ILuP; all of these changes resolved within 60 days of the perfusion. Transmission electron microscopy revealed minimal proliferation of type II pneumocytes in the perfused lung. Collectively, these experiments suggested that at a dose, which mediates antitumor effects by ILuP, melphalan induces no long-term histologic sequelae in the rodent lung.

Melphalan is often administered in conjunction with tumor necrosis factor-α (TNF-α) during isolated limb perfusion [28, 29]. Hendriks et al. [30] evaluated the effects of melphalan and TNF-α administered by ILuP in a rodent model of pulmonary metastases secondary to colorectal carcinoma. Rats were injected intrajugularly with adenocarcinoma cells and 7 days thereafter were randomized

Table 2 Isolated lung perfusion trials

Author	No. of patients	Agent	Dose	Technique	Duration	Mortality	Response
Hendriks et al. [31]	16	Melphalan	15–60 mg	Closed-circuit antegrade perfusion	30 min	0	N/A
Grootenboers et al. [32]	7	Melphalan	15, 45 mg	Closed-circuit antegrade perfusion	30 min	0	N/A
Johnston et al. [42]	6	Doxorubicin	1–10 mg	Closed-circuit, antegrade perfusion	50 min	0	N/A
	2	Cisplatin	14–20 mg	Closed-circuit, antegrade perfusion	60 min	1	N/A
Burt et al. [43]	8	Doxorubicin	40 mg/m^2	Closed-circuit, antegrade perfusion	20 min	0	N/A
			80 mg/m^2				
Putnam et al. [44]	12	Doxorubicin	60 mg/m^2	Single-pass, antegrade perfusion	20 min	1	1MR
			75 mg/m^2				Four stable disease
Ratto et al. [50]	6	Cisplatin	200 mg/m^2	Closed-circuit, antegrade perfusion	60 min	0	N/A
Schröder et al. [51]	4	Cisplatin	70 mg/m^2	Closed-circuit, hyperthermic, antegrade perfusion	20–30 min	0	N/A
Muller [52]	22	Cisplatin	30 mg/m^2	Torso perfusion	20 min×2	0	1 CR, 12 PR
		Mitomycin	10 mg/m^2				
		Navelbine	25 mg/m^2				
Pass et al. [71]	15	TNF-α	0.3–0.6 mg	Closed-circuit, hyperthermic antegrade perfusion	90 min	0	0
		IFN-α	0.2 mg				
Schrump et al. (unpublished)	8	Paclitaxel	100, 200, and 125 mg	Closed-circuit, hyperthermic retrograde perfusion	90 min	0	Four stable disease

to undergo sham thoracotomy or 25-min ILuP with either saline, melphalan, TNF, or melphalan/TNF. Additional animals received melphalan intravenously; tumor nodules were enumerated 7 days later. In additional studies, animals underwent contralateral pneumonectomy on day 21 and were euthanized 5 days later. Consistent with data reported by Nawata et al. [25], Hendriks and colleagues [30] observed a tenfold reduction in pulmonary metastases in animals receiving melphalan lung perfusions compared to control animals. Pulmonary tissue levels were 30–40-fold higher in perfused animals relative to those receiving comparable melphalan dose intravenously (Table 1). The cytotoxic effects observed following ILuP with 1 mg of melphalan were comparable to those seen after ILuP with melphalan at 2 mg. TNF-α had no appreciable antitumor effects when administered alone and did not appear to potentiate melphalan in this setting. Eighty percent of animals receiving melphalan (2.0 mg)/TNF (200 μg) lung perfusions tolerated contralateral pneumonectomy.

3.1.1 Clinical Trials

In a phase I trial, Hendriks et al. [31] performed 30-min ILuP with melphalan, administered at escalating doses (15, 30, 45, 60 mg) in 300 mL circuit volume using an extracorporeal circuit under normothermic or hyperthermic (42°) conditions (Table 2). Sixteen patients (seven colorectal, five renal, three

Table 3 Melphalan, doxorubicin, and cisplatin concentrations in perfusate, lung, and tumor tissue in patients

Drug	Dose or initial conc.	Perfusate (μg/mL)	N	Lung (μg/g of tissue)	Tumor (μg/g of tissue)	Reference
Melphalan	15 mg	23	12	4.6 (2.7)	5.3 (3.1)	[32]
	30 mg	83	6	3.3 (2.5)	3.6 (3.3)	
	45 mg	94	8	6.1 (2.3)	5.3 (2.6)	
	60 mg	122	3	13.4 (11.5)	8.7	
Doxorubicin	1 μg/mL	0.56[a]	1	0.72	0.64	[42]
	1 μg/mL	0.56[a]	1	0.79	0.25	
	1.5 μg/mL	0.98[a]	1	2.58	0.62	
	3 μg/mL	1.46[a]	1	1.58	2.19	
	5 μg/mL	2.76[a]	1	2.13	1.56	
	10 μg/mL	3.08[a]	1	2.81	2.81	
	40 mg/m^2	4.52[b]	1	0.58	NA	[43]
	40 mg/m^2	3.76[b]	1	4.64	5.03	
	40 mg/m^2	8.48[b]	1	10.1	6.62	
	40 mg/m^2	12.9[b]	1	18.6	14.5	
	80 mg/m^2	27.95	1	57.3	33.5	
Cisplatin	14 μg/mL		1	0.69	1.42	[42]
	20 μg/mL		1	0.68	1.09	
	70 mg/m^2	>100[a]	4	≤98.30		[51]
	200 mg/m^2	>250[c]	6	75	68	[50]

[a]Perfusate peak concentration during the perfusion
[b]Mean perfusate concentration
[c]Calculated from data provided in manuscript

sarcoma, one salivary gland) underwent 21 ILuP procedures followed by complete metastasectomy. Operative mortality was zero; no major systemic toxicities were observed. Grade 3 pulmonary toxicity (pneumonitis) was observed in two patients undergoing normothermic ILuP with 60 mg melphalan. In an extension trial [32], eight additional ILuP procedures were performed in seven patients using 15 and 45 mg melphalan under hyperthermic conditions. Overall, pharmacokinetics of melphalan were linear with dose during ILuP [32]. Considerable interindividual variability was observed, possibly due to different pulmonary blood volumes that added to the initial volume in the circuit. Peak concentrations and area under the concentration curve (AUC) in perfusates at the maximal tolerated dose (MTD) were 108.6 and 53.4 μg/mL, respectively. Normal lung tissue melphalan levels ranged from 1.1 to 26.6 μg/g; tumor tissue concentrations ranged from 0.8 to 11.5 μg/g depending on dose. At the MTD of 45 mg, C_{max} and AUC of perfusates were 101 ± 41 and 53 ± 15 μg/mL, respectively, compared to 0.46 ± 0.37 μg/mL and 6.6 ± 4.7 μg/mL, respectively, in systemic circulations. Normal lung and tumor melphalan concentrations were 6.1 ± 2.2 μg/mL and 5.3 ± 2.6 μg/mL, respectively (Table 3). In this extension trial, three of eight ILuP procedures performed with 45 mg melphalan under hyperthermic conditions were complicated by empyema, postoperative bleeding, or rhabdomyolysis [33]. In total, 29 procedures were performed in 23 patients. After a median follow-up with 62 months, 6 of 23 patients (26 %) were alive and free of disease. Sixteen patients developed recurrent disease, 11 of whom died; 5 of these 16 patients exhibited only extrathoracic disease recurrence. The 5-year overall survival rate was approximately 55 %; median survival time was 84 months. No significant survival differences were noted for patients with carcinomas versus those with sarcomatous lung metastases [34].

Table 4 Doxorubicin concentration in perfusate and lung tissue

Animals	Route	Dose or initial conc.	Perfusate (μg/mL)	Lung (μg/g)	Tumor (μg/g)	Extraction (%)	Reference
Dog	ILuP		0.27–64.1	1.6–65			[35]
Dog	ILuP	1.95 μg/mL		3.9			[36]
	ILuP	2.95 μg/mL		8.8 (1.2)			
	ILuP	4.39 μg/mL		16.9 (1.7)			
	ILuP	5.79 μg/mL		19.2 (0.8)			
	ILuP	7.61 μg/mL		20.6 (4.5)			
Rat	ILuP	80 μg/mL	72.1 (6.9)	107.8 (30.2)		38.3 (13.2)	[37]
	ILuP	160 μg/mL	118.4 (12.1)	172.2 (64.4)		38.9 (15.2)	
	ILuP	320 μg/mL	255.2 (12.8)	498.1 (180.6)		58.3 (13.1)	
	ILuP	480 μg/mL	384.1 (46.2)	418.5 (69)		57 (9.8)	
	ILUP	640 μg/mL	457.6 (32.5)	663.8 (350.2)		41.4 (9.3)	
	IV	5 mg/kg		19.9 (4.4)			
	IV	5 mg/kg		25.5 (1.5)			
Rat	ILuP	80 μg/mL		170.5		5.5	[38]
	ILuP	320 μg/mL		46.2		4.3	
Rat	ILuP	100 μg[a]		13.8 (4.3)		3.9 (2.5)	[41]
		400 μg[a]		58.5 (20.1)		36.9 (10.4)	
		100 μg[b]		2.0 (0.7)		0.8 (0.5)	
		400 μg[b]		5.2 (3.7)		3.2 (3.5)	
Pig	ILuP	50 mg/m^2		21.9			[40]
	IV	50 mg/m^2		3 (0.8)			

Numbers in parentheses are standard deviations. *ILuP* isolated lung infusion, *IV* intravenous perfusion
[a]Free doxorubicin
[b]Liporubicin

3.2 Doxorubicin Preclinical Studies

A series of animal experiments have been conducted to examine the toxicity and potential efficacy of doxorubicin administered by ILuP techniques. Minchin et al. [35] examined the pharmacokinetics of doxorubicin administered by 50-min ILuP in dogs using a closed, oxygenated, extracorporeal circuit; an in-line heat exchanger maintained a physiologic temperature of the perfusate that contained 1–80 mg of doxorubicin in 1 L of whole blood. Uptake of doxorubicin in the canine lung appeared uniform, time-dependent, and saturable, suggestive of either facilitated transport or tissue-binding mechanisms. Maximal tissue to perfusate blood ratios were 10–15 at low doses of doxorubicin; however, with higher perfusate doses, doxorubicin tissue to blood ratios were <2 (Table 4). Doxorubicin was undetectable in the systemic circulation; a systemic to pulmonary circulation leak attributable to bronchial arterial blood flow approximated 10 mL/min.

Baciewicz et al. studied the pharmacokinetics of ILuP with doxorubicin in dogs [36]. Perfusate concentrations ranged from 1.95 to 7.61 μg/mL, and lung concentrations of doxorubicin ranged from 3.9 to 20.6 μg/g (Table 4). A plateau of doxorubicin concentration in lung tissue appeared to be reached at a perfusate concentration of 5.79 μg/mL, suggestive of either saturation of transporters or direct toxicity impeding further uptake.

Weksler et al. [37] evaluated doxorubicin pharmacokinetics of ILuP and intravenous injection in rats. Lung doxorubicin concentrations after ILuP were 107.8–663.8 μg/mL and were significantly higher than those after intravenous doxorubicin (19.9–25.5 μg/mL) (Table 4). Lung doxorubicin concentrations reached a plateau at a perfusate concentration of 255.2 ± 12 μg/mL of doxorubicin. Extraction ratio (the percent of doxorubicin extracted by the lung from the perfusate) appeared to be

related to perfusate concentration, ranging from 38 to 58 %. The optimal perfusate and other pharmacokinetic factors for ILuP using doxorubicin were also investigated in rats [38]. The mean lung concentrations of doxorubicin were <100 and <300 μg/g, respectively, for perfusate concentrations of 80 and 320 μg/mL. Extraction ratios were 5.5 and 4.3, respectively, which were lower than those previously reported by these investigators [37]. The latter study suggested that perfusate concentration and duration of perfusion—but not dose per kilogram or per square meter of body surface area, total infused dose, or the rate of infusion—were the primary factors determining final lung concentrations of doxorubicin [38]. These investigators [39] also observed that rats undergoing single-pass ILuP with 1.6 mg of doxorubicin (320 μg/mL) over 10 min tolerated contralateral pneumonectomy 21 days later. In additional experiments, rats were injected intravenously with MCA-induced sarcoma cells 7 days prior to ILuP with either doxorubicin as described above or normal saline. Three weeks following ILuP, extensive tumor metastases were present bilaterally in all animals undergoing saline lung perfusions and in non-perfused lungs of rats receiving doxorubicin ILuP; no tumor metastases were identified in lungs perfused with doxorubicin; histopathologic analysis revealed moderate interstitial fibrosis in doxorubicin-perfused lungs.

Furrer et al. [40] evaluated the pharmacokinetics and immediate toxicities of doxorubicin (50 mg/m^2) administered either by 15-min single-pass or normothermic recirculating blood perfusion using similar flow rates (~100 mL/min), as well as intravenous systemic infusion in a porcine model. Doxorubicin lung tissue concentrations following single pass were comparable to those observed after recirculating blood perfusion (~18 μg/g vs. 22 μg/g, respectively); in contrast, pulmonary doxorubicin levels were only 3.0 μg/g of tissue following intravenous drug administration. Wet to dry ratios were significantly lower following single pass relative to recirculating blood perfusions, suggesting that doxorubicin administered by single pass induced less acute perfusion-related edema than the same dose of doxorubicin delivered by recirculating blood perfusion techniques.

Yan et al. [41] evaluated distribution of free and liposomal doxorubicin (Liporubicin) administered by ILuP techniques in a rodent pulmonary metastasis model. Briefly, sarcomas were generated in rat lungs, following which either free or liposomal doxorubicin was administered by normothermic, single-pass, antegrade ILuP over 20 min. Heterogeneous drug distribution was observed in the perfused lungs. Liposomal doxorubicin levels in normal lung and tumor tissues were approximately 6-fold and 11-fold lower than free doxorubicin levels following ILuP with 100 μg and 400 μg of doxorubicin, respectively. Furthermore, tumor Liporubicin levels were lower than tumor doxorubicin levels.

3.2.1 Clinical Trials

Several phase I studies have been performed to examine the toxicities and clinical efficacy of doxorubicin lung perfusions in patients with unresectable pulmonary malignancies (Table 2). Johnston et al. [42] treated six individuals with escalating doses of doxorubicin (1–10 μg/mL of perfusate in a closed extracorporeal circuit) via 45–50-min, normothermic ILuP. Three patients underwent unilateral lung perfusions, and three individuals had bilateral simultaneous lung perfusions. Flow rates were adjusted to maintain physiologic pulmonary arterial pressures. Following ILuP, residual perfusate was flushed from the lungs with either blood or low-molecular-weight dextran. Isolated lung perfusion circuits provided excellent separation of pulmonary and systemic circulations even under bilateral simultaneous perfusion conditions. Maximum doxorubicin levels in normal lung equaled or exceeded those observed in tumor tissues following lung perfusion (Table 3) [35, 42]. In two individuals, doxorubicin was detected in mediastinal lymph nodes following lung perfusion, indicating transport of drug through the pulmonary interstitium to the regional lymphatics. One patient developed pneumonia that was fatal. No objective responses were noted in this pilot study in which MTD was not determined.

Burt et al. [43] utilized a closed, oxygenated, extracorporeal circuit to administer doxorubicin via ILuP to eight patients with unresectable sarcomatous metastases. Seven patients were treated at a dose

of 40 mg/m^2, and one patient received 80 mg/m^2 doxorubicin via 20-min perfusion (300–500 mL/min) at ambient temperatures (Tables 2 and 3). Following ILuP, doxorubicin was flushed from the lungs with Hespan. Approximately 14 % of the total dose of doxorubicin in perfusates was extracted by the lungs. Consistent with what was reported by Minchin et al. [35], uptake of doxorubicin in tumors tended to be less than that observed in normal lung tissues (average drug concentrations following ILuP with 40 mg/m^2 of doxorubicin were 11.1 μg/g of tissue for normal lung and 8.7 μg/g of tissue for tumor nodules). A modified toxicity grading system was implemented by these investigators to assess pulmonary toxicity related specifically to drug exposure rather than the thoracotomy procedure itself. Six of the eight perfused patients experienced grade II pulmonary toxicity, defined as >20 % diminution in diffusion capacity for carbon monoxide (DLCO), or dyspnea at rest or with exertion. The single patient receiving 80 mg/m^2 of doxorubicin exhibited complete destruction of the perfused lung resulting in empyema and suppurative pericarditis requiring surgical intervention. Although none of the seven individuals perfused at a dose of 40 mg/m^2 experienced clinically significant pulmonary symptoms, postoperative pulmonary function tests revealed diminished forced expiratory volume in 1 s (FEV_1) as well as DLCO values, indicative of subacute interstitial toxicity.

Putnam et al. [44] treated 12 sarcoma patients with doxorubicin administered via single-pass isolated lung perfusion. Eight patients received 200 mg/mL (approximately 60 mg/m^2) and four patients received 250 mg/mL (approximately 75 mg/m^2) doxorubicin in 1 L of crystalloid solution administered over 20 min. One patient experienced a major response, and four individuals exhibited stabilization of disease. Acute, pulmonary toxicity (interstitial pneumonitis) occurred in two individuals, both of whom were in the high-dose cohort; pneumonitis was fatal in one of these patients. Late pulmonary toxicity evidenced by diminution of ventilation and perfusion was observed in several patients in this study. Although extensive pharmacokinetic data were not published, doxorubicin levels in normal tissues exceeded those in tumor nodules (median 592 μg/g [range 74–2,750] vs. 153 μg/g [range 12–1,294], respectively). These observations, which were consistent with those reported by Minchin et al. [35] and Burt et al. [43], may have accounted for the short- and long-term pulmonary toxicities observed following doxorubicin perfusions in this study.

In a phase I study, Otterson et al. [45] treated 53 patients with inoperable pulmonary malignancies (23 sarcoma, 16 lung cancer, 12 miscellaneous) with escalating doses (0.4–9.4 mg/m^2) of doxorubicin administered via inhalation techniques. Doxorubicin was delivered by an OncoMyst model CDD2A inhalational device, which aerosolized compounds to particles of 2–3 μM and prevented escape of exhaled aerosol. Deposition efficiency of TC99M was used to predict deposition of doxorubicin and predict patient doses. Two of four patients treated with 9.4 mg/m^2 developed dose-limiting pulmonary toxicities. One of eleven patients treated at the 7.5 m^2 dose level experienced >20 % diminution of pulmonary function attributable to study drug. One sarcoma patient exhibited a partial response to therapy, and eight patients had stabilization of disease lasting five or more courses (range 5–15). No pulmonary drug levels were measured in this study. However, systemic doxorubicin levels were, in general, considerably lower than those typically observed following systemic administration of standard doses of doxorubicin.

3.3 Cisplatin Preclinical Studies

ILuP with cisplatin has been evaluated by several investigators. Li et al. evaluated ILuP with cisplatin in a rat lung tumor model [46]. The results demonstrated significantly higher platinum concentrations in pulmonary tumor in rats undergoing ILuP with 0.1 mg/mL of cisplatin than rats receiving a 1 mg intravenous injection (6.7 ± 1.6 vs. 2.5 ± 0.6 μg/g of tissue [$p < 0.05$]) (Table 5). In accordance with the findings of Wang et al. [47], a lower cisplatin level was observed in tumor nodules than in normal lung tissue in the perfused rats, but almost the same levels were seen in the animals treated with

Table 5 Cisplatin concentration in perfusate, lung tissue, and tumor tissue in rats

Animals	Tumor	Route	Dose or initial conc.	Lung (μg/g)	Tumor (μg/g)	Reference
Rat	Sarcoma	ILuP	0.1 mg/mL		6.67 (1.64)	[46]
		IV	1 mg		2.51 (0.60)	
Rat	Sarcoma	ILuP	25 μg/mL	~4	4.76 (0.60)[a]	[48]
		ILuP	50 μg/mL	~11	4.95 (0.80)[a]	
		ILuP	100 μg/mL	~21	4.84 (0.74)[a]	

Data are presented as means and SD. *ILuP* isolated lung perfusion, *IV* intravenous infusion

[a]Concentration at 60 min of ILuP

intravenous cisplatin. These studies suggested that ILuP with cisplatin was pharmacokinetically superior to intravenous injection.

In another study, Li et al. [48] investigated the pharmacokinetics of cisplatin in rat tumor and lung tissues after ILuP using different perfusion times and perfusate drug concentrations. Isolated lungs were perfused over various times with cisplatin at 25, 50, or 100 μg/mL. Total cisplatin concentrations in lung tissues increased significantly with perfusion time and increasing cisplatin perfusate concentration. Cisplatin concentrations in normal lung tissues after a 60-min perfusion ranged from approximately 4–21 μg/g of tissue. However, cisplatin concentrations in the perfused tumor nodules (4 mm in diameter) ranged from 4.17 ± 0.82 to 4.95 ± 0.80 μg/g of tissue and did not change significantly with the perfusion time or perfusate cisplatin concentration (Table 5). Furthermore, cisplatin concentrations in tumor tissue were inversely related to the weight of tumor nodules after ILuP. The results suggest that ILuP may not be beneficial for bulky metastatic disease.

In additional studies, Ratto et al. [49] utilized a porcine model to evaluate the pharmacokinetics of cisplatin administered via 15-min infusion distal to a pulmonary artery tourniquet (stop-flow), 15-min infusion into a lung isolated by tourniquets on the ipsilateral pulmonary artery and pulmonary veins (stop-flow/occlusion), or by 4-h ILuP under normothermic conditions using a closed, oxygenated extracorporeal circuit. Cisplatin (AUC) values in pulmonary tissues were approximately threefold higher in the stop-flow/occlusion animals compared to the stop-flow group [11,538 =/–4,586 μg/(min·mL) vs. 3,658 ± 824 μg/(min·mL), respectively]. Interestingly, lung perfusions with 2.5 mg/kg of cisplatin did not increase pulmonary tissue AUC values relative to those observed following administration of the same dose by stop-flow/occlusion techniques; however, cisplatin AUCs in mediastinal lymph nodes were significantly higher following ILuP compared to stop-flow/occlusion, possibly owing to the duration of drug exposure in the perfusions. Drug uptake in lung tissues and mediastinal nodes following ILuP was dose dependent. Histopathologic analysis revealed no significant differences regarding acute toxicities in pulmonary tissues harvested 4 h after cisplatin administration by any technique.

3.3.1 Clinical Trials

Several phase I studies have been performed to examine toxicities and potential efficacy of cisplatin administered by ILuP techniques (Table 2). Johnston et al. [42] performed total lung perfusion using cardiopulmonary bypass techniques in two patients (one bronchoalveolar lung cancer and one sarcoma). The first patient underwent ILuP with 14 μg/mL cisplatin at 25 °C, whereas the second patient was perfused with 20 μg/mL cisplatin at 40 °C. Perfusion flow rates were adjusted to maintain physiologic pulmonary artery pressures. Perfusion durations approximated 60 min. Peak pulmonary circuit cisplatin levels approximated 10 μg/mL; peak cisplatin levels in systemic circulation ranged from 0.4 to 0.8 μg/mL, indicating a small leak between pulmonary and systemic circuits. Cisplatin levels in a normal lung were ~0.07 μg/g, compared with tumor drug levels of approximately 1.2 μg/g (Table 3). One of the two patients had detectable cisplatin in regional lymph nodes. One of the two patients developed respiratory failure and empyema, dying 81 days after the perfusion.

Ratto et al. [50] administered cisplatin (200 mg/m^2) to six patients with sarcomatous pulmonary metastases via 60-min normothermic ILuP using a closed, oxygenated extracorporeal circuit. Two patients developed reversible interstitial pneumonitis, one of whom required mechanical ventilatory support. No systemic toxicities were observed. Cisplatin levels in normal lung and metastatic lesions were comparable, ranging between 65 and 75 μg/g tissue (Table 3). In all likelihood, the low protein content of the perfusate (approximately 1/7 that of normal serum) enhanced drug delivery during ILuP. Indices of interstitial injury (DLCO, pO_2, and pCO_2) assessed at 10, 30, and 90 days postoperatively were essentially unchanged from baseline values. Response to therapy was not evaluated in this trial.

Schröder et al. [51] performed hyperthermic ILuP with cisplatin in four sarcoma patients. Following metastasectomy, patients underwent isolated lobar or unilateral whole-lung perfusion with 70 mg/m^2 cisplatin administered at a temperature of 41 °C for 20–30 min at a rate which maintained a mean pulmonary artery pressure less than baseline values (approximately 300–500 mL/min). One individual underwent staged bilateral lung perfusions 1½ months apart. Maximal cisplatin concentrations at the completion of the perfusions approximated 98 μg/g of tissue, values which were considerably higher than those observed by Johnston et al. [42]. All patients experienced transient pulmonary toxicity manifested as non-cardiogenic pulmonary edema and desquamation of perfused bronchial mucosa. The one patient who had undergone unilateral whole-lung perfusion exhibited grade II pulmonary toxicity (>20 % decrease of FEV and DLCO relative to baseline values) 3 weeks post-ILuP that gradually resolved over the next 9 weeks. Two additional patients exhibited grade I pulmonary toxicity 3 weeks following ILuP; these toxicities resolved in both patients within 6 weeks of their procedures. One patient undergoing lobar perfusion experienced no clinically significant diminution in pulmonary function. Three of the four patients undergoing metastasectomy and perfusion were alive and free of disease with a median follow-up of 13 months. Collectively, this limited clinical study demonstrated that hyperthermic lung perfusions with cisplatin are feasible in patients with pulmonary metastases.

Muller [52] evaluated the effects of combined regional and systemic chemotherapy for the treatment of inoperable non-small-cell lung cancer. Twenty-two chemo-naive patients underwent 20-min regional perfusion of the thorax with 10 mg/m^2 of mitomycin, 25 mg/m^2 of navelbine, and 30 mg/m^2 of cisplatin. Regional perfusion was accomplished by balloon catheter occlusion of the aorta above the celiac axis and the inferior vena cava at the cavoatrial junction, as well as pneumatic tourniquets on the upper extremities. Three hundred micrograms of GM-CSF were administered intravenously during the perfusion. Thereafter, patients received 250 mg/m^2 of 5-fluorouracil (5-FU) and 20 mg/m^2 of cisplatin via continuous intravenous infusion over 4 days. Two cycles of regional and systemic chemotherapy were administered 4 weeks apart. The overall response rate was 59 % (4.5 % CR, 54.5 % PR). Six additional patients exhibited minor responses. Nearly all patients responding to therapy experienced either improvement or stabilization of pulmonary function. No dose-limiting toxicities were observed during 45 cycles of therapy. Sixteen of twenty-two patients underwent surgery, thirteen of whom had complete (R0) resections. Overall 1-year survivals were 87 % and 68 % for patients with bulky IIIA and IIIB/IV disease, respectively.

3.4 Gemcitabine Preclinical Studies

Several studies have been performed recently to evaluate pharmacokinetics and toxicities of gemcitabine administered by lung perfusion techniques (Table 6). van Putte et al. [53] delivered escalating doses of gemcitabine (20, 40, 80, 160, or 320 mg/kg; approximately 5, 10, 20, 40, and 80 mg, respectively) or buffered starch to rats via 25-min ILuP at a rate of 0.5 mL/min followed by 5-min washout. Pulmonary gemcitabine levels following perfusion/washout with 160 or 320 mg/kg were 1.5 ± 1.6 μg/g and 2.5 ± 1.8 μg/g, respectively. Levels of gemcitabine in systemic circulation were undetectable following ILuP. Lung levels of gemcitabine were 0.2 ± 0.1 μg/g with serum levels of 92.2 ± 63.6 μg/mL following IV administration of 160 mg/kg gemcitabine. In additional experiments, resection of the

Table 6 Concentration of gemcitabine and paclitaxel in perfusate and lung tissue

Drug	Animals	Route	Dose or initial conc.	Perfusate (μg/mL)	Lung (μg/mL)	AUC [μg (h·mg)]	Reference
Gemcitabine	Rat	ILuP	160 mg		1.5 (1.6)		[53]
		ILuP	320 mg		2.5 (1.8)		
		IV	160 mg		0.2 (0.1)		
Gemcitabine	Rat	BFO^{a}_{10}	13.3	2,700	0.62 (0.37)		[55]
		BFO^{a}_{20}	26.7	2,700	0.90 (0.53)		
		BFO^{a}_{30}	40	2,700	0.76 (0.38)		
		BFO^{a}_{40}	53.3	2,700	1.19 (0.77)		
	Rat	BFO^{b}_{10}	5.3	2,700	0.63 (0.13)		[55]
		BFO^{b}_{20}	10.7	2,700	0.94 (0.21)		
		BFO^{b}_{30}	16	2,700	0.97 (0.41)		
		BFO^{b}_{40}	20.4	2,700	1.35 (0.6)		
Gemcitabine	Pig	SPAP	1,250 mg/m^2		2,700 ± 1,800	43,179	[58]
		IV	1,250 mg/m^2		<50	3,180	
Paclitaxel	Sheep	ILuP	40 mg	11.9[a]	15[b]	26.2	[61]
		ILuP	200 mg	69[a]	59.9[b]	78.9	
		ILuP	800 mg	289.8[a]	90.1[b]	183.8	
		IV	200 mg		25.4[b]	73	

[a]Flow rate = 0.5 mL/min
[b]Flow rate = 0.2 mL/min

contralateral (non-perfused lung) was performed 3 weeks following ILuP. Animals (67–100 %) undergoing ILuP at doses of 20–320 mg/kg followed by delayed contralateral pneumonectomy were alive 90 days following lung perfusion. Mortality following pneumonectomy did not appear to correlate with perfusion doses.

In a subsequent study, van Putte et al. [54] further examined toxicity and potential efficacy of gemcitabine delivered by ILuP techniques in a rodent pulmonary metastasis model. Rats with unilateral pulmonary metastases underwent ILuP as described above using gemcitabine at doses of 160 or 320 mg/kg, or buffered starch, whereas animals with bilateral metastases received either a single intravenous dose of gemcitabine (160 or 320 mg/kg) or buffered starch. All rats receiving 320 mg/kg gemcitabine IV compared to 40 % of animals receiving 160 mg/kg IV died within 1 week. The overall survival rate for animals having gemcitabine perfusions was 83 %. Animals undergoing ILuP with either 160 or 320 mg/kg gemcitabine exhibited a twofold increase in pulmonary interstitial fibrosis compared to animals receiving intravenous gemcitabine; the extent of fibrosis induced by perfused gemcitabine was similar for these two doses. Rats with unilateral metastases undergoing ILuP with 320 mg/kg gemcitabine had a median survival time of 38 ± 4 days compared to 28 ± 3 days for animals with unilateral metastases treated with intravenous gemcitabine. These data suggest that gemcitabine administered via ILuP techniques may prolong survival in preclinical animal models yet induces interstitial fibrosis that could be significant in humans.

An additional study [55] was performed to evaluate pulmonary gemcitabine levels following administration of this drug during blood flow occlusion (BFO) for 10, 20, 30, or 40 min. Gemcitabine was delivered at rates of 0.2 or 0.5 mL/min (Table 6). Pulmonary uptake was saturated after 20-min BFO; no significant differences in pulmonary gemcitabine levels were observed using flow rates of 0.5 mL/min relative to 0.2 mL/min. Furthermore, no significant differences in wet to dry ratios were observed between different flow rates and perfusion times. Pulmonary gemcitabine levels were three- to sixfold higher following delivery of drug via BFO compared to IV administration of gemcitabine at the MTD of 160 mg/kg.

In subsequent experiments van Putte et al. [56] examined the effects of delayed washout following ILuP on pulmonary gemcitabine levels in a rodent model. In this study, doses of gemcitabine (1.3–6.7 mg/mL in 25 mL perfusate) were administered via ILuP. An additional cohort of rats underwent 6-min ILuP with 6.7 mg/mL gemcitabine followed by 5-min flush and 30 or 60 min of reperfusion; another cohort of animals had 6-min perfusion followed by delayed cross-clamp release for 30 or 60 min followed by a 5-min flush. Interestingly, whereas pulmonary gemcitabine levels after 30-min ILuP exceeded those observed following 6-min perfusion, the wet to dry ratio (indicative of tissue edema) was higher in the 30-min perfused lung. Tissue drug levels after 6-min perfusion were 70 % of levels observed following 30-min ILuP. Although the lung was not saturated, 6-min perfusion resulted in pulmonary gemcitabine levels of 2.3 ± 0.34 mg/g; these levels were comparable to what had been observed in a previous toxicity study (2.5 ± 1.8 mg/g) using 30-min ILuP with 320 mg/kg (5.3 mg/mL inflow concentration). A linear relationship was observed between perfusate concentration and tissue drug levels, suggesting that uptake of gemcitabine into lung parenchyma occurs primarily by diffusion rather than active transport mechanisms. Overall 43–51 % of the drug in the perfusate was absorbed into the lungs. These findings suggest that decreased ILuP times, with delayed cross-clamp release, result in comparable tissue drug levels with less interstitial edema (hence potentially reduced long-term pulmonary toxicity). Efficacy studies using such modified ILuP techniques have not been published.

In additional studies, van Putte et al. [57] utilized a porcine model to evaluate pharmacokinetics of gemcitabine delivered via selective pulmonary artery perfusion (SPAP) techniques. Briefly 16 pigs underwent SPAP with gemcitabine (1 g/m^2). Three groups underwent SPAP for 2 min with either normal, 50 %, or 90 % reduced pulmonary blood flow. An additional group received systemic administration of a comparable dose of gemcitabine over 30 min. C_{max} and AUC values for 2-min and 10-min SPAP were eleven- and sixfold and two- and threefold higher, respectively, than those observed following IV gemcitabine infusions. Flow reduction led to inhomogeneous pulmonary drug delivery. The relatively high C_{max} and AUC values achieved during SPAP may be attributable to efficient first-pass uptake into the lung, resulting in systemic AUC levels comparable to those observed following systemic administration of gemcitabine. The fact that uptake of gemcitabine after 2-min SPAP was only five- to sixfold higher than IV administration despite a 30-fold difference in drug concentration at the catheter tip suggests that SPAP at 2 min may saturate uptake mechanisms in the lung. SPAP for 10 min appeared to be optimal for pulmonary drug uptake.

In a related study, these investigators [58] used a porcine model to examine pharmacokinetics of gemcitabine (1.25 g/m^2), carboplatin (AUC 5), or both administered by 2-min SPAP followed by 30-min blood flow occlusion to delay drug washout from the lung. Additional animals received similar doses of gemcitabine or carboplatin IV. Gemcitabine and carboplatin lung levels 8 min after completion of SPAP exceeded 2,500 μg/g and ~17 μg/g, respectively, tapering off linearly over the next 20 min. Pulmonary gemcitabine levels, when this drug was administered with carboplatin, were 750 μg/g, suggesting that carboplatin adversely affected gemcitabine uptake. In contrast, carboplatin lung levels, when this drug was perfused with gemcitabine, were somewhat higher (65 μg/g) than when carboplatin was administered alone. Serum levels following SPAP of gemcitabine and/or carboplatin were also somewhat higher than when these drugs were administered intravenously.

3.4.1 Clinical Trials

To date, no data pertaining to regional delivery of gemcitabine for treatment of pulmonary malignancies have been reported.

3.5 Paclitaxel Preclinical Studies

All of the animal and human lung perfusions described thus far have utilized antegrade (inflow via PA, outflow via PV) perfusion techniques that may not be optimal for drug delivery to pulmonary neoplasms, which frequently derive their blood supply from the bronchial arteries [14, 16, 59]. The fact that chemotherapeutic agents administered by selective bronchial artery infusion can mediate significant regression of pulmonary neoplasms [15, 60] attests to the relevance of this circulatory system regarding growth of pulmonary malignancies. By exploiting venous collaterals between the pulmonary and bronchial arterial systems, retrograde perfusion (inflow via pulmonary vein; outflow via pulmonary artery) may enhance the efficiency of drug delivery to primary as well as metastatic tumors in the lung.

Schrump et al. [61] utilized a sheep model to evaluate the feasibility, pharmacokinetics, and immediate toxicities of paclitaxel administered via retrograde, hyperthermic ILuP techniques. Adult sheep underwent 90-min hyperthermic, retrograde ILuP using a closed, oxygenated, extracorporeal circuit with a 3 L perfusate containing crystalloid and packed red blood cells (pRBC) to a final hematocrit (Hct) of 10 and escalating doses of paclitaxel (2–800 mg). Paclitaxel levels in perfused tissues increased with escalating perfusate doses; drug levels in high-dose perfusates declined more slowly, suggesting that uptake of paclitaxel into pulmonary tissues was saturable. The average C_{max} (50 ng/mg) in lung tissues obtained when 200 mg of paclitaxel was utilized in the perfusion (78 μM paclitaxel) was approximately twofold higher than that observed following systemic infusion of the same dose of paclitaxel over 1 h; tissue AUCs under these conditions were relatively comparable (Table 6). The plasma C_{max} and AUC following 1-h infusion of 200 mg of paclitaxel (approximately 150 mg/m^2) in sheep were essentially comparable to those reported by Maier-Lenz et al. [62] following 1-h infusion of 225 mg/m^2 of paclitaxel in cancer patients. When the dose of paclitaxel in the perfusate was increased to 800 mg (approximately 325 μM), C_{max} of paclitaxel in perfused tissues was 86 ng/mL, and AUC was 165 (ng·h)/mg. Paclitaxel levels in the systemic circulation were undetectable at all perfusate doses during the ILuP; following restoration of circulation to the perfused lung (after washout), systemic levels were either undetectable or extremely low, indicating that retained drug was not rapidly released from the perfused lung into the systemic circulation. Histopathologic examination of lung tissues obtained 3 h following completion of the ILuP revealed no pulmonary hemorrhage, alveolar edema, or interstitial thickening. Survival was not evaluated in these experiments, nor did the sheep model allow assessment of antitumor activity of paclitaxel administered in this manner.

In a more recent study, Tanju et al. [63] compared early effects of paclitaxel and docetaxel on pulmonary physiology in rats undergoing ILuP. Briefly, rats underwent ILuP with paclitaxel (140 mg/kg), docetaxel (70 mg/kg), or normal saline delivered at a rate of 0.5 mL/min with perfusion pressures of 20 mmHg. Ventilation pressures, compliance, and blood gases were evaluated 5 min after restoration of pulmonary blood flow, prior to resection of the perfused lung. Ventilatory pressures were higher, compliance was lower, and pO_2 values were lower in drug-treated rats compared to controls. ILuP with docetaxel resulted in less CO_2 retention than paclitaxel. Docetaxel-treated lungs exhibited intra-alveolar hemorrhage and mononuclear cell infiltration without perivascular edema. In contrast, animals perfused with paclitaxel exhibited dense perivascular and intra-alveolar edema. These data suggest that docetaxel might be preferable to paclitaxel for ILuP. Delayed toxicities associated with these agents were not assessed in this study.

3.5.1 Clinical Trials

Schrump et al. conducted a phase I study of hyperthermic, retrograde isolated lung perfusion with paclitaxel in patients with unresectable pulmonary malignancies (Schrump, unpublished). Ten lung

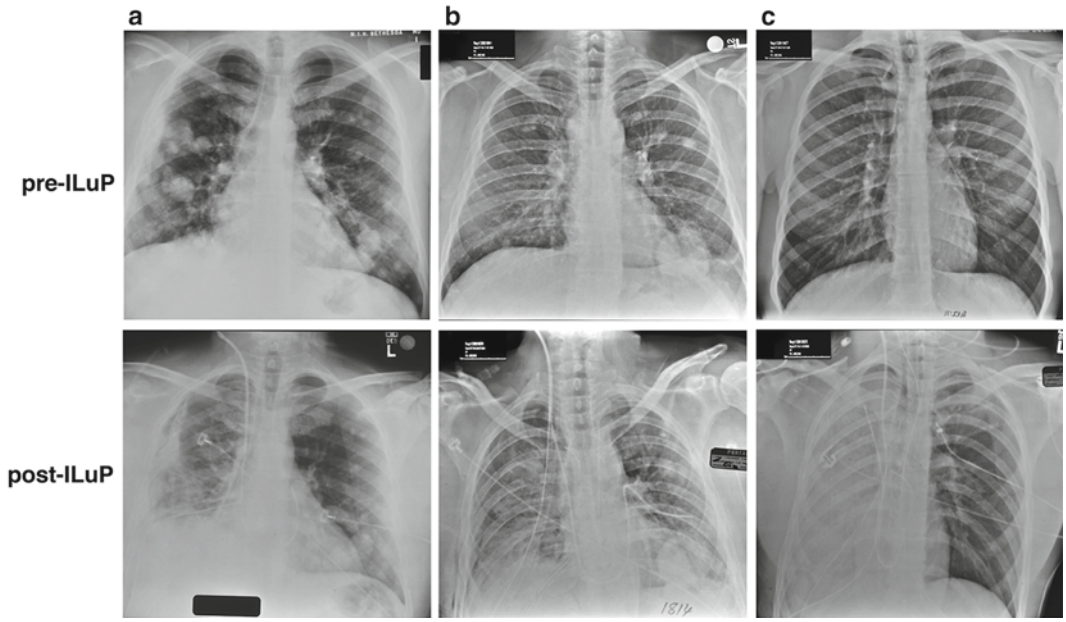

Fig. 1 Preoperative and 24-h postoperative chest X-ray films from three representative patients undergoing hyperthermic retrograde ILuP with 100 mg of paclitaxel (**a**), 125 mg of paclitaxel (**b**), or 200 mg of paclitaxel (**c**). Corresponding pharmacokinetic data for these patients are summarized in Table 7 (*use figure from Fig. 1 of Chap. 21 on page 359 of the first edition*)

perfusions (two left, eight right) were performed in eight patients with refractory pulmonary metastases. Five of those perfusions were performed in the context of complete pulmonary metastasectomy. All patients received intravenous decadron, diphenhydramine, and cimetidine prior to ILuP. Inflow was achieved by a single retrograde cardioplegia cannula placed into the isolated left atrial cuff or by dual cannulation of the ipsilateral superior and inferior pulmonary veins. Outflow was established by cannulation of the ipsilateral main pulmonary artery. Flow rates were adjusted to maintain a pressure of 14–16 mmHg within the pulmonary veins; under these conditions flow rates ranged between 500 and 1,000 mL/min. Temperatures of the perfusate were adjusted via in-line heat exchanger to maintain a temperature of 39.5–41 °C in the lung, assessed by temperature probes placed into the upper and lower lobes.

Prior to initiation of the perfusion, the isolated lung was flushed with 1 L of Ringer's lactate containing 250 μg of prostaglandin E to dilute the pulmonary vasculature. Thereafter, the lung was perfused for 90 min with paclitaxel using a closed, oxygenated circuit containing a dilute blood perfusate (Hct = 10). Following completion of the paclitaxel perfusion, the lung was flushed with 2 L of Ringer's lactate prior to reestablishing normal blood flow to the isolated lung.

No dose-limiting toxicities were observed in three patients undergoing four perfusions with 100 mg of paclitaxel. However, significant pneumonitis requiring mechanical ventilation was observed in three patients perfused with 200 mg of paclitaxel. Although the pneumonitis was dramatically reversible in all three patients, the severity of the acute pulmonary injury warranted dose reduction. As such, three additional patients underwent four lung perfusions with 125 mg of paclitaxel; no pulmonary toxicity was observed in these individuals. Representative chest X-ray films and pharmacokinetic data for three individuals are depicted in Fig. 1 and Table 7. Uptake of paclitaxel in tumor tissue equaled, if not exceeded, that in normal lung parenchyma. Although no objective responses were observed, prolonged disease-free interval was observed in four individuals who underwent ipsilateral metastasectomy at the time of ILuP; these individuals underwent contralateral metastasectomy shortly after ILuP yet recurred in the non-perfused lung.

Table 7 Paclitaxel concentrations in perfusate, plasma, and lung tissues

	C_{max} (mg/L)			Normal lung (ng/mg of tissue)		Tumor (ng/mg of tissue)	
Patient	Dose (mg)	Perfusate	Plasma	90 min	120 min	90 min	120 mg
1	100	30	0.059	6.2	10.9	4.8	9.8
7	125	40	0.1	17.3	12.9	25.6	19.0
6	200	51	0.16	24.4	14.7	26.8	24.2

3.6 TNF-α Preclinical Studies

Although the macrophage-derived cytokine TNF-α exhibits potent antitumor effects [64, 65], systemic administration of tumoricidal doses of recombinant TNF-α is not tolerated in cancer patients [66, 67]. However, due to the effects of TNF on tumor vasculature, this cytokine has been utilized with melphalan in hyperthermic isolated limb and liver perfusions resulting in complete response rates approximating 75 % in melanoma and sarcoma patients [68, 69]. Weksler et al. [70] evaluated the antitumor effects of TNF-α in a rodent perfusion model. Preliminary in vitro experiments revealed that 42 μg/mL of murine or human TNF-α inhibited in vitro proliferation of MCA-induced sarcoma cells by 20–40 % relative to untreated cells. Tumor-bearing rats undergoing ILuP with 420 μg of TNF-α exhibited a five- to sevenfold reduction in the number of metastases in the perfused lung compared to the non-perfused lung. These data suggested that when administered by ILuP techniques, TNF-α can mediate significant antitumor effects without apparent systemic toxicity.

3.6.1 Clinical Trials

In a phase I trial, Pass et al. [71] treated 15 patients with pulmonary metastases from a variety of malignancies by 90-min hyperthermic ILuP using a closed, oxygenated extracorporeal circuit containing 0.2 mg of interferon-α and escalating doses (0.3–0.6 mg) of TNF-α (approximately 7 μg/mL in the highest cohort of patients). There were no operative deaths, and reduction of disease (not meeting criteria for partial response) was observed in three patients; TNF-α levels in pulmonary tissues were not ascertained in this study (Table 2). One patient experienced reversible interstitial pneumonitis requiring mechanical ventilation. FEV and DLCO, as well as ventilation and perfusion in the treated lung, were diminished 10–20 % relative to baseline values 8 weeks following ILuP, suggesting subclinical pulmonary toxicity following ILuP.

3.7 Interleukin-2 Preclinical Studies

Aerosolization is an appealing method for administration of cytokines such as interleukin-2 (IL-2) for inoperable pulmonary malignancies. In a series of dog experiments, Khanna et al. [72] evaluated the toxicities and potential efficacy of either free or liposomal IL-2 in normal dogs. Free IL-2 (5×10^6 units) was administered twice daily by inhalation techniques. Additional dogs received aerosolized saline. Leukocyte counts in bronchoalveolar lavage (BAL) were significantly higher, and immune effector populations including leukocytes and eosinophils were higher in dogs receiving liposomal IL-2 compared to those receiving free IL-2.

An additional canine study [73] was performed to examine the characteristics and distribution of nebulized IL-2 liposomes. The mass median aerodynamic diameter of the liposomes was 1.98 ± 2.02 μm. Aerosolized IL-2 was deposited homogeneously throughout the lungs of

anaesthetized dogs. Approximately 24 h after inhalation, most of the liposomes remained in the lungs, whereas some were taken up into spleen.

In subsequent studies, Khanna et al. [74] treated dogs with pulmonary metastases [7] or spontaneous lung cancers [2] with aerosolized liposomal human IL-2. Two of four dogs with metastatic osteosarcoma exhibited complete regressions lasting >12 months and >20 months. One dog with lung cancer had stable disease for 8 months. Numbers of immune effector cells (eosinophils and lymphocytes) were significantly increased in BAL, and mean BAL effector lytic activity was significantly increased 15 days after commencing IL-2 inhalations compared to pretreatment values. Interestingly, this lytic activity was not evident 30 days following commencement of aerosolized IL-2 therapy. No pulmonary toxicities were observed in this study.

3.7.1 Clinical Trials

A number of trials have been performed to evaluate the toxicity and efficacy of inhaled IL-2 in patients with potentially inoperable pulmonary malignancies. Lorenz et al. [75] treated 16 patients with refractory pulmonary malignancies with five daily administrations of aerosolized IL-2. Reversible, dry nonproductive cough was dose limiting. Mild decreases in pulmonary function tests and pO_2 were seen in all patients. One complete response, one partial response, and one mixed response were observed in 14 patients with metastatic renal carcinomas. Dose-dependent expansion of immune effector cells was observed in BAL fluids. Increased systemic levels of soluble interleukin-2 receptors were observed. No other systemic effects of aerosolized IL-2 were evident. Melichar et al. [76] observed no increase in urinary neopterin levels following inhalational IL-2 therapy in 13 patients with metastatic renal cell carcinoma, consistent with a lack of systemic immune activation.

Huland et al. [77] reviewed results of inhaled IL-2 therapy for nearly 300 patients with pulmonary metastases, 188 of whom had metastatic renal cell carcinoma. A variety of doses and schedules were used for IL-2 administration. Overall, inhaled IL-2 was well tolerated. Among 188 patients with renal cell carcinoma treated at a single European clinic, progression of pulmonary metastases was prevented in nearly 70 % of patients with a median duration of 7 months. Overall survival appeared to be improved relative to historic controls (17.2 vs. 5.3 months).

In a small single-institution study, Enk et al. [78] treated seven melanoma patients with pulmonary metastases with inhalation IL-2 for 6 months. Patients also received periodic bolus administrations of DTIC every 4 weeks. Therapy was well tolerated. No significant systemic toxicities were observed. Six patients developed cough. The overall response rate was 71 %; two patients exhibited complete response, two patients had partial remissions, and one had stable disease. Cough and dyspnea induced by inhaled IL-2, which appears to be consistent with an asthma-like syndrome [79], could ameliorate induction of accessory cell function of alveolar macrophages [80], thereby attenuating antitumor immunity mediated by this cytokine.

4 Summary and Future Directions

Only 3 of 106 patients undergoing ILuP procedures in the aforementioned trials died as a direct result of perfusion (perioperative mortality = 3 %); these data indicate that ILuP can be performed safely in properly selected individuals with unresectable pulmonary neoplasms. Data from Schröder et al. [51], as well as our experience (Schrump unpublished), indicate that cisplatin and paclitaxel lung perfusions can be performed in the context of aggressive pulmonary metastasectomy (including lobar resections) without apparent significant long-term sequelae.

In a recent study Nowak et al. [81] examined alterations of tumor and normal lung tissues following ex vivo ILuP. Briefly, lobectomy and pneumonectomy specimens from lung cancer patients were ventilated and perfused ex vivo using a physiologic crystalloid solution for 10, 60, 90, 120, and 240 min. Perfusions up to 120 min could be performed without disruption of histologic or physiologic parameters. However, perfusions greater than 120 min in duration resulted in progressively severe lung edema, with increased inspiratory and pulmonary artery pressures. Perfusions more than 240 min in duration were associated with loss of cell viability and associated histologic abnormalities; these ex vivo studies using human lungs provide potentially useful information regarding the development of ILuP regimens for inoperable pulmonary malignancies.

In an additional study Schumann et al. [82] evaluated the effects of reperfusion of isolated lungs following low and high perfusion pressures or low and high positive end-expiratory pressure (PEEP) (4 vs. 8 mmHg). Lung weights were lower following reperfusion with low PA relative to high PA pressures (mean 27 vs. 40 mmHg). Pulmonary edema (reflective of total lung weight) was lowest, whereas compliance was highest, and lungs exhibited lowest amounts of alveolar inflammation/destruction when reperfusion was performed using low perfusion pressures and high PEEP. These studies, which were designed primarily for lung transplant purposes, may have direct implications regarding reperfusion techniques used for future ILuP trials for cancer.

At present, the major limitation of ILuP relates to the lack of specificity regarding uptake and cytotoxicity of drugs in normal lung parenchyma relative to tumor tissues; this phenomena has been well established for doxorubicin, and agents such as melphalan or TNF may have limited use in ILuP owing to their potential for inducing significant interstitial injury. Continued efforts should focus on the identification of novel agents that mediate cytotoxicity preferentially in cancer cells. Furthermore, efforts should be undertaken to elucidate the pathophysiology of perfusion-related pneumonitis [83, 84] and to identify agents that can ameliorate such injury. For instance, depletion of tissue plasminogen activator or administration of *N*-acetyl-cysteine attenuates reperfusion injury in transplanted lungs [85, 86]; conceivably, similar strategies could be used to minimize pneumonitis observed following ILuP. In addition, a standardized system should be utilized for all future clinical trials to enable objective assessment of pulmonary toxicities following ILuP. At present, ILuP appears most effective when performed in the context of pulmonary metastasectomy, and future trials should focus on the use of ILuP or inhaled drugs as an adjuvant to aggressive resections. Continued efforts should also be directed toward refining minimally invasive techniques for delivery of chemotherapeutic agents via torso perfusion, SPAP, or selective bronchial artery infusion, as well as the development of inhalation agents for the treatment of inoperable pulmonary malignancies.

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