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Mild hypothermia attenuates lung edema and plasma interleukin-1β in a rat mechanical ventilation-induced lung injury model

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ABSTRACT

Recent data suggest that deep hypothermia has protective effects on experimental induced lung injury. It is not well known if these effects persist with mild hypothermia. The authors hypothesized that mild hypothermia may attenuate lung injury and decrease local and systemic proinflammatory cytokines in a rat model of injurious mechanical ventilation (MV). Twelve Sprague-Dawley male adult rats were anesthetized, intubated, and randomly allocated to normothermia group (37°C) (NT) or mild hypothermia group (34°C) (MH). After 2 hours of deleterious MV (peak inspiratory pressure [PIP] 40 cm H_2O, zero end-expiratory pressure [ZEEP], and inspiratory fraction of oxygen [Fio_2] 100%), arterial blood gases, lung gravimetry, and histological study were obtained. Protein content, interleukin (IL)-1β, and tumor necrosis factor (TNF)-α were measured in plasma and bronchoalveolar lavage (BAL) fluid. Subjects that underwent MH had a significant lower wet-to-dry lung weight ratio (8.32 ± 0.28 vs. 10.8 ± 0.49, $P = .01$), IL-1β plasma concentration (0.6 ± 0.6 vs. 10.27 ± 2.80 pg/mL, $P = .0048$) and $Paco_2$. There were no differences in terms of Pao_2, histological injury, or BAL protein content. In this model of injurious mechanical ventilation, subjects treated with mild hypothermia had less lung edema and lower plasma IL-1β. Some of known beneficial effects of deep hypothermia can be obtained with mild hypothermia.

KEYWORDS therapeutic hypothermia, ventilator-induced lung injury

Injurious mechanical ventilation (MV) was first described more than 30 years ago [1]. Lung damage due to MV (ventilation-induced lung injury [VILI]) is the result of many potential insults (volutrauma, atelectrauma, rheotrauma), but positive pressure is considered the cornerstone of MV detrimental effects. This mechanical *noxa* leads to local production of inflammatory mediators that may translocate into systemic circulation and induce distant organ failure [2–7].

Induced hypothermia consists of a controlled decreased in body temperature for therapeutic purposes [8]. Its use has been extended to several clinical scenarios where tissue dysoxia is an important issue, such as post cardiac arrest, cardiopulmonary bypass for heart surgery, and organ protection for transplant, among others [9–13].

Recent experimental studies suggest that hypothermia might have a beneficial role on lung injury [14]. In different models of acute lung injury (ALI) and injurious MV, hypothermia-treated animals have shown improved gas exchange and lung mechanics, as well as less pulmonary edema and inflammation, histological evidence of injury, and oxidative stress damage [15–19]. However, these experimental studies used deep hypothermia ($\leq 30°C$). The possibility to obtain the same benefits of deep hypothermia with temperatures closer to normothermia has aroused special interest recently. Mild hypothermia (defined as $34°C \pm 0.5°C$) is a feasible clinical goal

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that can be applied to large mammals. Contraindications and adverse reactions to this degree of hypothermia are uncommon, contrary to deeper hypothermia in which hemodynamic tolerance is poorer and life-threatening consequences (cardiac arrhythmias, coagulopathy, dyskalemia, severe infections) are far more frequent. In experimental ALI, mild hypothermia (33°C to 34°C) has shown some protective effects, but only when compared to hyperthermia (39°) [14, 20–22]. In respect to normothermia (37°C), usefulness of mild hypothermia is unclear.

We hypothesized that the protective effects of deep hypothermia can be obtained with mild hypothermia, attenuating lung injury and decreasing local and systemic inflammatory mediators in a rat model of injurious MV.

METHODS

Animal Anesthesia

Our institutional Bioethics Committee approved the study protocol. Twelve Sprague-Dawley 400-g male rats were used for this study. In our animal research facility rats were maintained in a humidity-, light-, and temperature-controlled environment. Food and water were provided ad libitum.

After inhalatory induction with 2% sevofluorane, rats were anesthetized by intraperitoneal injection of ketamine (50 mg/kg; Drag Pharma Invetec, Santiago, Chile) plus xylazine (10 mg/kg; Alfasan, Woerden, Holland). Tracheal intubation was performed with a 16-G BD Angiocath (Angiocath, Becton Dickinson, NJ, USA) catheter for MV. An adequate level of anesthesia was assumed if pedal reflex was absent. Otherwise, a second ketamine (25 mg/kg) plus xylazine (5 mg/kg) dose was administrated.

Experimental Groups and Mechanical Ventilation

Rats were randomly allocated to normothermia group (NT; central temperature 37°C ± 0.5°C, $n = 6$) or mild hypothermia group (MH; 34°C ± 0.5°C, $n = 6$). Temperature was measured at the thoracic esophagus (YSI Reusable Temperature Probe; Yellow Spring Instrument, OH, USA). In the NT group, body temperature was maintained using an external heater. In the MH group, body temperature was reduced to 34°C over a 10-minute period using cold gel-bags. Room temperature was maintained at 25°C.

Injurious ventilation strategy settled for this model was a peak inspiratory pressure (PIP) of 40 cm H_2O, zero end-expiratory pressure (ZEEP), inspiratory time (tI) of 0.3 seconds, respiratory rate (RR) of 60 breaths per minute, and an inspiratory fraction of oxygen (FIO_2) of 1, delivered by a Siemens 900C (Munich, Germany) ventilator in a pressure control mode.

Ventilatory parameters were kept for 2 hours after target temperature was achieved.

Sample Collection, Gravimetry, and Histological Grading

Animals were sacrificed at the end of the study by intravenous administration of a lethal dose of thiopental (50 mg/kg; Richmond Laboratories, Buenos Aires, Argentina), after extracting 5 mL of whole blood. Arterial blood gas was measured and corrected according to body temperature by a device-incorporated algorithm (i-STAT Cartridges G3+; Abbott Laboratories, NJ, USA). The remaining blood was immediately centrifuged at 3000 rpm for 10 minutes and plasma stored at −80°C.

Chest was opened via sternotomy, right hilum was clamped, and bronchoalveolar lavage (BAL) of the left lung was performed using 6 mL of cold (4°C) saline solution. Recovered fluid was centrifuged at 3000 rpm for 10 minutes and the supernatant was frozen at −80°C. The right lung was removed and gravimetry was performed according to the method described by Pearce et al. [23]. The left lung was fixed using a 10% formaldehyde endobronchial instillation and embedded in paraffin for histological preparation. The lung was cut from the lung apex toward the base at 3-mm interval and stained with hematoxylin and eosin. A blinded pathologist examined 10 randomly selected fields at 200× magnification. For each field, histological findings were graduated on a scale previously validated by Hong et al. [15] that includes (a) alveolar neutrophilic infiltrate; (b) interstitial neutrophilic infiltrate; (c) perivascular neutrophilic infiltrate; (d) lung congestion; and (e) alveolar bleeding (total score, 0–15).

Determination of Lung Injury Variables in BAL Fluid and Plasma

Total protein concentrations in BAL fluids, measured by Bradford method, were used to assess alveolar capillary permeability. To evaluate local and systemic effects of MH, tumor necrosis factor alpha (TNF-α) and interleukin 1 beta (IL-1β) cytokines were measured on BAL fluid and sera using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Biosource International, Camarillo, CA).

Statistical Analysis

The study was powered to detect a 30% reduction in plasma and BAL cytokines according to previous experimental data [17, 18]. Sample size per group needed to achieve an 80% study power was 6, with a .05 2-sided significance level. Data are expressed as average and standard error of the mean (SEM). Statistical analysis was performed using SPSS 7.5 program (SPSS, Chicago, IL) with Student's t test and significance was set at $P < .05$.

RESULTS

All animals completed the protocol and body temperature was kept within target range throughout the study period. No adverse effects due to temperature control convective methods were noted.

Blood Gases

After 2 hours of MV, the MH group had a significantly lower $Paco_2$ ($P = .004$) and higher pH ($P < .01$). There was not significant difference on Pao_2 ($P = .098$) (Table 1).

Vascular Permeability Markers

Gravimetry (wet-to-dry lung weight ratio) was significantly higher in the NT group in relation to the MH group ($P \leq .01$). The BAL protein concentration did not show a significant difference between both groups (Table 1).

Histological Markers

No histological differences were found between both groups, neither overall score nor specific histologic variables examined (Table 2).

Inflammatory Markers

We did not find differences in IL-1β and TNF-α BAL concentrations (Figures 1 and 2). However, IL-1β plasma concentration in NT group was 10.27 ± 0.49 pg/mL, whereas the MH subjects had 0.6 ± 0.6 pg/mL ($P = .0048$). For TNF-α, the NT group had a median plasma concentration of 49.14 ± 5.57; the MH group 57.64 ± 13.07 pg/mL ($P = .28$).

DISCUSSION

This experimental model in rats demonstrates that mild hypothermia may attenuate some consequences of injurious MV when compared to normothermia. This statement is supported on 2 main findings. First, animals that underwent hypothermia had lower serum IL-1β levels. Second, subjects under hypothermia showed significantly less lung edema, per gravimetry assessment, than the normothermia group.

The effect of body temperature on lung injury has been a subject of study in recent years. Hyperthermia has been shown to increase ALI when compared with normothermia and hypothermia [14, 22]. There is a number of experimental information of the protective effect of deep hypothermia on ALI [15–19, 24, 25], but the evidence of protective and anti-inflammatory effects of mild hypothermia is scarce [14, 20–22]. In addition, only negative results have been reported when comparing mild hypothermia and normothermia [14, 20–22]. We measured plasma and BAL TNF-α and IL-1β, because previous experimental studies have usually addressed the effect of hypothermia and lung injury with these 2 cytokines [15–18]. We found that the proinflammatory cytokine IL-1β was drastically reduced in plasma of animals exposed to injurious MV and treated with hypothermia. Like a previous study, IL-1β and TNF-α concentrations in BAL did not differ in temperature groups [22]. In BAL fluid, these cytokines have been found to be decreased only in animals with lipopolysaccha-

TABLE 1 Arterial Blood Gas Analysis, Wet-to-Dry Lung Weight Ratio, and Bronchoalveolar Lavage Protein Concentration of Study Groups After 2 Hours of Injurious Mechanical Ventilation

	NT		MH		
	Mean	SEM	Mean	SEM	P value
pH	7.41	±0.012	7.52	±0.009	<.01
Pao_2 (mm Hg)	321.0	±32.04	387.3	±35.53	.098
$Paco_2$ (mm Hg)	38.72	±1.58	21.15	±0.78	<.01
Wet-to-dry-ratio	10.80	±0.49	8.32	±0.28	<.01
BAL proteins (mg/mL)	1.18	±0.17	1.03	±0.17	.267

Note. BAL = bronchoalveolar lavage; MH = mild hypothermia; NT = normothermia; SEM = standard error of the mean.

TABLE 2 Lung Injury Histological Score of Study Groups After 2 Hours of Injurious Mechanical Ventilation

	NT		MH		
	Mean	SEM	Mean	SEM	*P* value
Alveolar neutrophilic infiltrate	2.00	±0.26	1.72	±0.48	.30
Interstitial neutrophilic infiltrate	1.50	±0.23	1.70	±0.38	.32
Perivascular neutrophilic infiltrate	1.50	±0.43	1.33	±0.31	.35
Lung congestion	0.67	±0.21	0.63	±0.26	.40
Alveolar bleeding	0.83	±0.31	0.72	±0.46	.41
Total histological score	6.50	±0.31	6.10	±0.41	.22

Note. MH = mild hypothermia; NT = normothermia; SEM = standard error of the mean.

ride (LPS)-induced lung injury treated with deep hypothermia. [15, 16, 18].

Lung gravimetry is a well-established method to assess lung water content and, thus, could be used as a surrogate index for pulmonary edema. As previously described with deep hypothermia, we found that animals treated with mild hypothermia had a reduced wet-to-dry lung weight ratio. However, one of the known effects of hypothermia is cardiac output reduction, which in turn may influence the development of lung edema [26]. With our data and data from previous experiments it is not possible to determinate if wet-to-dry lung weight ratio reduction is a primary effect of hypothermia or if it is secondary to a reduction in cardiac output. In addition, hypocapnia in hypothermic subjects probably reflects a decrease in the metabolic rate and not a better lung function or gas exchange [14, 26, 27]. Future large-animal studies are needed with better monitoring techniques to determine this. The discrepancy observed between lung gravimetry and histology is not surprising, because the former is a more sensible method, currently considered gold standard for lung edema.

The MV protocol designed for this study is based on previous studies in rats that demonstrated that VILI could be experimentally generated with peak inspiratory pressure (PIP) greater than 30 cm H_2O for at least 1 hour. ZEEP is a known factor for amplification of this damage [1, 24, 28, 29]. A recent experimental study failed to demonstrate differences on plasma proinflammatory cytokines in rats exposed to injurious MV and treated with mild hypothermia compared to normothermia [22]. Their ventilatory protocol included high PIP (35 cm H_2O) and 2 cm H_2O of peak end-expiratory pressure (PEEP). This low-grade PEEP may have significant protective effects in the absence of other lung noxa.

No histological differences were observed between groups. This finding may indicate that plasma proinflammatory cytokines preceded severe lung damage. However, with our data we are unable to conclude

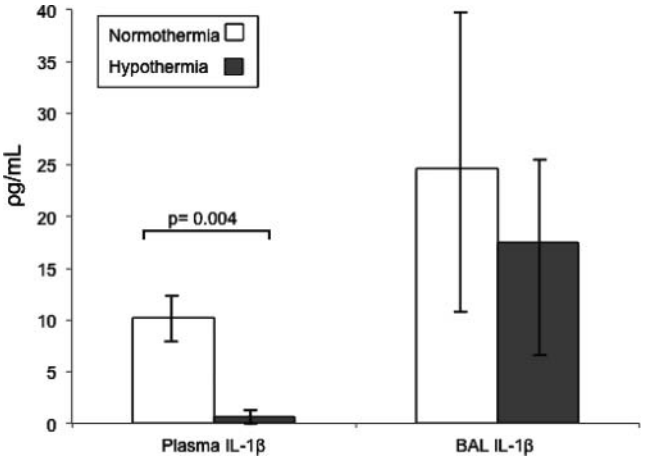

FIGURE 1 Plasma and bronchoalveolar lavage (BAL) concentrations (mean ± SEM) of IL-1β of normothermia and mild hypothermia subjects after 2 hours of injurious mechanical ventilation.

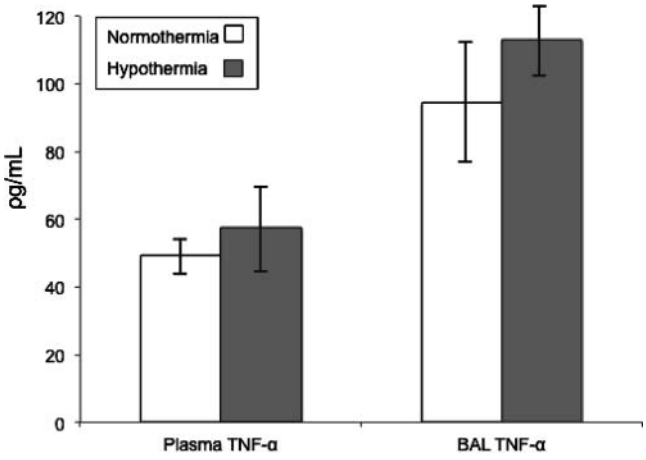

FIGURE 2 Plasma and bronchoalveolar lavage (BAL) concentrations (mean ± SEM) of TNF-α of normothermia and mild hypothermia subjects after 2 hours of injurious mechanical ventilation.

if this reflects a diminished systemic response to the lung insult or a reduced translocation of cytokines from pulmonary to plasma. In this way, plasma proinflammatory cytokines may be an early biomarker of lung injury, an issue that has been addressed in recent studies [30].

There are a few case reports of hypothermia and ALI in human subjects. One noteworthy study was performed by Villar and Slutsky over 15 years ago [31]. This small, concurrent-controlled, prospective nonrandomized study included 19 adults with severe acute respiratory distress syndrome (ARDS; predicted mortality 100%). They found an improvement of lung function and a significant reduction of mortality of 33% in patients treated with conventional therapy plus mild hypothermia compared to standard care.

In our study, injurious MV was employed, something that is not accepted in clinical practice. This model does not allow us to foresee the effect of mild hypothermia in a lung-protective ventilation strategy (lower tidal volume ventilation and high PEEP). Second, hypothermia was induced at the same time as the ventilatory insult, very different from the usual clinical setting in which MV is initiated but therapeutic interventions may not be initiated until hours or days after the initial insult. Third, this study did not evaluate remote organ function, so we cannot state the direct effect of hypothermia on biotrauma. Plasma concentration of proinflammatory cytokines can estimate biotrauma only indirectly. Fourth, this study addressed only the potential beneficial effects of hypothermia. Description of potential detrimental effects, such as a reduction in cardiac output and coagulopathy, are needed. Oxygen consumption was not measured because it is known to be altered by changes of temperature. Finally, given the effect of hypothermia on metabolism, it may have a greater effect in animals with higher oxygen consumption, such as rats, than humans. Future studies must be designed to determine if the beneficial effects of hypothermia persist in an animal model with oxygen consumption equivalent to human beings. Also, they should include more severe lung noxa, MV strategy ad hoc to contemporary care (low tidal volume, high PEEP), and better potential adverse effects monitoring.

In conclusion, these data suggest that mild hypothermia may have a therapeutic role, decreasing lung edema and systemic inflammatory response induced by injurious MV. Nevertheless, clinical advantages, indications, and risks of hypothermia in this clinical setting are unknown to date. Further trials are needed to answer these questions.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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