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Feasibility of lung cancer hyperthermia using breathable perfluorochemical (PFC) liquids. Part I: Convective hyperthermia

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Clinical studies have shown that hyperthermia in combination with radiotherapy and/or chemotherapy may be effective in the treatment of advanced cancer. No method of lung hyperthermia, however, has been accepted as standard or superior. This investigation sought to demonstrate in animals the thermal and physiologic feasibility of lung hyperthermia induced using heated breathable perfluorochemical (PFC) liquids, a method termed liquid-filled lung convective hyperthermia (LCHT). The ability to use LCHT is rooted in the development of both PFC liquid ventilation, now in clinical development with the PFC perflubron (LiquiVent®), and a PFC blood substitute also in late Phase III trials (Oxygent™). As LCHT background, the PFC technologies and biology are first reviewed. The physical properties of a variety of PFCs were evaluated for LCHT and it was concluded that more than one liquid is suitable based on such properties. Using total liquid ventilation type devices, LCHT was shown to deliver successfully localized (lobar) lung heating in sheep, and bilateral whole lung heating and whole-body hyperthermia in rabbits, cats and lambs. During LCHT, lung parenchymal temperatures were uniform ($< 1^{\circ}C$) across heated regions. In addition, based on patterns relating lung tissue temperatures to inspiratory and expiratory PFC liquid temperatures in the endotracheal tube, LCHT may minimize invasive thermometry requirements in the lung. Based on acute experiments, it was concluded that LCHT appears feasible and may simplify lung hyperthermia. It was recommended that potentially synergistic combinations of LCHT with other whole-body hyperthermia or local heating modalities, and with chemotherapeutic lung drug delivery, also be explored in the future.

Key words: Lung cancer, hyperthermia, convection hyperthermia, perfluorochemical, perfluorocarbon, liquid breathing, pulmonary carcinoma, lobar hyperthermia, whole-body hyperthermia.

1. Introduction

1.1. *Challenge of lung hyperthermia*

Clinical studies show that hyperthermia in combination with radiotherapy and/or chemotherapy may be effective in the treatment of advanced cancer[1–5]. When the current project was first proposed[6], it had been previously reported[7] that of approximately 20 000 hyperthermia treatments, fewer than 300 had been targeted to the lung, in spite of lung carcinoma being the leading cause of cancer mortality. The challenge of lung hyperthermia still persists, with no methods demonstrating superiority.

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A common approach to hyperthermia of the lung has been whole-body hyperthermia (WBH). Clinical WBH studies, the majority targeting 41–42°C core temperatures for 1–2 h, have demonstrated some responses in a variety of cancers but have, in general, had limited therapeutic gain due to heat-associated normal tissue and systemic toxicity[8,9]. To mitigate these problems, alternative WBH approaches using longer (up to 6 h) treatments at lower 'fever-like' core temperatures (39.5–40°C) have recently been explored[10]. The disappointing results for WBH may be attributed, in large part, to the inability of current modalities to heat rapidly the body (known to have a protective effect, e.g. Wust *et al.*[11]), and the complexity of maintaining thermal control of the treatment.

The use of WBH for lung cancer can be justified in cases of metastatic disease, but necessarily lacks lung-specificity. Some loco-regional electromagnetic approaches to lung heating requiring invasive thermometry, e.g. radiofrequency capacitive devices, have been shown to be clinically feasible for selected advanced lung cancers,[12] but also have had limited acceptance, for reasons of labor intensity and inadequate control and tailoring of heating patterns. More recently, isolated lung perfusion with chemotherapy, to concentrate drug in the lungs, has also been shown to be clinically feasible for co-administration of heat to one or both lungs[13]. Although an invasive procedure, studies of isolated dog lung perfusion heating have shown temperatures less than 44.4°C may be acutely tolerable for 2 h[14]. Furthermore, no histological evidence of lung or tracheal thermal damage was found in WBH canine studies employing external heating and concomitant airway heating with 42°C humidified gases for over 1.5 h[15]. The only negative observation was a temporary reduction in tracheal mucociliary transport.

The studies described here explore lung heating involving either partial or complete filling of the lung with heated breathable perfluorochemical (PFC) liquids, methods termed liquid-filled lung convective hyperthermia (LCHT). Related techniques of local lung hyperthermia induced by ultrasound propagated into the PFC-filled lung are described in Part II, a companion paper[16].

1.2. *Background: PFC liquid ventilation and blood substitutes*

LCHT techniques are made possible by a variety of technical advances, but most notably by the laboratory and clinical experiences with PFC liquid ventilation (LV) and PFC blood substitutes (oxygen carrier emulsions). Because PFC-mediated heating of the lung involves many of physiological and cellular processes of LV, a review of LV technology and the characteristics of PFCs in the body is offered next as background.

1.2.1. *Status of LV.* Beginning with the seminal experiments of Clark and Gollan[17], in which mice were first shown to survive sustained immersion breathing of oxygenated PFC liquid, the last three decades have produced a large body of information on the physiological and cellular biological responses to PFC LV, in both normal and injured lungs. The first two decades of LV research focused on total (or 'tidal') liquid ventilation (TLV), ventilation of the completely liquid-filled lung using a TLV system or 'liquid ventilator' to pump oxygenated PFC to and from the lung in a tidal flow manner (e.g. Shaffer[18]). In recent years, research interests have emphasized partial liquid ventilation (PLV) (e.g. Fuhrman *et al.*[19] and Tutuncu *et al.*[20]), gas ventilation of the PFC-filled lung, the only technique to reach commercially sponsored LV clinical trials. Thus far, over 400 acute lung failure patients of all

ages have been enrolled in PLV clinical trials with perflubron, (LiquiVent®, Alliance Pharmaceutical Corp., San Diego, CA, USA), the only PFC in US FDA-sanctioned human trials[21–24]. Recently, a Phase II/III 311-patient LiquiVent trial was completed in North America and Europe for the treatment of acute respiratory distress syndrome (ARDS).

1.2.2. *Status of PFC blood substitutes.* Regarding intravenous (i.v.) administered PFC oxygen carrier emulsions, Fluosol-DA® (perfluorodecalin and perfluorotripropylamine emulsion) (Green Cross Corp., Osaka, Japan), was approved by the US FDA in 1993 for use in percutaneous transluminal coronary angioplasty (PTCA). Certain PFC oxygen carrier emulsions have also been shown to sensitize tumor cells to radiation and chemotherapy (e.g. Teicher[25]), and Fluosol-DA was tested for oxygenating tumors in combination with radiotherapy for carcinoma of the lung (e.g. Lustig *et al.*[26]). The perflubron-based oxygen carrier Oxygent™ (Alliance Pharmaceutical Corp., San Diego, USA) has been used as a blood substitute in over 1400 surgical patients in studies similar to those described by Spahn *et al.*[27], demonstrating a reduction in donor blood in a 492 patient Phase III trial[28].

1.2.3. *PFC LV: background of safety and efficacy.* Improved pulmonary function (gas exchange and lung mechanics) and less lung damage in comparison to conventional mechanical ventilation, has been extensively demonstrated in lung-injured animals for both PLV[20,29,30] and TLV[31–33]. The mechanisms by which these benefits accrue are still being elucidated but are largely attributable to the unique physicochemical properties of these liquids. PFCs are synthetic fluorinated hydrocarbons in which the high strength of the carbon–fluorine bond imparts extreme chemical and physical stability, rendering them essentially inert and non-metabolized by the body[34]. As a class, PFCs have very high solubilities for gases (table 1), central to their ability to support ventilation, and have minimal solubility in aqueous material and only limited lipid solubility. Perflubron appears to have the right degree of lipophilicity to promote rapid elimination[34].

PFCs have been used in the lung to treat respiratory distress syndrome (RDS) in both infants and adults (ARDS), disease states characterized by dramatically elevated alveolar surface tension due to the inactivation and depletion of native lung surfactant, and to the presence of edema, exudative proteins, inflammatory cells and debris. PFC surface tensions are considerably lower than those for water

Table 1. Physical properties of candidate perfluorochemical liquids. Gas solubilities, vapor pressure, surface tension at 37°C; others at 25°C.

Property	Water	FC-77	PF-alkanes	Perflubron	PF-decalin
O_2 solubility (ml gas/ml liquid)	3	50	52	53	49
CO_2 solubility (ml gas/ml liquid)	57	198	160	210	140
Density (g/cm^3)	1.00	1.78	1.78	1.92	1.95
Surface tension (dynes/cm)	72	15	15	18	19
Boiling point (°C)	100	97	101	143	142
Vapour pressure (Torr)	47	85	64	11	14
Specific heat (J/kg °C)	4174	1044	1044	1044	918
Viscosity (kg/ms)	1.0	0.8	0.8	1.1	2.9
Thermal conductivity (W/m °C)	0.600	0.063	0.063	0.063	0.067
Prandtl number	7	13	14	18	40

and biological fluids (table 1), and are thought to produce a 'surfactant-like' reduction in surface tension in gas-filled, PFC-lined alveoli in the injured lung. This trait contributes to their ability to open up atelectatic and debris-obstructed airways and alveolar spaces. Importantly, PFCs do not inactivate nor appreciably remove lung surfactant. The introduction of bulk PFC liquid into the lung also reduces surface forces because the gas–liquid interfacial area is either reduced (PLV) or eliminated (TLV).

In the injured lung, LV reduces perfusion shunt and likely improves ventilation to perfusion (V/Q) matching, both during TLV[32] and PLV[35]. These advantages are associated with restoration of alveolar ventilation, shifts in pulmonary blood flow to the non-dependent regions (thought to be caused by PFC density; table 1), with inhibition of hypoxic pulmonary vasoconstriction in mid-to-lower regions, reducing the vertical perfusion gradient in comparison with gas ventilation for TLV[36] and PLV[35,37].

Although high-purity PFCs are generally considered biochemically inert and are not metabolized, a growing number of LV studies support the presence of a variety of PFC associated anti-inflammatory cellular effects. Certain PFCs (e.g. FC-77 and perflubron) have also been shown likely to reduce bacterial adhesion to cells, perhaps avoiding increased risk of lung infection[38]. Some evidence supports a physical barrier mechanism for these PFC-mediated effects[39], whereas it has also been shown that cellular protective effects and reduction in inflammatory responses can occur independent of physical contact with the PFC liquid phase[40,41].

PFCs have also been shown useful in the lung for imaging. The bromine atom on the perflubron molecule ($C_8F_{17}Br$), for example, renders this PFC more radiopaque than others used for LV. In PLV studies, planar X-ray images[42] and X-ray CT[43] have been used to assess PFC filling and distribution. Due to the absence of hydrogen atoms, PFCs are also useful for magnetic resonance imaging (MRI), with perflubron having US FDA approval as an MRI contrast agent for the gastrointestinal tract[44].

1.2.4. *TLV devices.* LCHT requires the tidal delivery of temperature-controlled PFC liquid to all, or selected, portions of the lungs. The greater the fraction of lung volume treated, the greater the need for the device to perform the functions of ventilation. An LCHT system is, thus, a liquid ventilator (TLV system) capable of controlled hyperthermic breathing and having temperature data acquisition capability. Shaffer[18] summarizes early TLV use and devices, and Sekins *et al.*[45] present a brief review of TLV device development, including clinical and commercial requirements for such systems. Wolfson *et al.*[33] recently demonstrated consistent therapeutic results in a multicentre TLV preclinical study of ARDS, in which each center used computer-controlled double-piston pump TLV prototypes of identical design. Of particular relevance, Forman *et al.*[46] demonstrated that the lung is an excellent liquid–liquid (PFC-blood) heat exchanger by using TLV to induce systemic hypothermia (cooling) in animals, as a proposed adjunct to anaesthesia in surgery.

Comprehensive overviews of LV are found in the respiratory critical care literature (e.g. Wolfson *et al.*[47] and Fuhrman *et al.*[19]). For understanding PFC transport in the body and the safety of medically pure PFCs when administered i.v. in significant amounts, survey articles on the PFC blood substitutes (oxygen carriers) are also relevant[34,48].

1.3. *Current study objectives*

In the present investigation, we hypothesized that local (lobar), whole lung and WBH could be induced by convection heating (LCHT) in large and small animals using heated PFC liquids delivered in a tidal flow fashion. Further, it was hypothesized that LCHT could be performed under conditions that were physiologically tolerable, including maintenance of adequate ventilation, for periods appropriate to clinical hyperthermia treatments. The investigation first focused on the selection of appropriate PFCs, then involved the design and construction of an LCHT system providing desired ventilation, heating and thermal data acquisition capabilities. A series of *in vivo* tests, primarily in sheep, were then performed over a range of conditions to gain insights into LCHT thermophysiology, the relationship of PFC fluid temperatures to local and core temperatures, and the requirements for LCHT device design. The emphasis was first placed on delivering hyperthermia preferentially to the lungs, with the goal of minimizing systemic heating, then bilateral whole lung LCHT was explored for both selective lung heating and inducing WBH.

2. Materials and methods

2.1. *PFC liquids and thermophysical properties*

Several PFCs representing a wide range of molecular weights were compared based on their physical properties for convection heat transport and ventilation (table 1). The subset reported here are among the more favourable based on their respective combined properties, and include: (1) FC-77 (3M Corp., Minneapolis, MN, USA); (2) two nearly equivalent perfluoroalkanes, FC-75 (3M Corp.) and RM-101 (Miteni, Milan, Italy); (3) perflubron (Alliance Pharmaceutical Corp.); and (4) perfluorodecalin (PF-decalin) (Multifluor APF-140, Air Products and Chemicals, Inc., Allentown, PA, USA).

Although the affinity of PFCs for gases is paramount for ventilation, the liquids may be distinguished more on the basis of their solubility for CO_2 than O_2 (table 1), due to the much smaller arterial–alveolar concentration gradient driving exchange for CO_2. PFC liquids are dense, with specific gravities ranging from about 1.5 to 1.9.

PFC boiling should be avoided in the lung, both to minimize heterogeneity of fluid distribution and heat transfer, and to prevent vapor trapping during the repetitive tidal inflation of the lung, a potential source of lung trauma. The PFCs presented in table 1 are acceptable from this perspective, with vapor pressures both high enough to produce efficient evaporation from the lungs at the conclusion of the treatment, and low enough to prevent risk of intravascular gas–vapor embolism. For i.v.-administered PFC emulsion oxygen carriers, where embolism is most likely, vapor pressures below 20 Torr have been recommended[49].

Specific heat (c_p) and density (ρ) define thermal capacitance, ρc_p. Because there is little variation (table 1) between fluids in c_p (≈ 1040 J/kg °C), the denser have the higher thermal capacitance.

A key consideration is the minimization of flow resistance in the lung, a strong function of viscosity, μ. Flow dynamics may also be characterized in terms of the ratio of viscosity to density, or the 'kinematic viscosity'. Considering the higher densities of the PFCs presented in table 1, those with μ below that of PF-decalin are preferred and have flow dynamics similar to water.

The ability to transfer heat to the lung airway surfaces by convection may be characterized in terms of the 'Prandtl number', $\mathrm{Pr} = c_p \mu / k$, where k is thermal conductivity. The Prandtl number is a measure of the ratio of molecular momentum transport (viscosity effects) to thermal transport by diffusion, with lower Pr indicating more efficient heat transfer for a given flow resistance. Because c_p for PFCs is virtually constant, and their thermal conductivities only vary by about 20% ($k_{ave} \approx 0.064\,\mathrm{W/m\,^{\circ}C}$), their Pr comparisons (table 1) are dominated by differences in μ. Again, viscosities below PF-decalin are preferred on this basis as well.

Some variations in physical properties of PFCs can be seen with temperature over the hyperthermic range (37–45°C). Vapor pressures significantly increase ($\approx 40\%$), while smaller changes (decreases) occur for most other properties ($\approx 20\%$ for surface tension; $\approx 10\%$ for viscosity and Prandtl No; $\approx 5\%$ for CO_2 solubility; $< 2\%$ for density, thermal conductivity and O_2 solubility), with specific heat slightly increasing ($< 2\%$).

Based on the thermophysical properties of these PFC liquids, particularly the Prandtl number considerations, the PF-alkanes, perflubron and FC-77 were deemed essentially equivalent from a heat transfer perspective, and more suitable than PF-decalin for LCHT. Only the PF-alkanes and perflubron were used for the initial *in vivo* LCHT experiments described below and, due to their thermal equivalence, will not be distinguished from each other in the results reported. Of course, other factors, such as purity, vapor pressure and medical history will be relevant for clinical use.

2.2. *Laboratory hyperthermia systems*

The primary laboratory LCHT system used in these studies was a gravity pump-based circuit (figure 1). Another system, a positive displacement peristaltic pump-based liquid ventilator[50] was used for a few of the bilateral lung studies described below. In the gravity feed system, the inspiratory and expiratory flows

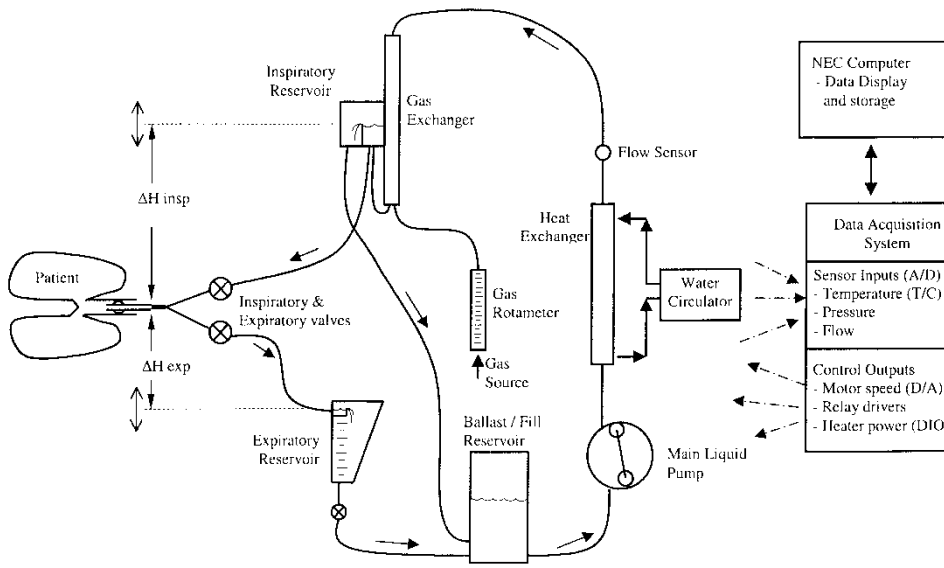

Figure 1. Schematic of gravity-pump based LCHT system.

were driven by hydrostatic pressure differences between the chest and the fluid free-surfaces of the inspiratory and expiratory reservoirs. The driving pressures were set by adjusting the reservoir heights above (ΔH_{ins}) and below (ΔH_{exp}) the chest, respectively. Fluid flow was controlled by pinch-valves at the proximal end of the cuffed endotracheal (ET) tube, through which PFC flowed to and from the subject. The source (inspiratory) liquid was oxygenated, scrubbed of CO_2, and heated to desired inspiratory temperature, T_{ins}, by a continuous fluid recirculation/regeneration loop. A ballast reservoir provided reserve liquid and added thermal capacitance to the system. The main liquid mechanical pump was used for the recirculation flow of the regeneration loop, and urethane tubing was used throughout the LCHT flow circuit. A liquid–liquid shell-and-tube heat exchanger served as the main system heat exchanger, with temperature-controlled water used for setting the PFC liquid temperature. The acrylic inspiratory and expiratory reservoirs each had integral overflow weirs to maintain ΔH_{ins} and ΔH_{exp} at fixed values. The expiratory reservoir also had a tapered chamber through which the weir overflow drained. By turning off the outlet flow from this reservoir between breaths, tidal volumes were periodically measured using volume graduation marks on the chamber.

The gravity-pump LCHT system provided automated control and data acquisition in regard to thermal parameters. System control was performed via a personal computer with peripheral data acquisition hardware (Keithley 500A ISA expansion card for analog voltage and thermocouple inputs). The real time control hardware (up to 16 channels of temperature data, 10 pts/s, 0.1°C resolution) and driver software provided for setting and monitoring all ventilation and thermal parameters, and for automatic control of T_{ins} by a proportional–integral–derivative algorithm. Invasive tissue temperature probes were all stainless steel 26-gauge type T thermocouple needle microprobes (models MT-26/4 and MT-26/6; PhysiTemp Instruments, Clifton, NJ, USA). Airway liquid temperatures (dictating inspiratory [T_{ins}] and expiratory [T_{exp}] liquid temperatures) were measured by a long thin (0.025-inch diameter) flexible, Teflon-sheathed Type T thermocouple probe (PhysiTemp model IT-18) whose tip was placed in the proximal end of the ET tube. These Teflon-sheathed probes were used rectally for recording animal core temperatures, and were checked by simultaneous use of a YSI 400 thermistor probe (YSI, Inc., Yellow Springs, OH, USA). The Keithley-thermocouple system had automatic cold-junction compensation and, before experiments, were two-point calibrated at 37 and 50°C in precision-controlled temperature water baths (CFT-75, Neslab, Portsmouth, NH, USA), with the bath temperatures measured using National Institute of Standards and Technology (NIST) traceable mercury-in-glass certified thermometers (0–50°C; 0.1°C resolution; VWR Scientific Products, Seattle, WA, USA). Based on post-experiment checks, including mid-range temperatures, overall calibration accuracy of the temperature data was approximately ± 0.2°C.

To reduce the loss of PFC in the vapor effluent from the spray–bubbler gas exchanger, cooling of the outlet gas/vapor flow was done with condensers. In most cases, measurable recapture of PFC liquid with efficiencies over 50% were achieved.

Tidal flow delivery of warm PFC required that the ET tube cuff seal the lobe, segment or whole lung, to prevent leakage of the PFC. Current conventional ET tubes which employ low pressure polyvinylchloride (PVC) cuffs were found not to produce adequate airway sealing against warmed PFC liquids. US FDA-approved latex rubber ET tubes (Rüsch, GMBH), although no longer widely used clinically

due to their tendency (with prolonged use) to produce bronchial wall ischemic damage, produced adequate sealing against the PFCs. When isolation of PFC delivery to specific lobes (in sheep) was desired, conventional clinical dual-lumen PVC ET tubes (Carlens tube, Mallinckrodt, St Louis, MO, USA) were modified to incorporate custom proximal and distal latex rubber cuffs, similar in design to the Rüsch tubes. Note that the thick-walled latex rubber cuffs required higher inflation pressures than the currently clinically preferred low-pressure PVC cuffs (6–12 versus < 1 psi). However, unlike the PVC cuffs, the majority of the inflation pressure in the latex cuffs went into the distension of the cuff itself, and not to coupling the cuff against the airway wall. To insure against injurious over-pressuring of the latex cuffs, they were inflated in small pressure increments until they occluded the airway sufficient to stop air leakage when pressurized by the gas ventilator or hand-operated transport ventilation bags.

2.3. In vivo *lung hyperthermia protocols*

A series of both local and bilateral (whole-lung) LCHT experiments were performed on normal animals, with hyperthermic plateau periods ranging from about 30 to 60 min. The studies were performed under the approval of the Temple University School of Medicine Institutional Animal Care and Use Committee and were managed according to the National Institutes of Health Regulations and the Guiding Principles in the Care and Use of Animals of the American Physiological Society. The local LCHT tests were performed targeting the right cranial (RC) lung lobe of sheep, using dual-lumen cuffed ET tubes. The bilateral lung heating experiments were performed in sheep, lambs and one cat. In all experiments, the animals were ventilated with O_2-saturated ($F_IO_2 = 1.0$) PFC, and in all but the one animal recovery experiment, blood gases were measured at 30-min intervals (blood gases corrected to animal body temperature). At the end of the acute experiments, animals were euthanized by i.v. sodium pentobarbital overdose (150 mg/kg).

2.3.1. *Local convective lung hyperthermia.* Lobar hyperthermia was performed in juvenile and adult sheep ($n = 7$, 15–30 kg), and each was subject to approximately the same surgical preparation, instrumentation setup and experimental protocol. Following intramuscular injection of ketamine (15–20 mg/kg), the animal was anaesthetized with 25–30 mg/kg i.v. sodium pentobarbital and secured in the supine position. A constant plane of anaesthesia was maintained (here, and in all studies) by assessing stability of vital signs (< 20% increase in either MAP or heart rate) in response to deep paw (or lower leg) pinch. The paw pinch involved hemostat clamping on soft tissue near hoof or toe pad, as applicable. After 1% lidocaine local infiltration, the right carotid artery and right jugular vein (or alternatively, femoral artery and veins) were each cannulated, and a tracheotomy of the proximal trachea was performed. Before suppressing spontaneous breathing efforts by continuous i.v. infusion of skeletal muscle paralytic (pancuronium bromide; 0.10 mg/kg bolus, followed by 0.10 mg/kg/h throughout the experiment), the paw pinch assessment was performed. The paw pinch response was also used following paralysis as criteria for adequacy of anaesthesia, maintained by supplemental sodium pentobarbital, 4 mg/kg/h. Each animal was intubated with the appropriate modified bifurcated ET tube through a tracheotomy of the proximal trachea. Placement of the ET tube was adjusted so that the RC lobe could be isolated and ventilated separately from the rest of the lung. This isolation was accomplished

first by guiding the tube with a bronchoscope and then securing it via inflation of both the proximal and distal cuffs, confirming ET tube sealing with pressure maintenance and pneumotachography of separate lung sections. If sealing was unsuccessful, usually due to airway branching architecture not matching cuff locations on the ET tube, positioning and securing the tube was performed intraoperatively via ligation of the ET tube in the trachea.

To permit placement of invasive temperature probes in the single RC lobe, a "window" in the right chest wall was surgically created by retraction of a single intercostal space following lidocaine infiltration (4 mg/kg, 0.50% lidocaine. Small (29 gauge) needle Type T thermocouple probes (model MT-25/5, PhysiTemp Instruments, Clifton, NJ, USA) were randomly positioned in the lobe with probe tip separations >1–2 cm.

To maintain stability of the animal before and after the hyperthermia portion of the experiment, it was mechanically gas ventilated (Harvard Large Animal Ventilator) at a tidal volume ≈ 15 ml/kg, $F_IO_2 = 1.0$, at 15–40 breaths/min, the rate set to maintain P_aCO_2 between 40 and 50 mmHg, and thus varying with animal size. Infusions of i.v. crystalloid solution (10% dextrose with 10 mEq sodium bicarbonate and 1 mg sodium pentobarbital/100 ml fluid) was maintained at 3 ml/kg/h throughout the protocol. Cardiopulmonary stability was confirmed with arterial blood gas tensions, pH, heart rate and mean arterial blood pressure (MAP) measurements.

Before induction of hyperthermia, the treated lobe was typically filled with a bolus of fully oxygenated PFC liquid. Typical RC lobar functional residual capacity (FRC) volumes were measured (via gas ventilation volume determination) to be from 15 to 20% of the entire FRC of the animal. In most cases, before LCHT a period of normothermic TLV of the lobe was performed to establish a baseline condition and to purge the lobe of residual gas. Extensive histology was not performed, but some tissue samples were examined to obtain a preliminary histologic evaluation.

2.3.2. *Local convective hyperthermia with recovery*. In a single 15 kg sheep, lobar LCHT was performed using perflubron with the goal of full recovery of the animal. To minimize surgical trauma, a right cranial lobe thoracic window was not created, nor were invasive temperature probes used in the lung. The lung temperatures were approximated based on tissue versus airway liquid (i.e. T_{ins} and T_{exp}) temperature relationships obtained from the non-recovery lobar experiments (see Section 3.1). To avoid potential recovery complications associated with catheter punctures for vascular access, anaesthesia was induced by intraperitoneal (i.p.) injection of sodium pentobarbital (30 mg/kg, introduced below the inferior margin of the liver) and maintained at subsurgical levels (5 mg/kg/h; two subsequent injections) for the duration of the experiment. A tracheotomy was performed under local anaesthesia (4 mg/kg, 0.50% lidocaine) and a customized double-lumen cuffed ET tube was placed to isolate the RC lobe. After cuff inflation, isolation was confirmed by pressure maintenance and independent flow profiles measured by pneumotachography. Post-experiment radiography was also used to confirm isolation of perflubron to the lobe. Pulse oximetry was monitored by an ear probe.

The lobe was filled and normothermic (38°C) LV of the lobe was performed until the transition to hyperthermia was initiated. The remainder of the lung was ventilated throughout the experiment by spontaneous (non-assisted) air breathing. After returning the treated lobe to normothermic LV, the lobe was mechanically gas

ventilated (Harvard Large Animal Ventilator, $F_IO_2 = 1.0$; peak inspiratory pressure = 20 cm H_2O, positive end-expiratory pressure, PEEP = 5–8 cm H_2O, rate = 30 breaths/min) for 10 min with frequent suctioning. The trachea and skin were closed as the animal was extubated, after which it was supported for 20 min with a blow-by gas flow (8 l/min, $F_IO_2 = 0.5$) delivered by nasal cone. Lateral and anterior–posterior chest X-rays were taken to assess the distribution of perflubron in the lung at this time. Post-LCHT management included both systemic and local antibiotics (intramuscular penicillin, 125 000 units; gentamicin, 20 mg; topical neomycin sulphate and isoflupredone acetate) and analgesics (2% tetracaine HCl, 1 ml subcutaneous infiltrate bilateral to trachea; fentanyl, intramuscular 5 μg/kg given twice, every 4 h). Analgesics were discontinued according to veterinary care monitoring affirming responsiveness and good clinical parameters, including no signs of respiratory distress.

2.3.3. *Bilateral lung hyperthermia.* Animals (lambs) treated with bi-lateral LCHT ($n = 4$) were acute studies, not involving recovery, and were surgically prepared and anaesthetized in a fashion similar to that described for the sheep lobar LCHT, including paw pinch assessment of plane of anaesthesia before and after paralysis. Regarding the single cat experiment, the animal (1.82 kg) was anaesthetized with sodium pentobarbital (i.p.: 30 mg/kg) and, following local anaesthesia (1% lidocaine), the ET tube (4 mm) was inserted though a tracheotomy. The carotid artery was cannulated for continuous monitoring of blood pressure and calculating heart rate, and the femoral vein was cannulated for continuous infusion of metabolic substrate, anaesthesia and paralytic agents. Animals were liquid ventilated at 5 breaths per minute and tidal volume of 15 ml/kg.

In some cases a thermodilution catheter (Arrow International, Redding PA, USA) was used, both for sheep (7.5 Fr) and the cat (5 Fr), being inserted for the measurement of central venous blood temperature, T_{cvp}. Some experiments were performed with invasive thermometry, as above, but others used projected lung tissue temperatures (see Section 3.1) based on airway liquid temperatures (i.e. T_{ins} and T_{exp}). Blood gases and MAPs were recorded. In addition to preferential heating of the lungs, the bilateral technique was also explored as a means for inducing WBH.

The focus of the hyperthermia studies series reported herein was on developing and comparing LCHT techniques and parameters and on technical feasibility. As such, the studies did not require specific time-temperature thermal dose requirements be met across all experiments. However, thermal dose was used for specific comparisons in select experiments to illustrate the comparative efficacy anticipated from different methods and different anatomical sites. The dosimetry employed for this purpose was that of the well known thermal isoeffect dose of Sapareto[51] and Dewey[52], whereby tissue temperatures measured over a treatment time interval were converted to cumulative equivalent minutes (CEM_{43}) for an isoeffect at a reference temperature of 43°C. The dose calculation used was:

$$CEM_{43} = \sum_{t=0}^{t=\text{final}} R^{(T-43)} \Delta t, \tag{1}$$

where, in a specific treatment time interval Δt (here sampled every 5 min), the tissue mean temperature is T; the constant $R = 4$ for $T < 43°C$ and $R = 2$ for $T > 43°C$[52].

3. Results

3.1. *Local lung hyperthermia*

Figure 2(a) and (b) demonstrate, over the course of a lobar LCHT experiment, here with a minimum liquid temperature target of 42°C, the typical dynamic temperature pattern measured in the ET tube as the heated liquid alternately flows into and out of the lung. When heating from normothermic TLV to a hyperthermic plateau (figure 2a) the temperature maxima become the inspiratory liquid temperature (T_{ins}). Similarly, when returning to normothermia (figure 2b), T_{ins} flips from maxima to minima as the liquid cools the lung. Corresponding temperature oscillations of lesser magnitude (not shown) were exhibited by the invasive needle probes in

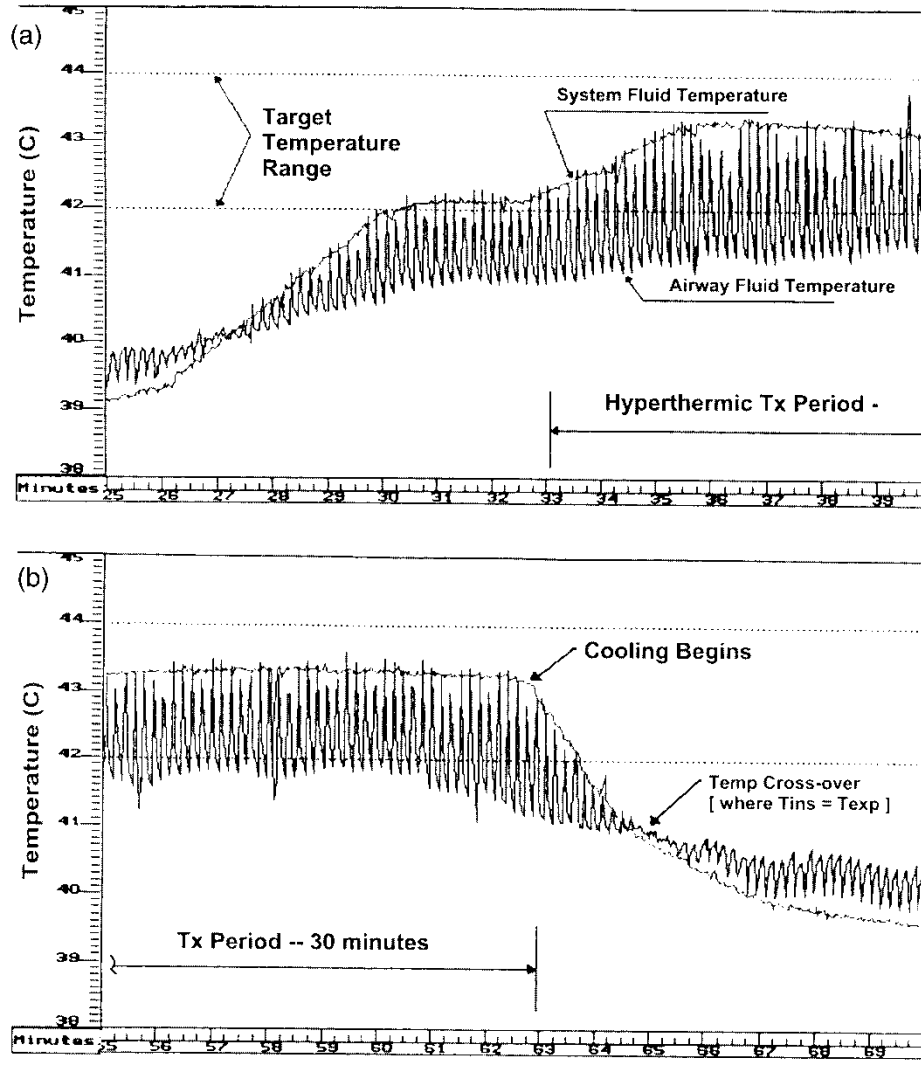

Figure 2. (a) Typical temperature waveform during LCHT treatment of a RC lobe of a sheep. Temperatures measured at the distal end of an ET tube (airway temperature). Breath rate = 5 breaths/min. (b) Waveform for the final portion of the treatment of (a). Six-minute cool-down to normothermia shown.

treated lung tissue, and represented the individual probe-tips located variously in small airways (presumedly dominated by PFC temperature), pulmonary blood vessels (dominated by local perfusion), or in tissue airway walls and lung interstitium.

Figure 3 shows, for the seven different sheep treated at different intensities of lobar hyperthermia, the relationship between the steady-state temperature (i.e. 'envelopes' of the oscillating waveform amplitudes) for both the airway liquid (measured in the ET tube) and the corresponding local tissue sites in the heated lobes (these envelopes encompassing the temperature maxima and minima from all four needle probes). As shown, lung tissue temperatures were most often constrained to narrow ($< 0.5^{\circ}$C) bands near, and slightly above, the minimum airway liquid temperature (during heating this corresponded to T_{exp}). The maximum airway temperatures corresponded to T_{ins}. In the animal treated at the highest temperatures (LCHT-7), some tissue temperatures fell slightly below T_{exp}, possibly indicating some probes resided in thermally significant vessels, and/or significant cooling from pulmonary blood flow.

3.2. *Local lung hyperthermia with recovery*

In the single sheep recovered from lobar LCHT, hyperthermia was induced in the RC lobe by ramping T_{ins} from normothermia to 43.5°C and then maintaining constant T_{ins} for a treatment period of 30 min. Based on the approximate relationship derived from figure 3, lung tissue temperatures were estimated to fall in the range between T_{exp} and $T_{exp} + 0.3\,(T_{ins} - T_{exp})$, (figure 4). This resulted in projected minimum lung temperatures $>41^{\circ}$C, sustained for at least 30 min, with maximum lung temperatures from 42 to 42.5°C for more than 20 min.

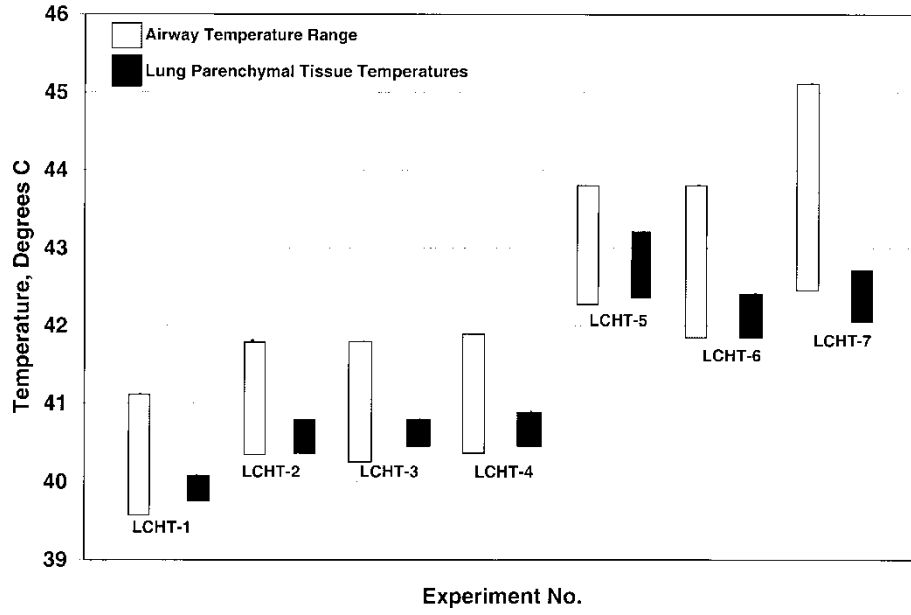

Figure 3. Comparison in isolated LCHT experiments of upper airway liquid temperature envelopes (dark bars) (measured in ET tube) with lung tissue temperature envelopes (lighter bars) (measured by randomly located needle probes).

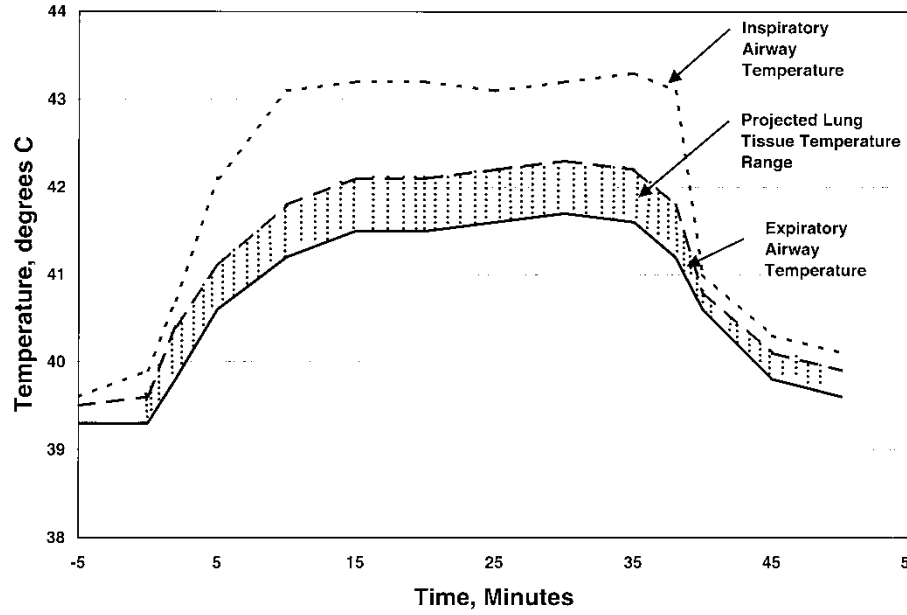

Figure 4. Projected tissue temperature envelope in RC lobe of a sheep in a recovery experiment animal.

Post-LCHT chest radiographs (figure 5) confirmed that perflubron, and therefore the treatment, had been localized to the targeted RC lobe. The animal was extubated based on ability to breathe room air spontaneously. After the return to room air spontaneous breathing and surgical closure, fentanyl was discontinued as assessed by the animal facility that the animal was as responsive and active as for pre-experimental conditions, with no clinical signs of distress and with full movement of all extremities and neck. The animal received routine (in our practice of pulmonary recovery of sheep) chest physical therapy post-treatment (intermittent aggressive massage and percussion). During recovery, there were no signs of hypoxia assessed by ear pulse oximetry (> 90% on room air). A temporary (2.5-h) episode of tachypnea was observed, but subsided and the animal was able to stand unassisted 6 h post-closure. Within 8 h of extubation, the animal was drinking and urinating. At 24 h post-experiment (day 2), the animal was active, the eyes, nose and wound were dry, and there were no signs of respiratory distress at rest. Also on day 2, lateral chest X-rays were again taken. These radiographs revealed significant clearing of the RC lobe, but with some residual perflubron in the dependent caudal segment. By day 9, substantial clearing of the apical portions had occurred, with some caudal residual fluid persisting, but only minor residual perflubron persisting until the last radiographic measurement, taken on day 49. The animal was observed in the vivarium for 7 weeks, with frequent veterinary assessment of status and intermittent chest X-rays and high-resolution CT examinations. After the first 48 h, no symptomatic evidence of the treatment was noted.

3.3. *Bilateral whole-lung hyperthermia*

Various bilateral LCHT heating protocols were performed in three sheep and one cat to explore ways of preferential lung heating. Figure 6 shows the thermal

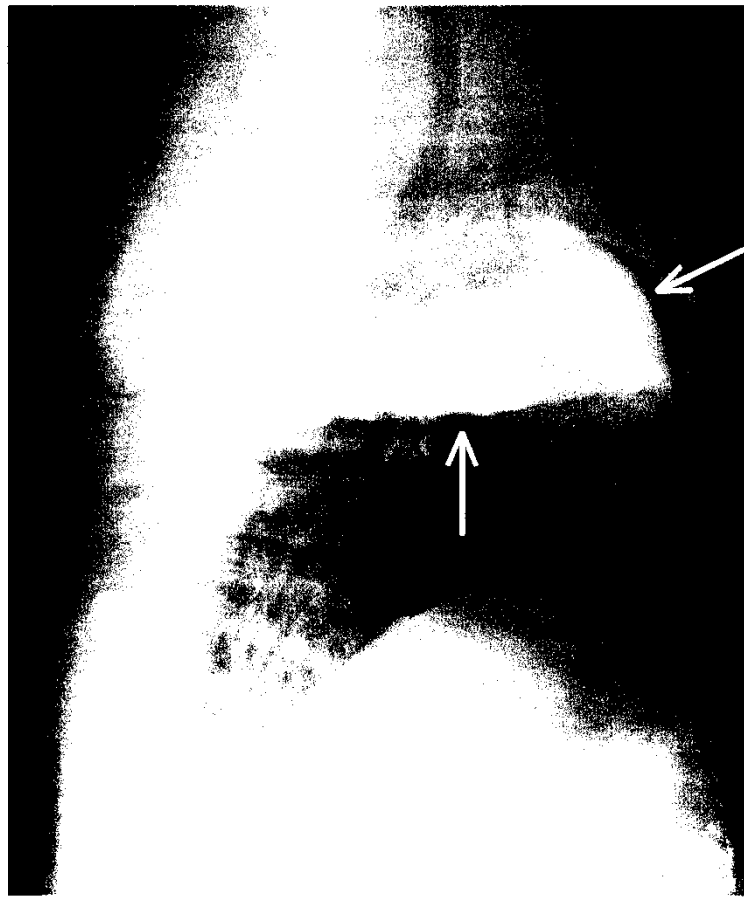

Figure 5. Lateral view chest radiograph in recovery animal immediately after completion of the LCHT experiment and at the outset of the recovery period.

behaviour in a sheep using a strategy of first lowering core temperature with bilateral convective precooling, and then heating with LCHT. During the heating portion, a lung temperature excursion from about 32°C (hypothermia) to 41°C (hyperthermia) was imposed over 2 h, with the driving T_{ins} ranging from 31.5 to 42.5°C. Similar to the lobar LCHT results, T_{exp} reasonably approximated that of lung tissue (from needle probes) during both the transient and steady-state portions of bilateral heating and cooling. In the cooling interval of figure 6, core temperature lagged (above) lung tissue temperatures by only about 1°C. Similarly, the heating phase produced core temperature lags also on the order of 1°C below lung tissue. The protocol thus created only modest preferential lung heating, with temperatures not reaching therapeutic thresholds in the treatment time used.

Other bilateral LCHT experiments in large sheep with similar rates of heating and involving surface cooling (e.g. with cold water-perfused blankets, or with placing ice around the animal) during heating were also unsuccessful in producing sustained significant temperature differences between core and lung. Figure 7, however, shows a bilateral LCHT response where more rapid convective heating of a

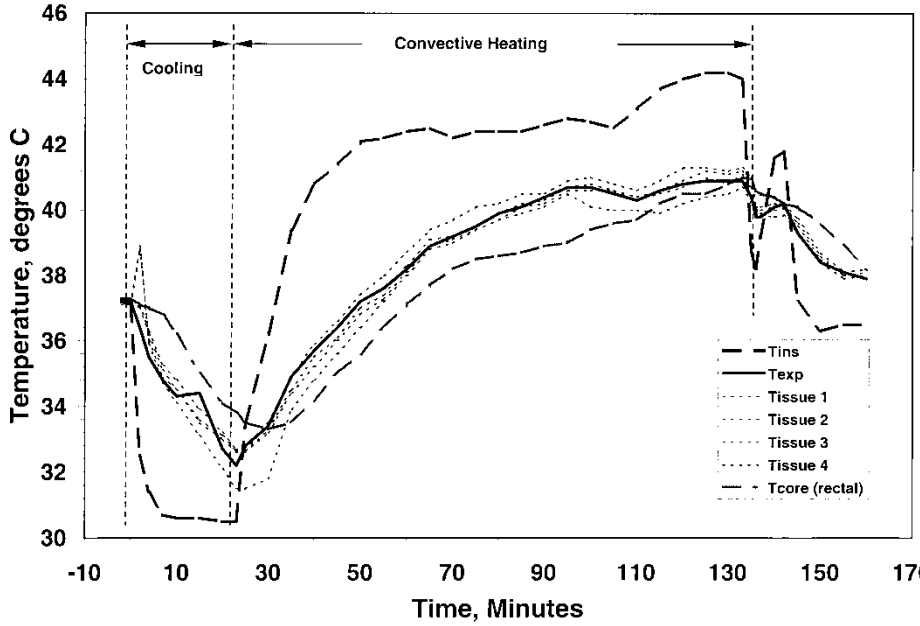

Figure 6. Temperature-time profiles of a bilateral whole-lung hyperthermia experiment in sheep (16.5 kg), preceded by whole-lung cooling via hypothermic TLV.

sheep was performed starting from normothermic TLV, and which did produce therapeutic lung temperatures. In this protocol, after ramping T_{ins} just above 45°C it was decreased to adjust steady state lung temperatures to the target therapeutic range (42–44°C). Without invasive probes, lung parenchymal temperatures were again assumed roughly to approximate T_{exp}, which reached therapeutic temperatures ($T > 42$°C) within 12 min from normothermia, whereas WBH ($T_{core} > 42$°C) was effectively delayed until 30–35 min. These two sites (lung versus core) were substantially differentiated based on administered thermal dose, with $CEM_{43} = 81.6$ and 30.1 min, respectively, in the lung (T_{exp}) and core at the completion of the experiment ($t = 70$ min). Not unexpectedly, as T_{core} and central venous temperature (T_{cvp}) exceeded 42°C, hemodynamic decline was registered (figure 7b via MAP measurements, reflecting potential reduction in systemic vascular resistance associated with heat-induced vasodilation.

As shown in figure 8, an even more rapid heating protocol was performed by pre-heating the system reservoir and starting bilateral LCHT with $T_{ins} = 43.5$°C, rather than ramping T_{ins} up from normothermia. Here rapid lung heating was achieved ($T_{exp} > 42.5$°C within 6 min) while T_{cvp} and T_{core}, although elevated, were maintained below 42°C for the entire 30-min LCHT period. At the end of the 30 min, the animal was cooled back to normothermia within 10 min via cooled inspiratory PFC, with no apparent hemodynamic compromise in the experiment. Adequate arterial oxygenation was achieved throughout the treatment (mean arterial partial pressure of oxygen, P_aO_2, approximately 130 mm Hg) and towards the completion of LCHT arterial oxygenation further improved as the animal temperatures declined toward normothermia (mean $P_aO_2 \approx 180$ mm Hg at completion). There was, however, an ongoing combined respiratory and metabolic acidosis

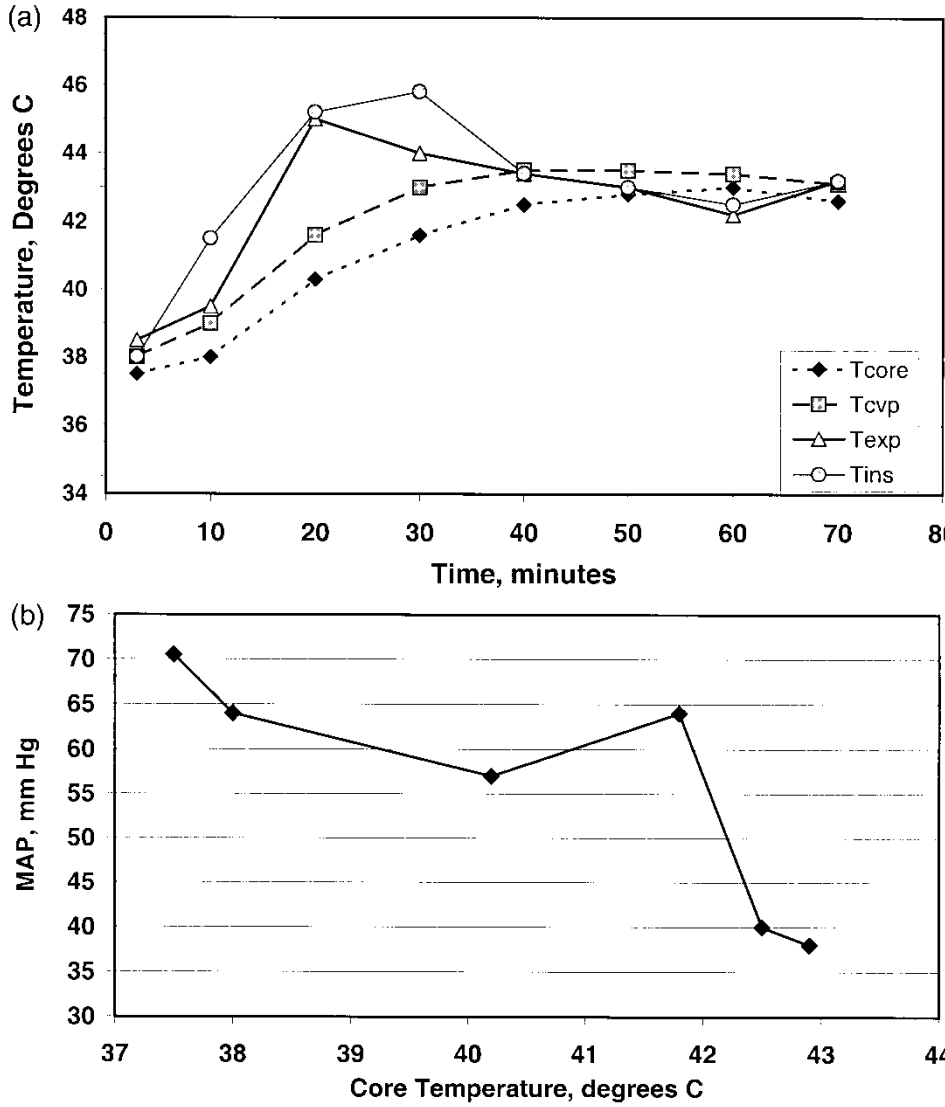

Figure 7. (a) Bilateral whole-lung hyperthermia in lamb (3.2 kg). LCHT begun from normothermic TLV and ramped to hyperthermia ($T_{ins} \approx 45°C$) in 12 min. (b) Mean arterial blood pressure as a function of core temperature in the same animal as in (a).

(minimum arterial pH $\approx$ 7.1) during much of the LCHT period. Hypercapnia ($P_aCO_2 \approx 80$ mm Hg) from inadequate ventilation is believed to have played a major role, noting that ventilator settings were fixed in the protocol, with optimization of TLV ventilation not permitted. Before LCHT the animal had been gas ventilated for 20 min, and after the treatment was acutely recovered and managed under gas ventilation for another 5 h. Arterial oxygenation post-hyperthermia on gas ventilation returned within several minutes to pretreatment levels ($P_aO_2 > 200$ mm Hg), as did P_aCO_2.

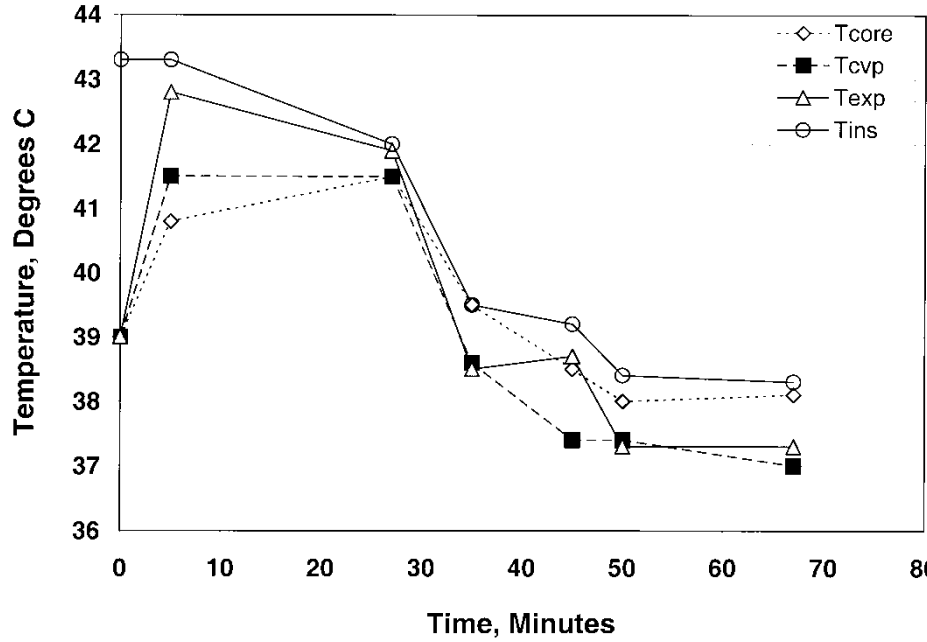

Figure 8. Bilateral whole-lung hyperthermia in lamb (4.2 kg). LCHT begun with preheated inspiratory liquid temperature of 43.3°C.

While gas exchange during bilateral LCHT was adequate in most experiments, as suggested, transient reductions were seen with increasing lung and body temperature, and/or with LCHT duration, again with TLV/LCHT settings held constant. Figure 9(a–c) represent a prolonged (4.25 h) TLV/bilateral LCHT period in the adult cat, preceded and followed by conventional gas ventilation, both with $F_IO_2 = 1.0$. A 42°C plateau in lung temperature (approximated by T_{exp}) and 41°C WBH, was maintained for about 30 min while oxygenation (figure 9b) and carbon dioxide removal (figure 9c) declined slightly over the hyperthermia period. Pulmonary mechanics measurements, performed in this animal before and after LV, showed that lung compliance was not adversely affected after the prolonged LV/LCHT treatment.

4. Discussion

4.1. *LCHT thermal characteristics and control*

The measurements from the lobar LCHT experiments indicated a reasonably uniform temperature distribution across the lobe, falling within a limited thermal envelope (range < 1°C), in spite of the uncertainty as to the medium (airway, interstitium, or blood vessel) in which individual lung tissue needle thermocouple probe tips resided. Even when slowly moving a needle probe along a linear track during steady-state LCHT, similarly small temperature excursions were found as the lobe was traversed. LCHT lung temperatures were found, in general, to be bracketed by T_{ins} and T_{exp}, and tended to concentrate near T_{exp}. This pattern was used in some experiments to project lung tissue temperatures in the absence of invasive thermometry. With a more extensive LCHT data set in the future, including

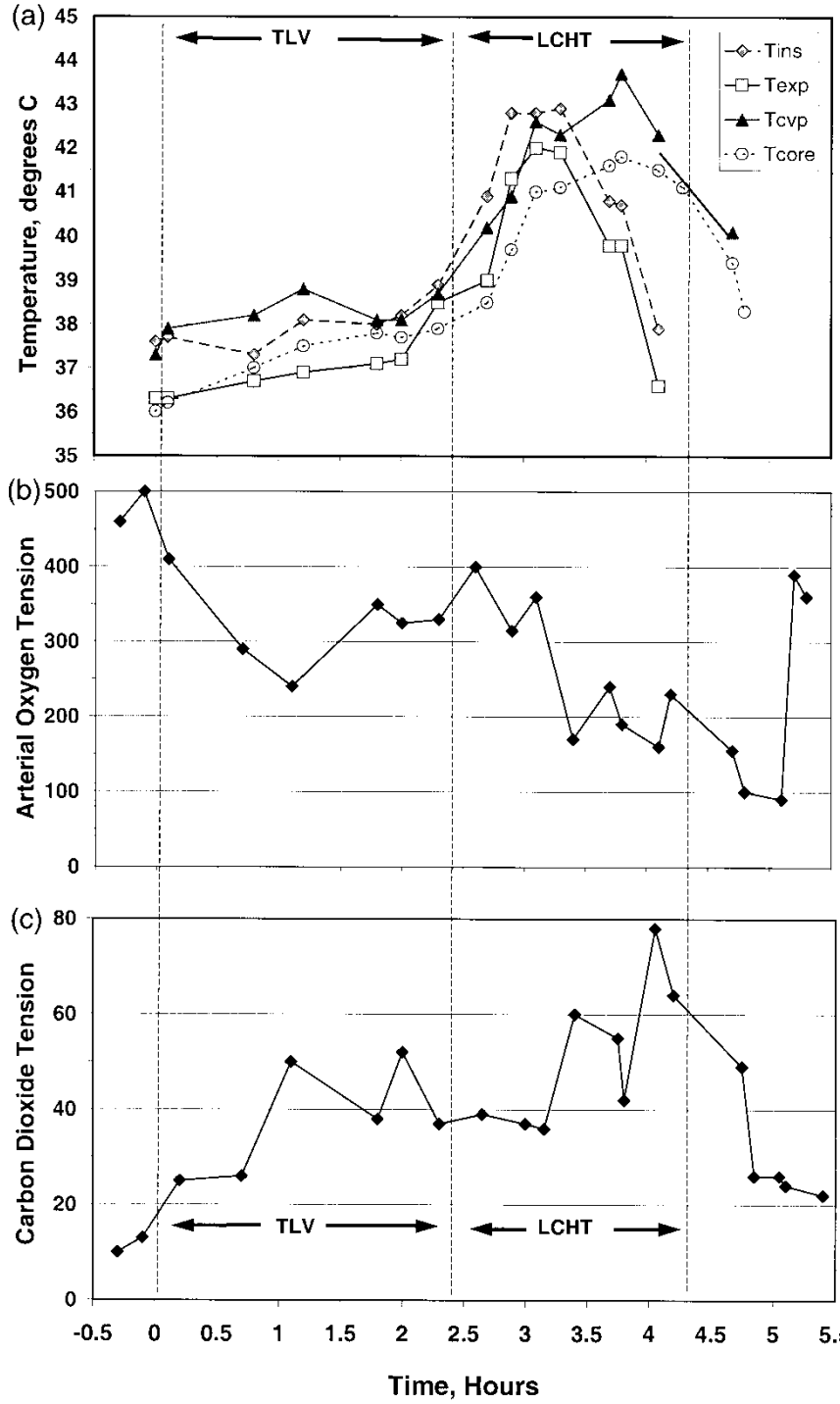

Figure 9. (a) Bilateral whole-lung hyperthermia in adult cat (1.82 kg). LCHT was preceded by a prolonged normothermic TLV period. The animal returned to gas breathing ($F_IO_2 = 1.0$) after return to normothermic TLV; (b) arterial oxygen partial pressure; and (c) arterial CO_2 partial pressure during the TLV/LCHT period.

examining the effect of variations in tidal volume (V_t), 'breathing' rate, [$T_{ins} - T_{exp}$] gradient, etc., tissue temperatures may perhaps ultimately be predicted with confidence solely from liquid temperatures measured within the ET tube. This may reduce the need for invasive lung thermometry, at least in the treatment of diffuse microscopic lung disease or in lung cancer involving small tumors in the lung.

Lung temperature is influenced significantly by the rate (breaths/min) and V_t of the liquid "breaths". Varying the residence time of breaths, governed by I:E, the inspiration (t_i) to expiration (t_e) time ratio, may also be used to manipulate tissue temperatures. These parameters, along with T_{ins}, are the primary settings available for LCHT control. In the current studies some changes in V_t and T_{ins} were made (not shown) to adjust lung temperatures to desired values; rate being kept constant. Clearly more flexibility exists in LCHT settings for local heating than for whole-lung treatments, since ventilation requirements constrain the latter. It should follow, however, that tidal volumes and rates sufficient for good gas exchange are likely to produce good heat transfer, and conversely.

Although most animals were ventilated successfully throughout the hyperthermia and TLV periods in the bilateral experiments, trends showing decreased gas exchange and temporary acidosis occurred (e.g. for peak P_aCO_2 values of figure 9(c) minimum pH ≈ 7.15 was noted). The degree to which these could have been reversed was unknown since, as stated, LV settings were maintained constant during hyperthermia. Although a small portion of the LCHT-associated decreases in gas exchange may be explained by known decreases in PFC gas solubility and hemoglobin O_2 saturation with increasing temperature, the largest contribution likely came from declines in haemodynamic performance at the higher core temperatures.

An intriguing combination LCHT method, designed to achieve preferential lung heating potentially without inducing WBH, is that of simultaneously cooling one lung while performing LCHT on the target lung. This approach was not performed in these studies because of the need for two TLV systems in simultaneous operation, and the limitations of using human ET tubes in sheep.

4.2. *Bilateral LCHT and WBH*

Conventional WBH modalities mainly exploit heating of the skin surface, either by conduction/convection (heating pads or water baths[53,54]) heated humidity chambers[55] or infrared (IR) radiation (heat lamps)[11,56]. These surface heating methods must maintain safe skin temperatures and involve only limited transport area (exposed skin), and consequently produce relatively slow rates of energy delivery. Further, skin heating increases cutaneous blood flow and volume, accelerating heat loss in some cases. Water baths, heating pads and humidity chambers usually require from 1 to 3 h to bring core temperatures up to 42°C, and even modern infrared devices under ideal circumstances require 90 min or more for this transition[11]. Slightly more rapid heating can be achieved by extracorporeal heating of the blood (e.g. 35–40 min to 42°C)[57], but at the expense of increased complexity, invasiveness and patient risk.

Using the lung as a liquid–liquid heat exchanger, with its high surface area ($\approx 70\,m^2$) and with the entire cardiac output passing through it, LCHT should produce rapid and predictable WBH. For a 70-kg patient a net energy deposition of about 300 W will raise T_{core} from 37 to 42°C in approximately 1 h, including effects of increasing metabolic heating as temperature rises)[11]. Assuming an

initial elevated $T_{ins} = 43^{\circ}C$ (e.g. in figure 8) and perflubron, LCHT power delivery to the patient would begin at approximately 500 W under typical settings: power = (0.5 l/breath) × (5 breath/min) × (1 min / 60 s) × (1.92 kg/l) × (1040 J/kg°C) × (43–37°C) × (1 W/J/s) ≈ 500 W. At steady-state the $[T_{ins} - T_{exp}]$ gradient (here 83 W/°C) driving heating would balance other heat loss mechanisms.

Conversely, because the lung heat exchanger is so efficient, very rapid heating of T_{ins} (e.g. figure 7) or even preheating of the liquid (e.g. figure 8), may be required to induce a lag in core versus lung temperature, to focus a bilateral treatment on the lungs. As shown, with no modification of surface heat loss from the animals, WBH can be effectively delayed (about 30 min in figure 7) using accelerated T_{ins} heating.

WBH implemented by LCHT may offer a more simplified control of patient hyperthermia. For example, to maintain appropriate thermal balance while avoiding thermal toxicity, some IR systems and other regional surface heating modalities require repetitive patient repositioning, vigilant power monitoring and adjustment, and maneuvers to address surface heat loss (e.g. evaporative sweat losses)[11]. For LCHT, manipulation of T_{ins} and V_t likely will serve as the main control variables for WBH and, ultimately, could be controlled through automated algorithms based on feedback from airway PFC and body core temperatures. Even manual thermal adjustments may be effective for simple control. More straightforward control of lung and body temperatures could also simplify complex heating protocols. For example, step-down heating (Henle[58]), whereby a high temperature is applied for a short time and then followed by milder temperatures for the remainder of the treatment, might be implemented by timed changes in T_{ins}, V_t and/or breathing rate. The use of skin surface insulation would further improve rates of WBH heat-up, or permit a lower T_{ins} to sustain T_{core} in target regions, and may further simplify control by limiting surface heat loss.

Further, combining LCHT with surface heating modalities may present advantages, in spite of increased complexity. By enclosing the patient in a high-humidity, low-power IR environment[56], and simultaneously performing bilateral LCHT, very rapid induction of WBH would appear possible.

4.3. *LCHT thermophysiology and safety*

The effects of normothermic liquid ventilation have been extensively characterized in animals, in treatments lasting up to multiple days, and are now being assessed in multiple-day treatment in patients (for perflubron PLV). The perflubron safety data to date, much of which is in unpublished US FDA regulatory submissions, is extensive, and was performed under Good Laboratory Practices for the purposes of supporting human trials, and is believed to indicate that minimal risks are associated with perflubron in the lung. Further, some boost in safety may accrue in hyperthermic applications from PFC-associated anti-inflammatory effects.

Although only sparse histology was performed on acute study lung samples in the present study (data not shown), these appeared morphologically similar to LV lung samples of animals supported normothermically on the same LV equipment. Regarding the effects of lung heating, per se, encouraging results have been obtained by Rickaby *et al.*[14], who performed sustained heating using hyperthermic blood perfusion of isolated dog lung lobes. Taking measurements of lung edema, compliance, perfusion pressure and serotonin uptake during 2 h of sustained

hyperthermia (time-averaged lung temperatures of 40.7°C and 44.5°C), they found no significant changes in these parameters versus normothermia (37.6°C), other than expected increases in perfusion pressure with temperature. They concluded that normal lung appears to tolerate the sustained heating regimens appropriate for cancer hyperthermia. In addition, in radiant heating WBH canine studies employing concomitant airway heating with 42°C humidified gases for over 1.5 h, Meyer *et al.*[15] showed no histological lung or tracheal thermal damage, although a temporary reduction in tracheal mucociliary transport was found.

Whether cardiopulmonary physiologic and lung injury effects associated with other WBH approaches are seen with bilateral LCHT is an open question. For example, although conventional WBH complications include pulmonary edema and intrapulmonary shunting[9] (see Section 1.2.3), these conditions have been shown to be improved by LV in lung injury models.

Even under WBH conditions, the uptake of PFCs into blood and tissue is projected to be small. Mandl *et al.*[59], have studied the uptake of perflubron in rabbits subjected to four continuous hours of perflubron PLV at either hypothermic (27°C), normothermic (37°C), or hyperthermic (41.5°C) core temperatures. They found that normothermic PLV blood and tissue perflubron concentrations were about 5 and 140 μg/ml, respectively, with these increasing as a function of temperature by approximately 0.5 and 10 μg/ml/°C, respectively. For perspective, perflubron i.v. emulsion doses of 2.7 g PFC/kg patient weight are currently being administered into surgery patients[34], corresponding to a PFC blood concentration > 30 mg/ml, more than three orders of magnitude above that expected from LCHT.

After a human PLV treatment, near complete evaporation of a volume of perflubron equivalent to the patient's entire FRC (≈2.0–2.5 l) usually occurs within 2 days, with only minimal levels in the airways persisting longer. The persistence of small amounts of perflubron in the lobe of the LCHT recovery animal in this study is believed associated with the right angle branching of the RC lobe off of the trachea, and the constant prone orientation of the animal. It is speculated that if selective gas ventilation of the isolated lobe had been continued after the LCHT treatment, a more rapid evaporation would have occurred. Shaffer *et al.*[60] have shown that postural changes might accelerate evaporative clearance of the PFC in sheep. It is noteworthy that detectable quantities of PFC have persisted in primate lungs for as long as 3 years without symptomatic evidence or observed toxic consequences[61].

4.4. *LCHT and chemotherapy of the lung*

LCHT may be used with adjuvent localized chemotherapy in the targeted lung region. By using the PFC to simultaneously deliver heat and carry cytostatic drugs to the region, systemic toxicity effects from the drugs may be mitigated. Several studies have now demonstrated the feasibility of utilizing PFC LV techniques to deliver to the lung, and to the body via the lung, aqueous or lipid-based pharmacologic agents (surfactants, antibiotics, adenoviral genes, vasopressors)[62–65] and gases (nitric oxide[66] and gaseous anaesthetics[67]). Although crude suspensions of cisplatin, cyclophosphamide and 5-fluorouracil in perflubron have been created[68], it is ultimately preferable that ready-to-use shelf-stable chemoactive agents in PFC carriers be available. The formulation of drugs in PFC emulsions (water-in-oil type) or via suspensions are possible, producing controlled, uniform delivery and bioavailability[69].

5. Conclusions

The production of sustained therapeutic temperatures in the lung exploiting convective heat exchange from liquid perfluorochemicals has been demonstrated in animals using hyperthermia methods based on LV technology. The LCHT procedure requires intubation with an ET tube, as normally occurs for mechanically ventilated patients, and affords the possibility of treating individual lobes, or treating the entire lungs or body.

The lung was found to be an excellent liquid–liquid heat exchanger, presenting LCHT as a potentially effective and rapid means of inducing WBH. The observation, however, that the lungs reside in the 'core' of the body, presents a challenge for selectively heating them. The thermal dosimetry data from this study, although preliminary, indicates that selective treatment of the lung may be possible, for example through rapid lung heating or local (lobar or perhaps single lung) treatment. Lung parenchymal temperatures were also found to be adequately uniform across LCHT treated lung regions, with temperatures clustering in somewhat narrow ranges within the band comprising inspiratory to expiratory liquid temperature, measured within the ET tube. Future studies will be needed to assess completely the adequacy of projecting lung interstitial, alveolar and tumor temperatures from proximal large airway liquid temperatures, but this appears possible and may minimize invasive lung thermometry.

From a thermal perspective, it appears that LCHT may be performed with a variety of PFC liquids, but preferably the PF-alkanes, perflubron, and FC-77. The extensive clinical history of perflubron may be advantageous for early human trials of LCHT. Further, perflubron's superiority as an X-ray imaging agent may also be helpful for confirming the targeting of LCHT to desired lung regions.

The laboratory TLV equipment used here has been adequate for preclinical studies, but additional development will be required before convective lung hyperthermia is appropriately safe and user friendly for the clinic. As an example, more automation (e.g. thermal feedback control based on T_{ins}, T_{exp} and T_{core}) will be useful. Further, new ET tubes may be needed to treat some regions of the lung.

Additional preclinical research is also needed to characterize more fully the physiology and safety of LCHT, particularly for longer (>1 h) treatments. In addition, *in vivo* efficacy (tumor regression) studies should be undertaken (e.g. LCHT in combination with adjuvant chemotherapeutics).

Lastly, the combination of LCHT with other local hyperthermia modalities (e.g. ultrasound, isolated lung perfusion chemotherapy, etc.) or with WBH systems (e.g. infrared enclosures) and with radiotherapy and chemotherapy present possibilities for synergy. These hybrid techniques should be included in future LCHT research.

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