[Frontiers in Bioscience E4, 2226-2232, January 1, 2012]

Isolated lung perfusion

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1. ABSTRACT

Isolated lung perfusion (ILP) has been historically used as a method to study basic lung physiologic concepts using animal models. More recently, ILP has been applied in lung transplantation and thoracic oncology. In lung transplantation, ILP has been used to assess physiological integrity of donor lungs after the organ is removed from the donor. This procedure is called *Ex vivo* Lung Perfusion (EVLP), and it has also been proposed as a method for active treatment and repair of injured unsuitable donor organs *ex vivo*. In oncology, ILP is an attractive method to deliver high dose chemotherapy to treat pulmonary metastatic disease. Since the lung vasculature is isolated *in vivo*, this technique is called *in vivo* lung perfusion (IVLP). This review will focus on the rationale, technical aspects, experimental and clinical experience of EVLP and IVLP. A perspective on the future use of these techniques is described.

2. *EX VIVO* LUNG PERFUSION (EVLP)

2.1. Introduction and experimental work to date

Lung transplantation (LTx) is a lifesaving therapy for patients suffering from end-stage lung diseases. However, the number of patients waiting for LTx greatly exceeds the number of donors available. One aggravating factor specific to LTx is the fact that, in general, only 15% of lungs from multi-organ donors are deemed usable for transplantation (1); the rest are generally considered unsuitable due to the lung injury that occurs with brain death and ICU related complications (i.e barotrauma or lung edema associated with fluid resuscitation). Since primary graft dysfunction (PGD) is a complication that leads to severe early and long term consequences to LTx recipients, transplant teams tend to be very conservative in selection of donor lungs. As a result, wait list mortality can be as high as 30 to 40% (2,3).

A novel strategy to overcome the shortage of donor lungs is the re-assessment and treatment of injured donor lungs using normothermic EVLP. The current clinical practice of organ preservation is cold static preservation (CSP). During retrieval, a cold pulmonary flush using low potassium dextran preservation solution (Perfadex®, Vitrolife, Sweden) is coupled with topical cooling and lung ventilation (4,5). Lungs are then transported at 4°C in a static inflated state. Hypothermia reduces metabolic activity to the point that cell viability can be maintained in the face of ischemia (5% of metabolic rate at 37°C) (6). Cold temperature preservation is therefore an important component of lung preservation (7, 8).

Physiological normothermic (37°C) *ex vivo* perfusion of organs has been originally described by Carrel and Lindbergh long before the field of transplantation emerged (9). Recently, normothermic perfusion became a research target as a preservation alternative in experimental models of lung, liver and kidney transplantation (10-15). One important advantage of normothermic perfusion is the maintenance of active metabolic functions which provides an opportunity for continued assessment of the organ during the *ex vivo* phase of organ preservation. Another advantage is the restoration of normal functions (using the organ's innate reparative mechanisms or through active therapeutic interventions). The use of EVLP as a potential important tool in lung transplantation started with the work from Steen *et al (*16). This group described the use of EVLP as a method to re-assess lungs from uncontrolled donation after cardiac death donors (DCD) (17), since these organs cannot be evaluated *in vivo.* A lung perfusion specific solution *(*Steen® solution) with optimal osmolarity and high dextran content was developed by this group (*16, 18)*. After these publications, other groups have demonstrated the feasibility of short-term EVLP (30 to 90min) using the technique described by Steen in order to evaluate lung function in animal models of donation after cardiac death (DCD) and experimentally using injured human lungs rejected for transplantation (19-25). Erasmus and colleagues, attempted to extend to 6h the EVLP duration using the same system and technique described by Steen. Although feasible, circuit-induced impairment of lung function became apparent by increased pulmonary vascular resistance (PVR) and increased airway pressures towards the end of the procedure (26).

In Toronto we have developed an EVLP system and strategy in order to be able to maintain lungs in the EVLP system for at least 12h without adding injury ("EVLP protecting mode"). The use of an acellular perfusate, a closed circuit with protective low perfusion pressure (pulmonary artery pressure (PAP) 10 to 13mmHg) and stable positive left atrial (LA) pressure (5 mmHg), and a protective mode of mechanical ventilation (tidal volume of 7 ml/kg, rate of 7 breaths per minute (bpm), with a positive end-expiratory airway pressure (PEEP) of 5 cmH_2O) were important modifications to achieve 12 hours of perfusion stability. In our first work, using normal pig lungs, lung function was stable during 12 hours of normothermic EVLP (27). This stability during prolonged EVLP translated into excellent post-transplant lung function, absence of edema formation and preserved lung histology after transplantation. The acellular perfusion assessment of lung function accurately correlated with post-transplant graft function and the addition of red blood cells did not provide additional functional information compared to acellular perfusate (27). This study provided the proof of concept that EVLP is able to maintain *normal* donor lungs for a prolonged period of time without damaging the organ. Further examination was then performed to determine the impact of prolonged EVLP using *injured* ischemic donor lungs (28). Pig donor lungs were cold preserved for 12 h and subsequently divided into two groups: cold static preservation (CSP) or normothermic EVLP for a further 12 h (total 24 h preservation). EVLP preservation resulted in significantly better lung oxygenation and lower edema formation rates after transplantation when compared to CSP. Alveolar epithelial cell tight junction integrity, evaluated by zona occludens-1 protein staining, was disrupted in the cell membranes after prolonged CSP but not after EVLP. Effective adenoviral green fluorescent protein (GFP) gene transfer and transgene expression by lung alveolar cells was also achieved during 12h EVLP (28) demonstrating preservation of metabolic functions during normothermia.

2.2. Toronto technique for EVLP

We have previously described the details for our acellular lung protective EVLP technique, including a detailed discussion about the rationale for the chosen ventilatory and perfusion strategies (27). The components and set up of the circuit are demonstrated in Figure 1. The EVLP perfusion and ventilation strategy is shown in Table 1. The preparation of the donor lungs occurs on the back table with the lungs immersed in a cold preservation solution. Specifically designed funnel-shaped cannulas are attached to the LA and PA. The lungs are then transferred from the back table to the XVIVO chamber™ (Vitrolife). The PA cannula is connected to the circuit and anterograde flow (PA-LA) is initiated at 150ml/min with the perfusate at room temperature. The temperature of the perfusate is then gradually increased to 37ºC over the next 30 minutes. Before increasing flow beyond this level, a careful check of the system is made. The PA and LA pressure readings are double-checked. When a temperature of 32-34ºC is reached (usually over 20 min), ventilation is started and the perfusate flow rate is gradually increased to the target flow (40% of estimated donor cardiac output) within 60 min. Once ventilation is started, the flow of gas ($86\%N_2$,$6\%O_2$,$8\%CO_2$, Praxair) that will de-oxygenate and provide carbon dioxide to the inflow perfusate via the gas exchange membrane is initiated (started at 1L/min) and titrated to maintain inflow perfusate pCO_2 between 35-45 mmHg. Steroids, antibiotics and heparin are added to the perfusate prior to EVLP initiation.

2.3. Clinical experience with EVLP

The first clinical use of an EVLP system was described by Steen in 2001 to briefly assess lung function from a DCD (29). The same group reported their experience with 60-90min of blood based perfusion to assess 6 high risk donor lungs prior to transplantation. Outcomes were acceptable, however, the mean time in the

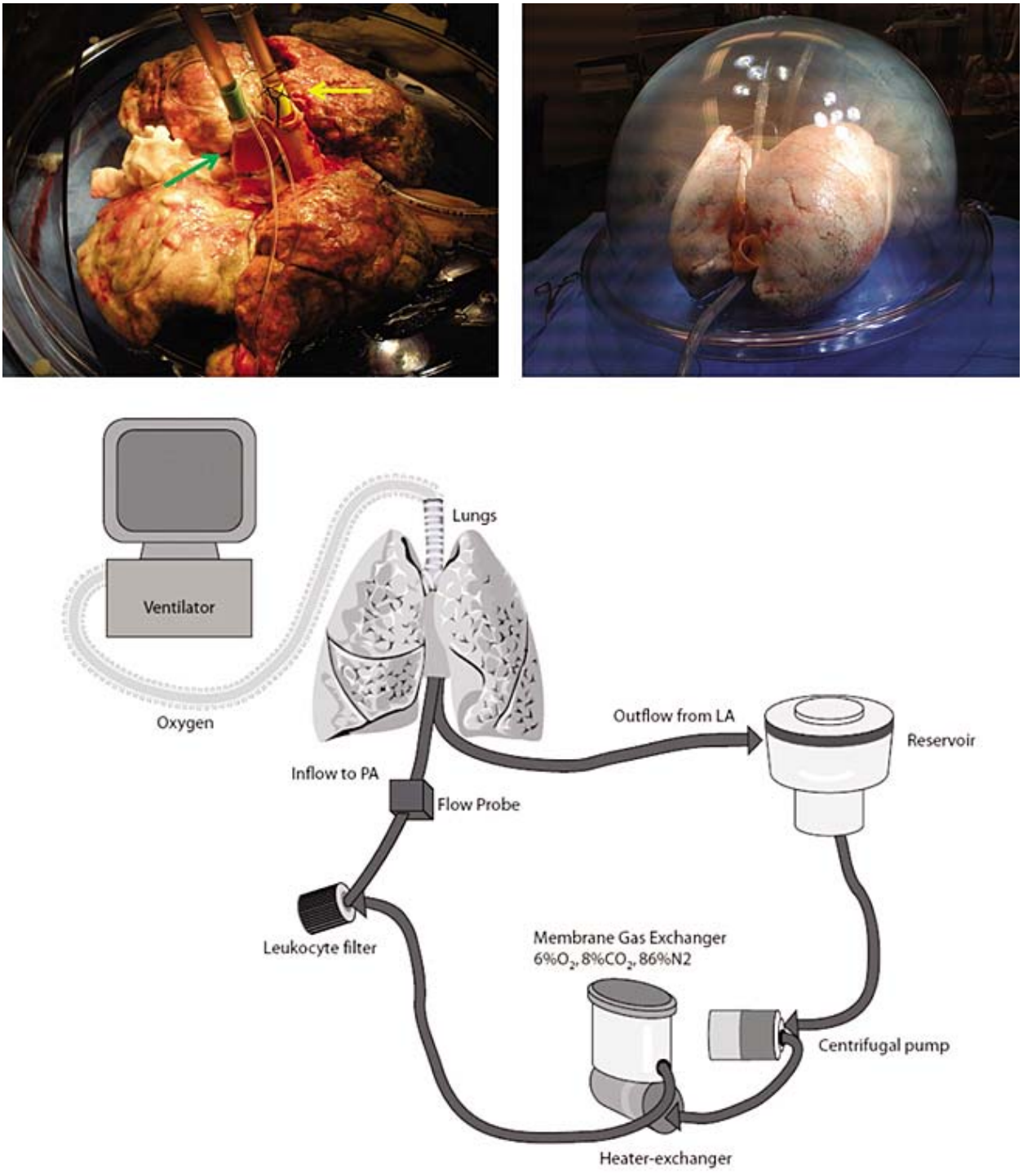

Figure 1. *Ex vivo* lung perfusion system (EVLP). Lungs are placed inside the Xvivo dome. The perfusate is circulated using a centrifugal pump passing through a leukocyte filter and a gas exchange membrane (to de-oxygenate and provide CO_2 to the perfusate) before entering the lungs through the pulmonary artery. A heater/cooler is connected to the membrane to maintain the perfusate at 37ºC. Pulmonary artery flow is controlled by the centrifugal pump and measured using an electromagnetic flow meter. Pulmonary artery and left atrial pressures are continuously monitored by built-in catheters connected to Xvivo cannulas: pulmonary artery (yellow arrow) and left atrial (green arrow). The lungs are ventilated with a standard ICU ventilator.

Table 1. Ventilatory and perfusion strategy for EVLP using the toronto technique

Ventilation	
Tidal Volume	7ml/kg
PEEP	5cmH_2O
Frequency	7 breaths per minute
I/E ratio	1/2
Recruitment	1 every hour to PawP 20cmH_20
Perfusion	
Pump flow	40% estimated donor CO
Pulmonary artery pressure	7-13 mmHg
Left atrial pressure	3-5 mmHg*
Perfusate exchange	250cc every hour
Perfusate composition	Steen solution, heparin, antibiotics, solumedrol
Perfusate pH	6.8 - 7.4
Perfusate pCO2	35 – 45 mmHg

* Left atrial pressure can be controlled by adjusting the height of the reservoir. CO=cardiac output.

Table 2. Outcomes in the EVLP and control groups

Variable	**EVLP (n=20)**	**Controls (n=116)**	**P Value**
Primary Endpoint			
PGD 2 or 3 at 72h (%)	15	30.1	0.11
Secondary Endpoints			
PGD 2 or 3 at ICU arrival (%)	25	30.3	0.30
PGD 2 or 3 at 24h (%)	15	36.2	0.07
PGD 2 or 3 at 48h (%)	30	35.3	0.46
ECMO (%)	0	3.5	0.37
P/F ratio T0h (mmHg) (Median)	424	372	0.51
Post-transplantation mechanical ventilation (days) (Median)	2	2	0.15
Post-transplantation ICU stay (days) (Median)	4	4	0.68
Post-transplantation hospital stay (days) (Median)	23	27	0.39
Bronchial complications requiring intervention (%)	5	4.3	1
30 Mortality (%)	10	5.2	0.33

Adapted with permission from (32)

intensive care unit was longer in recipients of perfused lungs compared to conventional transplantation (13 vs. 7 days) (30, 31).

The first prospective clinical trial using EVLP was recently completed at University of Toronto and the results were recently published (32). In this study, 20 EVLP lung transplants were performed after 4h of EVLP using the acellular protective ventilation/perfusion strategy developed by our group (27, 28). This trial demonstrated that extended acellular normothermic EVLP is safe for the assessment of high risk donor lungs, and similar early outcomes were obtained compared to conventionally selected and transplanted donor lungs. Table 2 demonstrates the outcomes of these patients compared to controls. Other centers in Europe have subsequently also successfully performed EVLP transplants using the Toronto strategy totalizing an experience of more than 60 EVLP LTx to date using this technique.

2.4. Pharmacological interventions during EVLP

Normothermic EVLP seems to be an ideal environment for therapeutic interventions in the donor lung prior to transplantation. The system provides the opportunity to better *select* which lungs to treat and the opportunity to *re-evaluate* to confirm a positive treatment effect. Furthermore, *side effects* of the treatment are greatly minimized by the targeted treatment of the organ and inflammatory responses during the repair process can theoretically be decreased due to the absence of circulating immune cells. Lastly, it provides flexible timing for treatment in contrast to *in vivo* treatment of the donor, where the time available for interventions is limited.

Several pharmacological investigations to target common donor lung injuries have been recently explored experimentally using EVLP as a platform.

Frank *et al.* demonstrated that optimal strategies for *ex vivo* perfusion and ventilation of human lungs have an important impact itself in enhancing alveolar fluid clearance (AFC) (33). The use of specific perfusate solutions with physiologic osmolarity and high oncotic pressures are important for the resolution of pulmonary edema. Pharmacological agents to remove pulmonary edema during EVLP by enhancing AFC have been studied. Sakuma *et al.* demonstrated the role of intra-tracheal epinephrine in enhancing AFC and removing pulmonary edema during EVLP. Treated lungs demonstrated an AFC of 84% above control clearance (34). The same group demonstrated the role of beta adrenergic stimulation on clearance of pulmonary edema using a human EVLP model (35). These drugs exert anti-edema effects by stimulating sodium channel activity and markedly increased the abundance of beta-and gamma-epithelial sodium channel (ENaC) in the plasma membrane of alveolar epithelial cells (36).

Since many donor lungs rejected for transplantation due to poor function have unrecognized pulmonary embolism (37, 38), Inci *et al* proposed the use of fibrinolytics during EVLP. This strategy might be especially important in controlled and uncontrolled DCD donors where donor heparinization might not be possible. Adding urokinase into the perfusate during EVLP resulted in improved graft function by reducing pulmonary vascular resistance and increasing oxygenation after 3 hours of

warm ischemia in a large animal DCD model (39). The same group demonstrated that intra-airway surfactant administration during EVLP improved *ex vivo* function donor lungs injured with acid aspiration (40).

A very large number of donor lungs are declined due to infection acquired during the brain death process and ICU period. EVLP provides a very attractive platform for high dose of antimicrobials without the risk of injuring other organs. In addition half-life of these drugs becomes much prolonged in the system. One recent study has shown that antibiotics during EVLP decrease the bacterial load (41) but further investigations are required to definitively prove this very promising concept. Perhaps, EVLP duration of several days might be required for the treatment of established pneumonias.

Inflammation related to brain death or other lung injuries during ICU stay greatly contribute to reperfusion injury after LTx (42-44). Anti-inflammatory drugs such as corticosteroids have been currently added to the EVLP perfusate. Other potential potent anti-inflammatory agents include activated protein C (45), alpha-1-antitripsin (46), inhibition of nuclear factor kappa B (47), and interleukin-10 (48-50).

2.5. Molecular interventions during EVLP

An exciting method of lung repair is using gene or stem cell therapy during EVLP. Gene therapy in lung transplantation is attractive because trans-tracheal delivery of gene vectors can localize the effect to the lung graft. Although *ex vivo* vector delivery is an attractive setting for clinical applicability (51), *ex vivo* gene transfer techniques have been traditionally ineffective in organ transplantation due to inhibition of metabolism during hypothermic preservation (52-54). Our group investigated the potential for new protein synthesis in the lung during normothermic EVLP and we demonstrated efficient gene transfer in alveolar epithelial cells and macrophages after 12 h of *ex vivo* gene delivery (28). Using large animals and injured human donor lungs rejected for transplantation, we showed that *ex vivo* adenoviral mediated interleukin-10 (a potent anti-inflammatory cytokine) gene delivery at the start of 12 hours of EVLP significantly improved lung function (50). hIL-10 gene therapy of injured human donor lungs reduced inflammation (downregulation of proinflammatory cytokines) and promoted cytoskeletal structural lung repair in association with improved function. Because EVLP preservation utilizes an acellular perfusate, there is an absence of neutrophils and inflammatory cells within the perfusate to respond to chemokines generated by the initial response to viral vectors. The superiority of adenoviral gene delivery *ex vivo* as compared to *in vivo* has also been recently demonstrated by our group (55).

Mesenchymal stem cells (MSCs) have been also used in the setting of experimental human EVLP. One recent study demonstrated that treatment with allogeneic human MSCs or its conditioned medium given 1 h following endotoxin-induced lung injury reduced extravascular lung water, improved lung endothelial barrier permeability and restored alveolar fluid clearance. Using siRNA knockdown of potential paracrine soluble factors, secretion of keratinocyte growth factor was essential for the beneficial effect of MSCs on alveolar epithelial fluid transport, by restoring amiloride-dependent sodium transport (56).

3. *IN VIVO* LUNG PERFUSION (IVLP) FOR TREATMENT OF CANCER METASTASES

3.1. Introduction and rationale

Another potential application of ILP is isolated perfusion of the organ *in vivo* for the purpose of delivering high dose chemotherapy to the lungs to treat cancer metastases. Pulmonary metastases occur in approximately 30% of patients dying of cancer. Most frequent metastatic pulmonary diseases suitable to surgical resection originate from soft tissue sarcomas and colorectal carcinoma. Although surgical resection is a widely accepted treatment for pulmonary metastases, a 5-year survival rate between 20 - 40% after complete resection remains disappointing (57). Unfortunately, most patients will develop recurrent disease even if complete resection is achieved, probably as a result of micrometastatic disease present at the time of initial operation. Most recurrences occur in the lungs themselves, suggesting that the lung is the major reservoir of occult metastatic burden. The relatively poor results of surgical resection of pulmonary metastases combined with intravenous systemic chemotherapy are probably due to drug resistance and the inability to achieve effective drug concentrations within the lung (58, 59). This implies that better chemotherapeutic agents and more efficient drug delivery as an adjuvant to surgery are needed. *In vivo* isolated lung perfusion (IVLP) has the theoretical advantage of selectively delivering an agent and diverting the venous effluent. This allows a drug to be delivered in a higher dose, while drug levels in critical organs that are relatively sensitive to the drug, are kept low enough to avoid severe side effects and toxicities (i.e. hematological, cardiac, renal etc.).

3.2. Experimental studies of IVLP

Pulmonary metastases from sarcomas have been the most studied disease in the setting of IVLP. IVLP with doxorubicin in a methylcholanthrene-induced sarcoma model in the rat was found to be a safe and effective method, and superior to intravenous injection. With a perfusate drug concentration that was well tolerated by the animals, lung tissue doxorubicin levels were 20-fold higher than after intravenous injection (60). After IVLP, a complete clearance of macroscopic and microscopic tumor was observed, whereas sham perfused lungs had massive tumor replacement (61). Perfusate doxorubicin concentration and the duration of perfusion were factors determining the final lung concentration of doxorubicin whereas flow rate of perfusion did not influence tissue uptake (62). In addition, this group compared 6 different perfusate solutions, and showed that Low Potassium Dextran (LPD) solution yielded the highest concentration of drug uptake in the lung tissue likely due its capacity to recruit the pulmonary microcirculation (62). Pulmonary artery pressures up to 25 mmHg seem to be the maximum

tolerated to prevent excessive lung damage with short term IVLP (63). Some studies also evaluated differences in tissue and tumor uptake with retrograde and anterograde perfusion. Retrograde IVLP did not confer a better doxorubicin uptake in the tumor as compared with antegrade lung perfusion despite the fact that the tumor vascularization in this model is greatly based on the bronchial artery circulation (64). Another study has shown that retrograde IVLP resulted in a higher melphalan concentration in the hilum and in the base of the lung, while anterograde perfusion induced a higher concentration at the apex of the lung (65).

Johnston provided a basis for further clinical and experimental studies by showing IVLP to be a safe technique in a large animal model (66).

3.3. Clinical studies of IVLP

In a pilot study by Johnston *et al.* four patients with unresectable pulmonary metastatic sarcoma and four patients with diffuse bronchioloalveolar carcinoma were treated with doxorubicin and cisplatin via IVLP and cardiopulmonary by-pass (67). Six patients were perfused with doxorubicin and two with cisplatin. Single left lung perfusion were performed in three patients and bilateral lung perfusion in five patients. Perfusion time ranged from 45 to 60 min at normothermic temperatures and whole blood was used as the perfusate. Pulmonary perfusate drug concentrations increased with higher doxorubicin dosages. Drug tissue levels also tended to increase with higher doses with only minimal systemic leakage. None of the eight patients had a partial or complete response to the regional chemotherapy and all died of progressive disease 23—151 days after lung perfusion. Burt *et al.* described their results of IVLP with doxorubicin after extensive laboratory research (68). Eight patients with inoperable lung metastases from sarcoma underwent single lung perfusion in a phase I protocol. Intrapulmonary concentrations of doxorubicin correlated with the dose given while systemic levels were minimal or undetectable. However, tumor levels were lower compared to lung levels. IVLP was performed in a deflated lung for 20 min using hetastarch + blood as perfusate. The maximal tolerated dose (MTD) in this study was defined at 40 mg/m^2 of doxorubicin since a significant chemical pneumonitis developed in one patient at a dose of 80 mg/m^2. There were also no partial or complete responses. One patient had stabilization of disease in the perfused lung, whereas the lesions in the untreated lung progressed markedly. Putnam *et al.* reported another phase I study of isolated single-lung perfusion with doxorubicin in 16 patients with unresectable pulmonary metastatic disease, also in sarcoma patients (69). Systemic levels were minimal or undetectable while two patients developed a grade 4 pulmonary toxicity at a dose of 75 mg/m^2, therefore defining the MTD at 60 mg/m^2 of doxorubicin in this study. Overall operative mortality was 18.8%. Only one major response occurred. Median survival time was 19.1 months in this study.

The group form Antwerp has recently translated their extensive laboratory work on IVLP to clinical trials. Their most recent phase I trial was performed using melphalan as the therapeutic agent in patients with resectable pulmonary metastases from sarcoma or colon cancer (70). IVLP was performed for 30 min at the time of pulmonary metastatectomy. Hetastarch was used as the perfusate. In total, 21 procedures of isolated lung perfusion with complete metastatectomy were performed. Operative mortality was 0%, and no systemic toxicity was encountered. Grade 3 pulmonary toxicity developed at a dose of 60 mg of melphalan at 37°C and therefore 45 mg was defined as the MTD. An extension of this trial was performed using hyperthermic 42°C perfusion, however pulmonary toxicity was significantly higher and therefore the authors recommended 37°C as the ideal temperature for IVLP (71). A recent publication described long-term results of IVLP in the phase melphalan I trial. No major long-term pulmonary toxicity was observed and the five-year overall and disease-free survival in this study was promising (72).

Investigation of different perfusion techniques, including minimally invasive lung cannulation and perfusion techniques (73), and the use of targeted molecular agents to treat cancer metastases to the lungs will be major goals of the future. To that end, gene therapy to up-regulate B7-1 (CD-80) (74) or interleukin-2 (75) during IVLP are potential targets to treat and prevent osteosarcoma metastases to the lungs.

3.4. Other techniques for delivery of high dose chemotherapy to the lungs

Less invasive techniques have been proposed in order to achieve high concentration of drugs in the lungs. The use pulmonary artery blood flow occlusion (BFO) using a catheter based technique has been recently proposed. While the advantage of this technique is the avoidance of a thoracotomy, the potential disadvantage is the absence of lung isolation from systemic circulation and therefore systemic exposure to the drugs. In a study by Van Putte and colleagues, significantly higher pulmonary gemcitabine peak concentrations were observed after gemcitabine and gemcitabine/carboplatin delivered by BFO compared to IV, while no differences were shown between serum, renal and lymph tissue (76, 77). Another described technique is chemo-embolisation using microspheres which proved to be safe in a large animal model (78).

4. CONCLUSIONS AND FUTURE PERSPECTIVES

By mimicking the lung's natural physiological environment and by providing oxygen and other substrates necessary for active metabolism, normothermic EVLP may offer the next step in lung assessment and preservation. Pre-transplantation treatment of donor lungs, such as the use of pharmacologic agents to reduce pulmonary edema and inflammation or the use of gene therapy and MSCs to better prepare the organ to deal with the reperfusion and subsequent immunologic insults will be the major goals of the future. Extended preservation time with acellular EVLP will also allow for improved and more accurate organ diagnosis using newly discovered biomarkers and a more personalized treatment for the organ including the induction of tolerance. Exciting spin offs from the current perfusion technology are the re-visitation of IVLP for the treatment of cancer metastases to the lung, and the use of

EVLP as a bioreactor for decellularization and regeneration of lungs (79, 80).

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Key Words: Lung transplantation, Preservation, Perfusion, Repair, Lung metastases, Review

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