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ORIGINAL ARTICLE

Mild hypothermia increases pulmonary anti-inflammatory response during protective mechanical ventilation in a piglet model of acute lung injury

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Summary

Background: The effects of mild hypothermia (HT) on acute lung injury (ALI) are unknown in species with metabolic rate similar to that of humans, receiving protective mechanical ventilation (MV). We hypothesized that mild hypothermia would attenuate pulmonary and systemic inflammatory responses in piglets with ALI managed with a protective MV.

Methods: Acute lung injury (ALI) was induced with surfactant deactivation in 38 piglets. The animals were then ventilated with low tidal volume, moderate positive end-expiratory pressure (PEEP), and permissive hypercapnia throughout the experiment. Subjects were randomized to HT (33.5°C) or normothermia (37°C) groups over 4 h. Plasma and tissue cytokines, tissue apoptosis, lung mechanics, pulmonary vascular permeability, hemodynamic, and coagulation were evaluated.

Results: Lung interleukin-10 concentrations were higher in subjects that underwent HT after ALI induction than in those that maintained normothermia. No difference was found in other systemic and tissue cytokines. HT did not induce lung or kidney tissue apoptosis or influence lung mechanics or markers of pulmonary vascular permeability. Heart rate, cardiac output, oxygen uptake, and delivery were significantly lower in subjects that underwent HT, but no difference in arterial lactate, central venous oxygen saturation, and coagulation test was observed.

Conclusions: Mild hypothermia induced a local anti-inflammatory response in the lungs, without affecting lung function or coagulation, in this piglet model of ALI. The HT group had lower cardiac output without signs of global dysoxia, suggesting an adaptation to the decrease in oxygen uptake and delivery. Studies are needed to determine the therapeutic role of HT in ALI.

Background

Acute respiratory distress syndrome (ARDS) and acute lung injury (ALI) are severe forms of respiratory failure with no etiological treatment; mechanical ventilation (MV) has been the cornerstone of supportive therapies for ARDS for several decades. Because injurious MV can amplify preexisting damage (1–6), ventilation with low tidal volume (V_T), moderate/high positive end-expiratory pressure (PEEP), and permissive hypercapnia is the current standard of care, also known as lung protective MV (7,8).

Induced hypothermia is the controlled lowering of body temperature for therapeutic purposes (9). Its use has been extended to several clinical situations where tissue dysoxia is an important issue, like management after cardiac arrest, during cardiopulmonary bypass and preservation of organ grafts, due to a nonspecific decrease in enzymatic activity, among others mechanisms (10–15).

Recent experimental studies suggest that hypothermia might have a beneficial role in lung injury. In rat ALI models, animals treated with deep hypothermia (≤30°C) had a better gas exchange and lung mechanics and less lung inflammation, edema, and signs of oxidative stress injury (16–21). Potentially serious and life-threatening adverse effects on hemodynamics, coagulation, and immune response prevent wide clinical use of deep hypothermia for long periods of time (9). Experimental studies in rats and rabbits using mild hypothermia (HT, 33–34°C) found also some protective effects on injured lungs, principally reduced lung edema and systemic inflammatory response, but significantly less than deep hypothermia (22–26). Beneficial effects described in these experimental models cannot be directly extrapolated to humans because induction of hypothermia, a hypometabolic state, may be more relevant in species with higher oxygen uptake (VO_2) related to body mass, like small mammals (27). Furthermore, some of these studies used injurious MV (high V_T and low PEEP) (22,25,26) that differs from the current standard of care. For these reasons, it is unknown whether HT has an additive beneficial effect in mammals with an indexed VO_2 similar to humans using a lung protective MV on ALI.

With these facts in mind, we designed this study to evaluate whether mild hypothermia would attenuate local and systemic inflammatory response in piglets with experimentally induced acute lung injury receiving protective MV. Secondary goals were to investigate the effects of mild hypothermia on gas exchange, lung mechanics and permeability, apoptosis in the injured lung and remote organs, and the potential adverse effects on hemodynamics and coagulation.

Methods

Animal preparation

This study used 38 2-week-old anesthetized Large White piglets (4.9 ± 0.2 kg). The Universidad del Desarrollo Ethics Committee and the National Bioethics Adviser's Committee approved the experimental protocol. All experimental procedures were in accordance with the Guiding Principles in the Care and Use of Laboratory Animals adopted by the American Physiological Society. Sample size was estimated by comparison of independent means using the statistical program EPIDAT® 4.0 (DXSP, Xunta de Galicia, Spain). The estimated sample size was 19 animals per group to observe a difference >10% in inflammatory response with an α error of 0.05 and β error ≤0.2, based on the lung function improvement reported in humans with mild hypothermia (28).

Surgical preparation and anesthesia:
Animals were premedicated with intramuscular acepromazine (1.1 $mg \cdot kg^{-1}$) and ketamine (20 $mg \cdot kg^{-1}$). After local infiltration with 1% lidocaine, each animal's trachea was cannulated via cut down with a cuffed tracheostomy tube (3.5 mm internal diameter; Mallinckrodt Shiley, St. Louis, MO, USA), the left jugular vein with a 4F double lumen catheter (Arrow, Reading, PA, USA) and the right axillary artery with a 4F thermistor-tipped catheter (PiCCO® PV2014L08; Pulsion Medical Systems, Munich, Germany). Anesthesia and neuromuscular blockade were maintained by continuous infusion of propofol (10 $mg \cdot kg^{-1} \cdot h^{-1}$), fentanyl (4 $\mu g \cdot kg^{-1} \cdot h^{-1}$), and pancuronium (0.2 $mg \cdot kg^{-1} \cdot h^{-1}$) throughout the experiment.

Mechanical ventilation
Animals were ventilated with an EVITA XL® ventilator (Dräger Medical, Lübeck, Germany) using the volume control mode. Initial settings were as follows: V_T = 10 $ml \cdot kg^{-1}$, PEEP = 5 cmH_2O, fraction of inspired oxygen (FiO_2) = 0.5, inspiratory time = 0.75 s, and respiratory rate (RR) = 20 $breaths \cdot min^{-1}$. After ALI was induced, ventilator settings were modified to noninjurious parameters (8): V_T = 6 $ml \cdot kg^{-1}$, PEEP = 10 cmH_2O, FiO_2 = 0.6, and the same inspiratory time. RR was adjusted (to a maximum of 40 $breaths \cdot min^{-1}$) to achieve a partial pressure of carbon dioxide ($PaCO_2$) 70 ± 10 mmHg. This $PaCO_2$ target was selected in base of the known protective effects of hypercapnic acidosis in various ALI models, with or without tidal volume limitation (29).

Acute lung injury
After surgical preparation, each animal was placed in lateral decubitus and a 10% solution of Tween® 20 [polyoxyethylene (20) sorbitan monolaurate; Sigma-Aldrich, St. Louis, MO, USA] in saline (1 $ml \cdot kg^{-1}$) was instilled in the airway of the dependent lung via a 2-mm catheter. The procedure was repeated with the animal rotated to the opposite side. Residual fluid was suctioned from the airway. Lung injury was targeted to achieve a partial pressure of oxygen (PaO_2) in arterial blood <200 mmHg with PEEP = 10 cmH_2O and FiO_2 = 1 in supine position at 20 min after Tween® 20

instillation. If this target was not met, Tween® 20 instillation was repeated (30).

Experimental groups

Immediately after ALI target was achieved, block randomization was used to allocate piglets to the mild hypothermia (33.5 ± 0.5°C; $n = 19$) or normothermia (NT, 37 ± 0.5°C; $n = 19$) group. Temperature was measured at the thoracic esophagus (YSI reusable temperature probe; Yellow Springs Instrument Co., Inc., Yellow Springs, OH, USA). In the HT group, body temperature was reduced to the target over a 20-min period using convective methods (cold gel packs). In the NT group, body temperature was maintained using an external heater. Room temperature was maintained at 25°C.

Measurements

Following ALI and the achievement of target temperature, hemodynamic and respiratory measurements were recorded at baseline and at 60, 120, and 240 min.

Pulmonary measurements:

PaO_2, pH, and $PaCO_2$ were assessed with an i-STAT® blood gas analyzer and EG6+ cartridges (Abbott Laboratories, Princeton, NJ, USA) in blood samples from the arterial catheter. The i-STAT device corrects the pH measurements for the provided body temperature. Oxygenation was assessed by the PaO_2/FiO_2 ratio and alveolar-arterial oxygen tension ($A\text{-}aPO_2$), defined as $(P_{bar}–P_{water}) \times FiO_2–(PaCO_2/0.8)$, where P_{bar} is barometric pressure and P_{water} is water vapor pressure. Minute ventilation (V_{min}) was computed from the product of expired V_T and RR. Static respiratory system compliance (C_{RS}) was calculated as $V_T/(P_{pl}–PEEP_{TOT})$, where P_{pl} is plateau pressure measured after a 4-s inspiratory hold and $PEEP_{TOT}$ is total PEEP measured after a 4-s expiratory hold. These variables were recorded from the ventilator display.

Hemodynamic measurements

Heart rate (HR), mean arterial pressure (MAP), and central venous pressure (CVP) were monitored (Infinity Delta XL®; Dräger Medical, Lübeck, Germany). Zero pressure was set at the midaxillary line. Cardiac output (CO), stroke volume (SV), systemic vascular resistance index (SVRI), and extravascular lung water (EVLW) were evaluated in triplicate by transpulmonary thermodilution using a commercially available device (PiCCO®; Pulsion Medical Systems, Munich, Germany) according to the manufacturer's instructions. The body surface area of each piglet was calculated by K/weight (in kilograms)$^{2/3}$, where $K = 0.112$ for pigs (31). Central venous hemoglobin oxygen saturation ($ScvO_2$) was assessed in blood samples from the jugular catheter using the i-STAT® analyzer and EG6+ cartridges. Oxygen delivery (DO_2) and VO_2 were calculated according to standard formulas. At 240 min after achieving target temperature, lactate was measured amperometrically using i-STAT® CG4+ cartridges (Abbott Laboratories).

Pulmonary vascular permeability markers

Bronchoalveolar lavage (BAL) was performed at baseline and at the end of the experiment with 10 ml normal saline; samples were centrifuged at 1000 RCF, and supernatant total protein content was determined with the bicinchoninic acid method (Pierce microplate BCA protein assay kit, reducing agent compatible; Thermo Scientific, Rockford, IL, USA) to avoid interference with Tween® 20. EVLW was measured by transpulmonary thermodilution as described above. Wet-to-dry ratio of the lung: Immediately after euthanasia, the lung was removed, drained of blood, and weighed. Then, the dry weight of the lung was determined after 3-day incubation in a heat chamber at 70°C (32). At the end of the study period, 5 ml arterial blood was extracted and plasma was frozen for subsequent analysis. While under anesthesia, the animals were euthanized by 10% potassium chloride infusion until the detection of ventricular fibrillation or asystole. After thoracotomy and laparotomy, lungs and kidneys were removed.

Measurement of cytokines and biochemical markers

Nondependent right lung (33) and kidney fragments were frozen in liquid nitrogen and stored at −80°C. Each frozen tissue sample (150 mg) was crushed with a mortar and pestle on dry ice and homogenized with 0.75 ml tissue protein extraction reagent (T-PER, Pierce, Rockford, IL, USA) supplemented with EDTA-free halt protease inhibitor cocktail (Thermo Scientific). After centrifugation at 10 000 ***g*** for 5 min at 4°C, pellets were discarded and supernatants were transferred to clean microcentrifuge tubes. Total protein concentrations in supernatants were determined using the reducing agent compatible microplate BCA protein assay kit (Thermo Scientific) and adjusted with T-PER buffer to 5 $mg \cdot ml^{-1}$ (lung) or 7 $mg \cdot ml^{-1}$ (kidney) and then frozen in liquid nitrogen. In lung and kidney supernatants and plasma samples, porcine tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-10 (IL-10) concentrations were quantified by ELISA using commercial kits (R&D Systems, Minneapolis, MN, USA). In lung and kidney supernatants, cellular apoptosis was evaluated by quantifying caspase-3 activity using a colorimetric caspase-3 assay kit (Sigma-Aldrich, St. Louis, MO, USA).

Coagulation

We evaluated the effects of mild hypothermia on coagulation at 240 min using prothrombin time (PT) and kaolin-activated clotting time (ACTk) cartridges (Abbott Laboratories) and an i-STAT® analyzer.

Statistical analysis

Data are expressed as means ± standard error of the mean (SEM). Normality was assessed with the Anderson–Darling test. The Wilcoxon signed-rank test and the Friedman test with Bonferroni correction were conducted to compare consecutive measurements of studied variables. Comparisons between groups were analyzed with the Mann–Whitney U-test. Significance was set at $P < 0.05$. All statistical analyses were performed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA). Figures were plotted with GRAPHPAD PRISM version 5.0c for Mac (GraphPad Software, La Jolla, CA, USA).

Results

All animals completed the experimental protocol. Three animals required a second Tween® 20 instillation to achieve the ALI target. Central temperatures were kept within target ranges throughout the study period (Table S1). No adverse effect of convective temperature control was observed.

Pulmonary measurements

In all animals, Tween® 20 instillation caused severe pulmonary dysfunction, reducing the PaO_2/FiO_2 ratio from 346 ± 10 mmHg to 155 ± 6 mmHg ($P < 0.01$) and C_{RS} from 1.58 ± 0.10 $ml{\cdot}cmH_2O^{-1}{\cdot}kg^{-1}$ to 0.93 ± 0.04 $ml{\cdot}cmH_2O^{-1}{\cdot}kg^{-1}$ ($P < 0.01$). No significant difference was found between the HT and NT groups in PaO_2/FiO_2 ratio, A-aPO_2, pH, $PaCO_2$, V_{min}, C_{RS}, or mean airway pressure at 60, 120, and 240 min (Figure 1, Table S1).

Hemodynamic measurements

After ALI, HR increased (133 ± 5 $beats{\cdot}min^{-1}$ to 162 ± 6 $beats{\cdot}min^{-1}$, $P < 0.01$), SV decreased (31 ± 1 ml to 27 ± 1 ml, $P = 0.01$), and $ScvO_2$ decreased (0.83 ± 0.01–0.77 ± 0.1, $P < 0.01$), with no significant change in MAP, CVP, CO, or SVRI. CO and HR decreased progressively only in the HT group (both $P < 0.01$, Figure 1). No significant difference in SV, MAP, or CVP was found between NT and HT groups in successive measurements. At 240 min, SVRI was higher in the HT group than in the NT group (Table S1).

At the end of the experiment, VO_2 and DO_2 were lower in the HT group ($P = 0.001$ and $P = 0.011$, respectively; Figure 2). Arterial lactate was 1.64 ± 0.34 $mmol{\cdot}L^{-1}$ and 2.32 ± 0.51 $mmol{\cdot}L^{-1}$ in the HT and NT groups at the end of the study, respectively ($P = 0.38$). $ScvO_2$ was also similar between groups (Table S1).

Pulmonary vascular permeability markers

As expected, EVLW increased in all subjects at 30 min after ALI, from 11.6 ± 0.3 $ml{\cdot}kg^{-1}$ to 14.8 ± 0.3 $ml{\cdot}kg^{-1}$ ($P < 0.01$). No significant difference

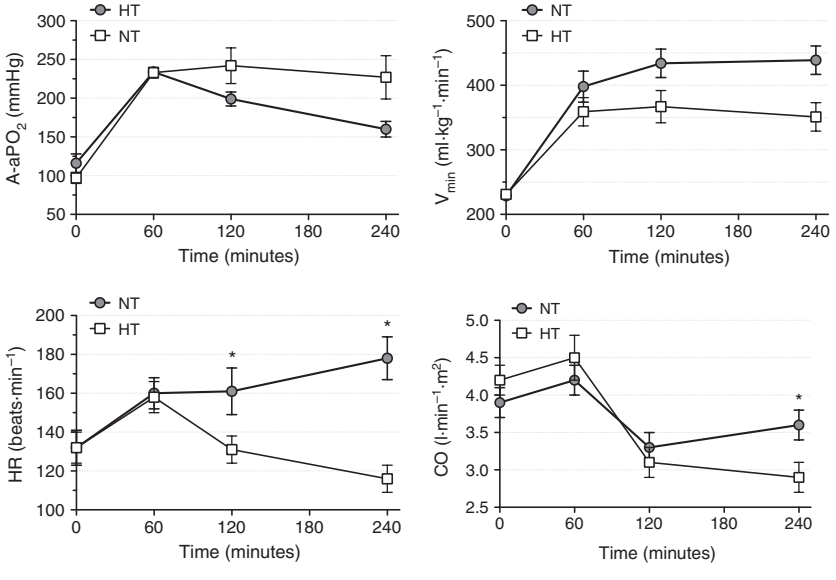

Figure 1 Pulmonary and hemodynamic measurements in the mild hypothermia and normothermia groups. A-aPO_2, alveolar-arterial oxygen tension; V_{min}, minute ventilation; HR, heart rate; CO, cardiac output. All data are presented as means ± SEM. *$P < 0.01$.

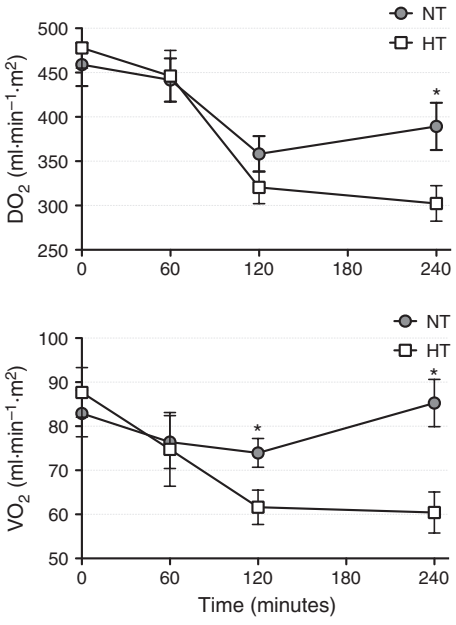

Figure 2 Oxygen delivery and oxygen uptake in the mild hypothermia and normothermia groups. DO_2, oxygen delivery; VO_2, oxygen uptake. All data are presented as means ± SEM. *$P < 0.05$.

in BAL protein content, EVLW, or wet-to-dry ratio was observed between HT and NT groups (Table 1).

Biomarkers: lung, kidney, and plasma cytokines and caspase-3 activity

We found no differences between groups in the plasma or kidney TNF-α, IL-1β, or IL-10 concentration at the end of the study period. However, in lung, IL-10 concentration was higher in the HT than in the NT group ($P < 0.001$), with no difference in TNF-α or IL-1β concentration. No significant difference in lung or kidney caspase-3 activity was found between groups (Table 2, Figure 3).

Coagulation

No significant bleeding occurred. At the end of the observation period, PT was 23 ± 9 s and 37 ± 12 s ($P = 0.1$) and ACTk was 65 ± 8 s and 85 ± 12 s ($P = 0.5$) in the HT and NT groups, respectively.

Discussion

The main finding of this study was that lung IL-10 concentrations were higher in piglets with ALI receiving protective MV under mild hypothermia than in subjects maintaining normothermia, supporting that mild hypothermia induces a local anti-inflammatory response in the injured lungs. Animals treated with hypothermia showed no difference in other systemic and tissue cytokines or in lung or kidney tissue apoptosis with respect to subjects that were maintained with NT during the observation period. Also, pulmonary mechanics, lung vascular permeability, and coagulation function were similar between both groups. Mild hypothermia reduced CO, VO_2, and DO_2 without modifying arterial lactate and $ScvO_2$.

The protective effects of hypothermia on ALI are well established. Direct effects of hypothermia on lung inflammation have been studied in depth and include suppression of cellular and innate immune response (16–21,23–25). Deep hypothermia inhibits ICAM-1 upregulation in injured lung tissue preventing neutrophil adhesion, activation, and alveolar sequestration (23). Also, it has been shown to decrease the release of inflammatory cytokines from alveolar macrophages and the activation of nuclear factor-κB in the lung, a pivotal pathway for the elaboration of inflammatory cytokines (18). Hypothermia induced in a mild to moderate degree also has been shown to decrease lung injury, but mostly when compared with hyperthermia (22,25,34,35). Indirect effects of hypothermia on lung inflammation are only partially known. As the metabolic rate decreases during hypometabolic state as hypothermia, VO_2 and VCO_2 also decrease. By virtue of this physiological advantage, hypothermia may ensure adequate gas exchange at lower minute ventilation than normothermia. In this regard, deep hypothermia has been used as a means for lung rest in a model of acute lung injury (16). However, the effect of mild hypothermia on gas exchange in humans appears to be minor (28) and

Table 1 Vascular permeability markers in the mild hypothermia and normothermia

	Baseline			240 min		
	NT	HT	*P*	NT	HT	*P*
BAL protein content (g·L^{-1})	0.23 ± 53	0.33 ± 14	1	6.15 ± 67	7.98 ± 98	0.171
EVLW (ml·kg^{-1})	11 ± 0.5	12 ± 0.5	0.141	17 ± 0.9	18 ± 1.1	0.265
Wet-to-dry ratio (ml·kg^{-1})				10.3 ± 0.4	10.3 ± 0.2	0.968

NT, normothermia; HT, mild hypothermia; BAL, bronchoalveolar lavage; EVLW, extravascular lung water.
All data are presented as means ± SEM.

Table 2 Plasma and kidney cytokines in the mild hypothermia and normothermia groups at the end of the study

	NT	HT	*P*
Plasma			
TNF-α (pg·ml^{-1})	513 ± 115	706 ± 190	0.93
IL-1β (pg·ml^{-1})	190 ± 130	102 ± 80	0.54
IL-10 (pg·ml^{-1})	12 ± 5	16 ± 4	0.34
Kidney			
TNF-α (pg·mg protein^{-1})	5.6 ± 2.9	5.3 ± 1.4	0.09
IL-1β (pg·mg protein^{-1})	46.3 ± 19.4	29 ± 11.3	0.45
IL-10 (pg·mg protein^{-1})	1.8 ± 0.1	2.0 ± 0.1	0.15
Caspase-3 activity (pmol pNA·μg protein^{-1})	62 ± 16	100 ± 28	0.32

NT, normothermia; HT, mild hypothermia; TNF-α, tumor necrosis factor alpha; IL-1β, interleukin-1 beta; IL-10, interleukin-10; pNA, p-nitroaniline.
All data are presented as means ± SEM.

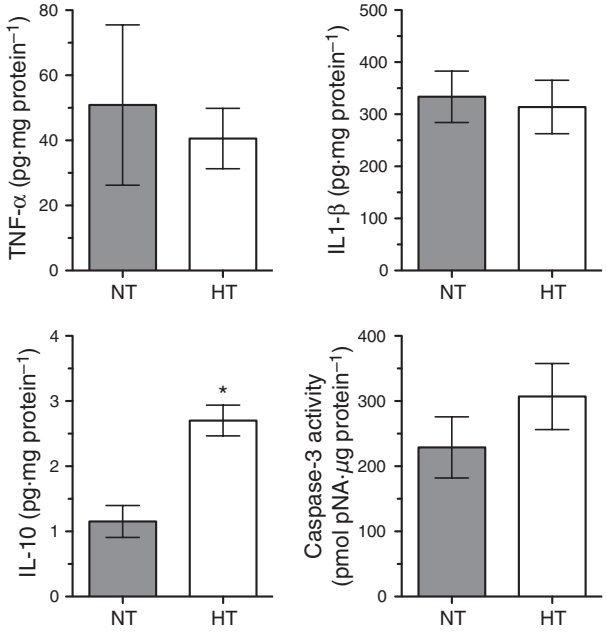

Figure 3 Lung concentrations of cytokines and caspase-3 activity in the mild hypothermia and normothermia groups at the end of the study. TNF-α, tumor necrosis factor alpha; IL-1β, interleukin-1 beta; IL-10, interleukin-10; pNA, p-nitroaniline. All data are presented as means ± SEM. *$P < 0.001$.

evidence for the anti-inflammatory effects of mild hypothermia versus normothermia is scarce (21,23–25).

Methodological issues such as the studied species, etiology of ALI, and MV strategy have limited the ability to extrapolate results of previous studies to humans. Using a mammalian species with similar VO_2 as the human, a chemically induced lung injury, and contemporary ventilation strategy, the current study overcomes some of the methodological flaws that limit the translational clinical benefit of previous experimental findings to the human ARDS setting. Because standard and maximal VO_2 adjusted to body mass are lower in large versus small mammals, we used piglets as an experimental pediatric model because their VO_2 is similar to that of humans (27,36). The effect of a hypometabolic state such as hypothermia may be magnified in species with higher VO_2, as seen in previous studies in rodents (27,37).

We used a model of lung injury induced by surfactant deactivation because it is the main pathogenic mechanism of acute and neonatal respiratory distress syndrome (38). Tween® 20, a nonionic detergent, deactivates pulmonary surfactant while sparing the alveolar–capillary membrane ultrastructure (39). Like previous studies, we observed a rapid development of ALI in piglets after tracheal Tween® 20 instillation, characterized by severe hypoxemia, loss of lung compliance, and pulmonary edema (40,41).

Our MV protocol was consistent with contemporary care of ARDS. MV strategy is relevant because inadequate MV settings, as used in previous experimental works, might increase inflammatory response in lungs and distant organs, commonly named VILI (ventilator-induced lung injury). We chose a MV strategy with low V_T plus moderate/high PEEP and hypercapnic acidosis. This protective MV synergistically reduces the inflammatory response in the injured lung (1,3,8,29,42), and it is now considered the standard of care of ARDS. We found no significant difference in lung IL-1β or TNF-α concentration between study groups. But it is important to consider that it might be difficult to demonstrate beneficial effects of new therapies, like mild hypothermia, using this protective MV. Others studies of mild hypothermia found no differences in these cytokines even using injurious MV settings (25,26). Noteworthy, we observed an increase in the anti-inflammatory cytokine IL-10. This cytokine has pleiotropic effects in immunoregulation and inflammation. The main biological function of IL-10 seems to be the limitation and termination of inflammatory responses. Also, it has an important regulatory role in the differentiation and proliferation of several immune cells such as T cells, B cells, natural killer cells, and antigen-presenting cells. A previous study in rats also found that IL-10 was involved in the protective effects of hypothermia on survival during endotoxemia (43). Thus, it is possible that the anti-inflammatory effect in the lung HT is regulated predominantly by increased anti-inflammatory mediators and not by decreasing pro-inflammatory mediators.

Deregulation of apoptosis has been implicated in many diseases, including lung injury (44). We measured lung and kidney caspase-3 activity as a marker of apoptosis to evaluate remote organ lesions (3). Despite the known pro-apoptotic effect of IL-10, we found no difference in lung and kidney apoptosis between experimental groups. We are aware that this single measurement is insufficient to evaluate functional cell survival, and future studies should include an apoptosis/necrosis index, but our results are relevant because tissue apoptosis in the lung and remote organs has not been studied in models of lung injury treated with hypothermia (3).

The effects of hypothermia on DO_2 and VO_2 are well known, as expected HT animals had lower CO, DO_2, and VO_2 (9). We found no difference in the arterial lactate and $ScvO_2$ between groups. Because there is a matching between DO_2 and VO_2, a low CO under hypothermia probably is, to some extent, an adaptation to a hypometabolic state.

The increased SVRI at 240 min in the HT group probably reflects mathematical coupling with CO, magnified by the mild (nonsignificant) hypertensive effect of hypothermia. Hypothermia increases blood viscosity and hemoglobin affinity for oxygen, which can result in tissue hypoxia. However, we did not detect low $ScvO_2$ and high lactate in HT group. Is possible that the permissive hypercapnia, secondary to protective MV, counteracts the effect of the hypothermia on hemoglobin affinity for oxygen.

The effect of hypothermia on coagulation is well known. Prolonged PT is evident in hypothermic patients and experimental animals, and in *in vitro* cooled plasma samples (45,46). Acidosis, frequently observed in patients with ALI/ARDS, increases the effect of hypothermia on coagulation (47,48). Despite the respiratory acidosis present in this model, we observed no differences between groups in coagulation test results or bleeding at the end of the observation period. However, we did not study the effects of HT on platelet function, the synthesis and kinetics of clotting enzymes, or plasminogen activator inhibitors, which may also be affected.

A few case reports have examined mild hypothermia and ALI in humans. Villar and Slutsky (49) conducted a small, nonrandomized, controlled study in 19 moribund adults with severe ARDS almost 20 years ago, finding improved lung function and outcomes in patients treated with mild hypothermia. As this study was performed prior to the use of currently accepted MV strategies, the results are difficult to apply in contemporary clinical practice.

Our study has some limitations. Hypothermia was induced shortly after ALI, which differs from the usual clinical setting where therapeutic interventions are initiated many hours or days after the initial insult. Second, differences between NT and HT groups in hemodynamic parameters (e.g., HR, CO, VO_2) did not achieve a plateau during the observation period. We think that the duration of the model (240 min) may also have influenced the lack of effect of hypothermia on pro-inflammatory cytokines, V_{min}, lung mechanics, and lung permeability, which could have been found in a longer period of observation or after hemodynamic/metabolic plateau have been reached. Future studies should thus employ an extended time period. Third, the results of this pediatric experimental model could not be generalized to other age-groups. Young mammals characteristically have higher oxygen uptakes than adults, posing a significant issue in therapies that induce hypometabolic states. Fourth, we evaluated biotrauma only partially using some cytokines and apoptosis markers implicated in local and remote organ injury. Potential positive or deleterious effects in distant organs cannot be discarded.

In conclusion, mild hypothermia had a therapeutic role in subjects with ALI and lung protective mechanical ventilation, increasing local anti-inflammatory response and decreasing VO_2 with no significant adverse effect on global tissue perfusion or coagulation in this experimental model. However, the clinical advantages, risks, and indications of prolonged mild hypothermia remain unknown and should be investigated in further studies.

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Conflict of interest

No conflicts of interest declared.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Pulmonary and hemodynamic measurements in the mild hypothermia and normothermia groups.

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