Potential therapeutic strategies aimed at reducing the intensity of mechanical ventilation in ARDS

Beurskens, Charlotte

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Potential therapeutic strategies aimed at reducing the intensity of mechanical ventilation in ARDS

Charlotte Beurskens

Potential therapeutic strategies aimed at reducing the intensity of mechanical ventilation in ARDS
van
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Potential therapeutic strategies aimed at reducing the intensity of mechanical ventilation in ARDS

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Potential therapeutic strategies aimed at reducing the intensity of mechanical ventilation in ARDS
Academic thesis, University of Amsterdam, the Netherlands

This thesis was prepared at the Laboratory of Experimental Intensive Care and Anesthesiology (L.E.I.C.A.) and the Department of Intensive Care, Academic Medical Center, Amsterdam, the Netherlands

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“Alles kump good”
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General introduction and outline of the thesis

Charlotte J.P. Beurskens and Nicole P. Juffermans
The problem: Ventilator Induced Lung Injury

Approximately 35% of the patients admitted to the intensive care unit (ICU) are mechanically ventilated during their stay [1]. Although mechanical ventilation is necessary to prevent the patient from dying, there are also drawbacks resulting from mechanical ventilation. In some patients, mechanical ventilation can even worsen the outcome by inducing ventilator-induced lung injury (VILI). VILI is thought to result from three types of trauma; baro-trauma, atelectotrauma and biotrauma [2, 3]. Baro-trauma is caused by overstretching of the alveoli, during mechanical ventilation with large tidal volumes or the use of high driving pressures. Atelectotrauma results from repetitive opening and closing of the airways. Biotrauma is the result of pulmonary inflammatory responses. In particular, lungs that are ‘primed’ by an inflammatory insult, such as in acute respiratory distress syndrome (ARDS), are susceptible to additional injury inflicted by the ventilator.

Approximately a decade ago, it became clear that restricting tidal volumes to 6 ml/kg reduced mortality in patients suffering from ARDS [4]. Also, high driving pressures are increasingly recognized to contribute to VILI [5-7]. Thereby, guidelines advocate to ‘do no harm’ and to practice protective ventilation, including application of low tidal volumes and low inspiratory pressures. In some patients however, it is not feasible to maintain normocapnia using this approach. Tolerating an increase in carbondioxide (CO₂) levels is called permissive hypercapnia. Permissive hypercapnia was shown to ameliorate lung injury. However, severe hypercapnia with acidosis may be detrimental, with severe consequences, including decreased host response to infection [8], impaired right ventricular function [9] and decreased diaphragm contractility [10].

Even in the era of protective ventilation and permissive hypercapnia, mortality rates of patients suffering from lung injury remain high [2, 3]. In a patient with ARDS, the demand of the ventilatory support intensifies and tidal volumes and pressures are increased to maintain an adequate gas exchange. However, this results in a vicious circle of increased baro- and atelectotrauma with a subsequent higher demand for potential injurious ventilation settings. Therefore new therapies are warranted to limit VILI. We investigated both induced hypothermia and heliox ventilation as potential therapeutic strategies to reduce the intensity of mechanical ventilation.
A possible new strategy to limit VILI: Induced hypothermia

Induced hypothermia (32-34°C) is applied as a therapeutic intervention in the operating room and at the ICU. Induced hypothermia is associated with reduced ischemia-reperfusion injury during operative procedures [11] and improved neurological outcome following cardiac arrest [12, 13]. In cardiopulmonary surgery, hypothermia is associated with a reduced risk of stroke [14, 15]. The underlying mechanism of mitigating harmful effects of ischemia-reperfusion by controlling body temperature is proposed to be inhibition of an exaggerated inflammatory response. In line, in a pig model of cardiac arrest, hypothermia reduced expression of pro-inflammatory cytokines within the brain [16]. Also in various models of (sterile) ARDS, hypothermia is associated with a reduction in lung injury by a decrease of the inflammatory response [17-23]. This suggests hypothermia may mitigate harm, caused by an ‘overshoot’ of the systemic inflammatory reaction in response to ventilation. However, the animal ARDS models often used ex vivo settings and non-physiological acid-base balances, which hamper extrapolation to the clinical situation.

Besides lowering inflammation, induced hypothermia also lowers metabolism with a concomitant decrease in CO₂ production. Thereby, hypothermia may allow for reducing the intensity of mechanical ventilation, with a reduction in applied driving pressures or tidal volumes needed for adequate ventilation. In line with this, retrospective data in our ICU patients suggest that induced hypothermia has a favourable effect on gas exchange, with a decline of the partial CO₂ tension (PaCO₂), at unchanged minute ventilation [24]. Thereby, in lung injury, hypothermia may be beneficial via two distinct mechanisms.

Reducing the inflammatory response may, however, also have a drawback. An adequate adaptive host immune response to pathogens and infection is crucial [25] and fever is considered an important factor for optimal antimicrobial host defence [26]. Hypothermia inhibits immune responses [27], with delayed generation of pro-inflammatory cytokines by monocytes [28] and reduction of neutrophil and monocyte migration [29, 30]. The consequence of inhibiting host response by induced hypothermia may be a higher infection risk. Clinical data on the risk of infection following hypothermia show conflicting results. Trials in cardiac arrest patients reported no increased overall infection rates associated with hypothermia [12, 31]. In addition, prolonged hypothermia (>48 hours) did not increase risk of infection in patients with brain injury, annotated all patients received
selective decontamination of the digestive tract [32]. However, a recent systematic review showed an association of hypothermia with increased prevalence of pneumonia and sepsis in patients enrolled in randomized controlled clinical trials of therapeutic hypothermia for any indication, although overall infection rate was not affected [33].

In this thesis, we explore the effects of induced hypothermia on VILI. We hypothesized that induced hypothermia may limit VILI in two ways. First, hypothermia may reduce metabolism, thereby reducing CO₂ production. This might enable lower minute ventilation with maintaining adequate gas exchange and lower respiratory rates and/or lower tidal volumes, thereby limiting barotrauma and VILI. Secondly, hypothermia may reduce the inflammatory response, resulting in reduction of biotrauma and VILI. Furthermore, we also investigated whether hypothermia affects the innate immune response to bacterial infection.

**A possible new strategy to limit VILI: Heliox ventilation**

Helium is an inert gas with a lower density than air and thus flow of helium through an airway is less turbulent, leading to lower resistance [34]. In patients, ventilated with helium in a mixture with oxygen (heliox), lower driving pressures are necessary to distribute oxygen to the distal alveoli and improve oxygenation [34]. Therefore heliox ventilation may have therapeutic potential in patients suffering from VILI, by improving ventilation and allowing for reduction in driving airway pressures.

At the ICU, heliox has been safely used to reduce the work of breathing in both paediatric and adult patients, in whom airflow was obstructed due to increased airway resistance during exacerbations of asthma and COPD [35-38]. In ARDS, data are generally limited to paediatric patient populations and paediatric animal models of acute lung injury. Here, heliox persistently was found to improve gas exchange during ventilation modes, such as high frequency oscillation ventilation, that are more frequently used in infants instead of adults [39-44]. Data on the use of heliox during conventional mechanical ventilation in adult models are scarce.

We hypothesized that the use of heliox could be a new strategy to reduce VILI, by allowing lower minute ventilation and lower driving pressures, as a result of improved compliance and improved CO₂ removal, thereby decreasing lung injury.
Outline of this thesis

The general aim of this thesis was to investigate induced hypothermia and heliox ventilation as potential therapeutic strategies to reduce the intensity of mechanical ventilation with the aim to avoid or limit VILI. This dissertation is divided in two parts, with part I describing induced hypothermia and part II discussing heliox ventilation, both as as a potential therapeutic strategies. In both parts a translational approach was chosen, using preclinical and clinical studies.

Part I focuses on the effect of induced hypothermia on VILI. In chapter 2, we reviewed the literature on energy expenditure in several critically ill patient populations. Since body temperature may influence energy expenditure, we not only compared the metabolic state and caloric need in the critically ill patient groups, but also summarized the effect of body temperature, use of sedation and severity of illness on energy expenditure.

In chapter 3, effects of induced hypothermia were investigated in a clinically relevant animal model of VILI. Adult rats were mechanically ventilated with injurious mechanical ventilation settings, combined with hypothermia (32°C) or normothermia (37°C). We hypothesized that induced hypothermia would protect from VILI and investigated whether reducing respiratory rates would enhance lung protection, since repetitive opening and closing of airways can attribute to atelectotrauma and thus VILI.

The effect of induced hypothermia was also studied in an infectious model of *Streptococcus pneumoniae* pneumosepsis in chapter 4. Hypothermia (32°C) was induced for 4 hours, during mechanical ventilation. Bacterial dissemination and mitochondrial function were investigated to substantiate the beneficial effects of hypothermia.

Having established that hypothermia reduces lung injury in both sterile and infectious experimental models of VILI and ARDS, we expanded our investigations to clinical studies. In chapter 5 body temperature of patients, admitted to the ICU after a cardiac arrest, was targeted to either 33°C (hypothermia) or 36°C (normothermia) [31]. In this population, we prospectively studied the levels of circulating mitochondrial DNA, since mitochondrial DNA is a marker of tissue damage and associated with adverse outcome [45].

In chapter 6 we focused on the effect of hypothermia on lung mechanics. Patients received a treatment with induced hypothermia for 24 hours and respiratory parameters were collected longitudinally. Patients served as their own control; hence no control group was required. In this study we hypothesized hypothermia could lower the intensity of mechanical ventilation.
Chapter 7 describes the results of induced hypothermia on immune response to several bacterial antigens. In cardiac arrest patients with a target temperature of 33°C or 36°C (identical design as in chapter 5), the effect of body temperature was investigated and compared within the groups and with healthy controls. We hypothesized that this study could be important to explore if the fear for higher infection risk, when using induced hypothermia, was legitimate.

In part II heliox ventilation was studied as a strategy to limit VILI. In chapter 8, we resumed available data on the effects of heliox ventilation in animal ARDS models and critically ill patients with ARDS or respiratory failure due to ARDS-like syndromes.

In chapter 9 an animal model of VILI, induced by barotrauma with tidal volumes of 15 ml/kg was executed. Rats were ventilated for 4 hours with either heliox or a standard gas mixture of oxygen-in-air. During mechanical ventilation respiratory data and blood gases were collected hourly, since we hypothesized that the use of heliox could facilitate CO₂ elimination, allowing for lower minute volume ventilation.

Chapter 10 describes a more severe animal model of ARDS. Lung injury was induced by intratracheal instilling 1 mg/kg lipopolysaccharide. Rats were mechanically ventilated with lung protective settings of 6 ml/kg tidal volumes and randomized to ventilation with heliox or oxygen-in-air. We expected more lung injury within this model and therefore hypothesized a more profound effect of heliox ventilation on the respiratory data.

In chapter 11 and 12, we evaluated in a clinical setting whether reduced intensity of mechanical ventilation could also be achieved by heliox administration during conventional mechanical ventilation in ICU patients. First, in chapter 11, safety and feasibility of heliox ventilation was tested in critically ill patients admitted to the ICU after a cardiac arrest. Secondly, the same patient population was studied in chapter 12, although the focus was shifted to the specific effect of heliox ventilation on gas exchange during the 3 hours the patients received heliox ventilation. We hypothesized heliox would allow a reduction in the intensity of mechanical ventilation. With that, this study could provide a proof of principle and thereby open doors for future research on heliox ventilation as therapeutic possibility in patients in whom protective mechanical ventilation is hampered by the development of respiratory acidosis.

The results of both the effects of induced hypothermia and heliox ventilation on VILI are summarized and discussed in chapter 13.


Part I

Induced hypothermia
Energy expenditure in different patient populations on the Intensive Care: “One size does not fit all”

Christanne M. Mooij; Charlotte J.P. Beurskens; Nicole P. Juffermans

Netherlands Journal of Critical Care, July 2013; 17(3): 3-9
Abstract

Objective: Adequate nutrition has an impact on outcome in critically ill patients. This descriptive literature search investigates whether there are differences in energy expenditure (EE) between specific subgroups of critically ill patients, including patients with sepsis, trauma, burns and cerebrovascular accidents. Also, we summarized specific factors, which may influence EE, such as the use of sedation, body temperature and severity of illness.

Design: a descriptive review of studies, which have measured EE or oxygen consumption with indirect calorimetry in critically ill patients. Studies were retrieved by a systematic search of the Medline database, using search terms referring to the measurement (energy expenditure); the patient population in general (critically ill patients) and to the four specific subgroups specific (sepsis, trauma, burns, stroke).

Results: EE in patients with sepsis, trauma and burns was increased (sepsis 102-198%; trauma 110-168%; burns 137-182%; stroke 149% for men and 120% for women) compared to reference values of EE in healthy individuals. Burn patients had the highest EE levels. There was no difference in EE between sepsis and trauma patients. Patients with a cerebrovascular accident had the lowest EE. Half of these patients had an EE that did not exceed EE levels in healthy adults. Use of sedation lowered EE, whereas fever increased EE. Uncertainty persists whether treatment of stroke patients with hypothermia decreases EE. According to most studies, higher disease severity scores are associated with higher EE, but one study found that severity of illness is negatively correlated with EE in sepsis.

Conclusions: Data for this review was limited, precluding definite conclusions. However, it is clear EE differs among critically ill patient populations. The use of a ‘one size fits all’ formula to estimate caloric need in the critically ill may not be appropriate in the design of studies on caloric need nor in patient care.
Introduction

In critically ill patients meeting caloric demand by adequate nutrition, is related to better outcome [1]. Thereby, adequately responding to the nutritional demands of patients admitted to the Intensive Care Unit (ICU) should be a daily goal in patient care. However, the optimal amount of calories that should be prescribed to critically ill patients has been a matter of debate [1].

It is thought that the consumption of calories, termed energy expenditure (EE), is increased in critically ill patients compared to the general hospital population, due to high metabolic demands during various inflammatory conditions [2]. A disbalance between high demands and limited energy supply may contribute to organ failure and adverse outcome in ICU patients [3, 4]. In this view, underfeeding could be detrimental. An alternative hypothesis relating to the optimal amount of calories holds that this hypermetabolic state might be harmful and that hypocaloric nutrition reduces hypermetabolism [5], thereby improving outcome [6]. In both strategies, measuring or estimating energy demands of patients is crucial in determining the optimal amount of feeding.

The EE can be measured in several ways, including indirect calorimetry. Alternatively, the Harris Benedict equation is used, which calculates the amount of calories needed in ICU patients and estimates an individual’s basal metabolic rate, multiplied by an activity factor [7]. A shortcoming of this formula is the controversy about what exactly this activity factor should be [8-10]. Also, the formula does not distinguish between specific ICU patient populations. Comparing caloric targets based on the calculated caloric need with use of this formula [6, 11] may therefore lead to inadequate conclusions in ICU patients. Given the relation between caloric supply and outcome, it seems paramount to be aware of possible EE differences between different subgroups. This paper summarizes data from all available studies which have directly measured EE in four specific subgroups of ICU patients, including sepsis, trauma, burns and cerebrovascular accident (CVA).

Methods

Medline database was used to identify medical subject’s headings (MeSH) and select search terms. In addition to MeSH terms, free text words were used. Search terms referred to the measurement: energy expenditure (calorimetry, indirect [MeSH]; energy metabolism [MeSH]; energy metabolism; energy expenditure; indirect calorimetry), to
the patient population in general: critically ill patients (critical illness [MeSH]; intensive care unit [MeSH]; critical care [MeSH]; intensive care [MeSH]; critical care; intensive care unit; critical illness; ICU patients) and to the four specific subgroups: (septic shock [MeSH]; bacteremia [MeSH]; sepsis [MeSH]; sepsis; pyemia; septicemia; blood poisoning; severe sepsis; bacteremia; septic shock); (wounds and injuries [MeSH]; trauma; wounds and injuries; severe trauma); (Burn, chemical [MeSH]; Burn [MeSH]; burn; chemical burns; electric burns; burn wounds); (brain ischemia [MeSH]; cerebral infarction[MeSH]; subarachnoid hemorrhage [MeSH]; intracranial hemorrhage [MeSH]; stroke [MeSH]; stroke; cerebrovascular apoplexy; CVA; cerebrovascular accident; brain infarction; cerebral ischemia; intracranial hemorrhage; subarachnoid hemorrhage; SAH). Search results were limited to adults. Studies were selected when data on EE (kcal/day) or oxygen consumption (VO₂ (ml/(min.m²))) were provided. If not mentioned otherwise, measured EE refers to resting EE. When EE or VO₂ of individual patients were given, mean and SD were calculated. Kilojoules were converted by using the equation 1 kcal = 4,184 kJ. When only EE/ kg body weight was given, EE was calculated by multiplying with mean body weight of the patient group. In that case, no SD could be calculated.

To facilitate comparisons of metabolism between groups, all measurements of EE in the subgroups of critically ill were compared with a reference value of EE or VO₂ as measured in healthy adult men and women [12] and expressed as a percentage of that reference value. If the proportion male/ female (M/F) of a study group was given, EE was corrected for the M/F ratio, by multiplying the number of male patients with the reference value of a healthy male and the number of female patients with the reference value of a healthy female, divided by the total number of patients. If no M/ F ratio was given, an M/F ratio of 3/1 was used, because this ratio corresponded best with the M/F ratios in the studies of EE in sepsis, trauma and burns groups where this ratio was given. The normal VO₂ range is 110 – 160 ml/(min.m²). The effect of several clinical conditions was investigated, including body temperature, use of sedation and disease severity. Severity of illness was estimated using either the “Acute Physiology and Chronic Health Evaluation” (APACHE) II or III score [24, 25], Injury Severity Score (ISS) [26, 27] or the percentage of body surface area (%BSA) that was burned for burn patients only.

Results

The Medline search yielded 361 studies. Of these, 338 were not suitable, because data on measured EE or VO₂ were not given for the specific patient subgroups under study,
leaving 23 studies with data on EE or VO₂ in patients with sepsis (n=10), sepsis and trauma (n=3), trauma (n=3), trauma and burns (n=1), burns (n=3) and CVA (n=3). In total, EE measurements in 448 patients were included.

**Comparison of EE and/or VO₂ between different patient groups**

In patients suffering from sepsis (table 1), all studies except one [13] found an increase in measured EE with values ranging from 102 to 198% compared to the reference value. Three out of the six VO₂ measurements were high, but within the normal range. The other VO₂ measurements slightly exceeded the normal range.

In the trauma group (table 2), all studies reported high EE measurements, ranging from 110 to 168% compared to the reference value. Of the VO₂ measurements, one study reported values within the normal range (153.6 ml/(min.m²) [14], other studies reported VO₂ values higher than 160 ml/(min.m²).

**Table 1: Caloric demand in critically ill patients with sepsis**

<table>
<thead>
<tr>
<th>References</th>
<th>Number of patients</th>
<th>Male/F</th>
<th>EE (kcal/day)</th>
<th>% of reference EE</th>
<th>VO₂ (ml/(min.m²))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frankenfield et al [22]</td>
<td>20</td>
<td>17/3</td>
<td>3395 ± 634</td>
<td>198 %</td>
<td>-</td>
</tr>
<tr>
<td>Koea et al [29]</td>
<td>4</td>
<td>3/1</td>
<td>2196 ± 440</td>
<td>132 %</td>
<td>-</td>
</tr>
<tr>
<td>Rusavy et al [30]</td>
<td>20</td>
<td>unknown</td>
<td>2116 (No SD)</td>
<td>127 %</td>
<td>-</td>
</tr>
<tr>
<td>Liggett et al* [9]</td>
<td>18</td>
<td>unknown</td>
<td>1982 ± 97</td>
<td>119 %</td>
<td>-</td>
</tr>
<tr>
<td>Clark et al [31]</td>
<td>11</td>
<td>unknown</td>
<td>1950 ± 175</td>
<td>117 %</td>
<td>-</td>
</tr>
<tr>
<td>Kiiski et al [32]</td>
<td>21</td>
<td>16/5</td>
<td>1960 ± 486</td>
<td>117 %</td>
<td>-</td>
</tr>
<tr>
<td>Uehara et al [33]</td>
<td>12</td>
<td>8/4</td>
<td>1859 ± 485</td>
<td>114 %</td>
<td>-</td>
</tr>
<tr>
<td>Basile-Filho et al [13]</td>
<td>15</td>
<td>11/4</td>
<td>1699 ± 271</td>
<td>102 %</td>
<td>-</td>
</tr>
<tr>
<td>Schaffartzik et al [34]</td>
<td>30</td>
<td>unknown</td>
<td>-</td>
<td>-</td>
<td>173 ± 30</td>
</tr>
<tr>
<td>Fernandes et al [35]</td>
<td>10</td>
<td>10/0</td>
<td>-</td>
<td>-</td>
<td>168.9 ± 63.1</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5/0</td>
<td>-</td>
<td>-</td>
<td>144.0 ± 30.5</td>
</tr>
<tr>
<td>Hanique et al [36]</td>
<td>14</td>
<td>9/5</td>
<td>-</td>
<td>-</td>
<td>163 ± 19</td>
</tr>
<tr>
<td>Natalini et al [37]</td>
<td>10</td>
<td>unknown</td>
<td>-</td>
<td>-</td>
<td>157 ± 35</td>
</tr>
<tr>
<td>Zauner et al [23]</td>
<td>14</td>
<td>8/6</td>
<td>-</td>
<td>-</td>
<td>135 ±26</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>204</strong></td>
<td></td>
<td></td>
<td><strong>102 – 198 %</strong></td>
<td></td>
</tr>
</tbody>
</table>

*Data are mean ± SD; M/F = proportion male/ female; *In Ligget et al, EE was measured using a pulmonary artery catheter.
In the patient population suffering from burns (table 3), EE measurements ranged from 137 to 182% of the reference values. Two studies reporting VO₂ measurements exceeded the normal range, with values of 131 and 209% compared to the upper limit of the normal range of oxygen consumption of 160 ml/(min.m²) [15, 16].

In stroke patients (table 4), increased levels of EE of 149% of the reference value for men and 120% for women were found in one study [17]. However, the two other studies [18, 19] found no increased EE levels compared to the reference value. In this patient group, no VO₂ measurements were available.

**Table 2: Caloric demand in critically ill patients with trauma**

<table>
<thead>
<tr>
<th>References</th>
<th>Number of patients</th>
<th>M/F</th>
<th>EE (kcal/ day)</th>
<th>% of reference EE</th>
<th>VO₂ (ml/(min.m²))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frankenfield et al [22]</td>
<td>13</td>
<td>10/3</td>
<td>2754 ± 401</td>
<td>168 %</td>
<td>-</td>
</tr>
<tr>
<td>Bruder et al [21]</td>
<td>24</td>
<td>19/5</td>
<td>2496 ± 574</td>
<td>148 %</td>
<td>203 ± 55</td>
</tr>
<tr>
<td>Stucky et al [38]</td>
<td>21</td>
<td>unknown</td>
<td>2263 ± 599</td>
<td>136 %</td>
<td>-</td>
</tr>
<tr>
<td>Kiiski et al [32]</td>
<td>25</td>
<td>17/8</td>
<td>2071 ± 430</td>
<td>126 %</td>
<td>-</td>
</tr>
<tr>
<td>Uehara et al [33]</td>
<td>12</td>
<td>9/3</td>
<td>1953 ± 416</td>
<td>117 %</td>
<td>-</td>
</tr>
<tr>
<td>Raurich et al* [10]</td>
<td>Total: 26</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>15/5</td>
<td>1900 ± 394</td>
<td>114 %</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>15/5</td>
<td>1840 ± 311</td>
<td>110 %</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Rauschak et al [14]</td>
<td>15</td>
<td>14/1</td>
<td>-</td>
<td>165.9 ± 21.2</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>13/5</td>
<td>-</td>
<td>-</td>
<td>153.6 ± 30.4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>154</td>
<td></td>
<td></td>
<td></td>
<td>110 – 168 %</td>
</tr>
</tbody>
</table>

Data are mean ± SD; M/F = proportion male/female; *In Raurich et al, 40 EE measurements are reported for 26 patients in total. One group of measurements was compared to 20 surgical patients, the other to 20 medical patients.

**Table 3: Caloric demand in critically ill patients with burns**

<table>
<thead>
<tr>
<th>References</th>
<th>Number of patients</th>
<th>M/F</th>
<th>EE (kcal/ day)</th>
<th>% of reference EE</th>
<th>VO₂ (ml/(min.m²))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gore et al [15]</td>
<td>6</td>
<td>unknown</td>
<td>3030</td>
<td>182 %</td>
<td>209 ± 27</td>
</tr>
<tr>
<td>Khorram-Sefat et al [8]</td>
<td>27</td>
<td>27/0</td>
<td>2878 ± 407</td>
<td>172 %</td>
<td>-</td>
</tr>
<tr>
<td>Royall et al [16]</td>
<td>22</td>
<td>17/3</td>
<td>2319 ± 553</td>
<td>139 %</td>
<td>335 ± 80</td>
</tr>
<tr>
<td>Stucky et al [38]</td>
<td>12</td>
<td>unknown</td>
<td>2284 ± 508</td>
<td>137 %</td>
<td>-</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>67</td>
<td></td>
<td></td>
<td></td>
<td>137 – 182 %</td>
</tr>
</tbody>
</table>

Data are mean ± SD; M/F = proportion male/female
Energy expenditure in different patient populations on the Intensive Care: "One size does not fit all"

Table 4: Caloric demand in critically ill patients with cerebrovascular accident

<table>
<thead>
<tr>
<th>References</th>
<th>Number of patients</th>
<th>M/F</th>
<th>EE (kcal/day)</th>
<th>% of reference EE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Badjatia et al [17]</td>
<td>50</td>
<td>16/34</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>male 2647 ± 1013</td>
<td>149 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>female 1622 ± 596</td>
<td>120 %</td>
</tr>
<tr>
<td>Bardutzky et al * [18]</td>
<td>10</td>
<td>4/6</td>
<td>1549</td>
<td>102 %</td>
</tr>
<tr>
<td>Bardutzky et al* [19]</td>
<td>34</td>
<td>20/14</td>
<td>1568</td>
<td>98 %</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>94</strong></td>
<td></td>
<td></td>
<td><strong>98 – 149 %</strong></td>
</tr>
</tbody>
</table>

Data are mean ± SD; M/F = proportion male/female; *In Bardutzky et al, total EE is given instead of resting EE.

Table 5: Energy Expenditure and the illness severity scores in different patient populations.

<table>
<thead>
<tr>
<th>References</th>
<th>% of reference EE</th>
<th>APACHE II score</th>
<th>Injury severity score</th>
<th>Injury severity score</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sepsis patients</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clark et al [31]</td>
<td>117</td>
<td>24</td>
<td>33 ± 9</td>
<td>29.83 ± 10.55</td>
</tr>
<tr>
<td>Uehara et al [33]</td>
<td>114</td>
<td>16 – 34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basile- Filho et al [13]</td>
<td>102</td>
<td>22.6 ± 7.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Trauma patients</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frankenfield et al [22]</td>
<td>168</td>
<td>33 ± 9</td>
<td>27 ± 9</td>
<td>36.7 ± 18.8</td>
</tr>
<tr>
<td>Bruder et al [21]</td>
<td>148</td>
<td>27 ± 9</td>
<td>22.18 ± 16.42</td>
<td></td>
</tr>
<tr>
<td>Stucky et al [38]</td>
<td>136</td>
<td>22 ± 20</td>
<td>22 ± 20</td>
<td></td>
</tr>
<tr>
<td>Kiiski et al [32]</td>
<td>126</td>
<td>26 ± 50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uehara et al [33]</td>
<td>117</td>
<td>26 ± 50</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Burn patients</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stucky et al [38]</td>
<td>137</td>
<td>29.83 ± 10.55</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>References</th>
<th>% of BSA burned</th>
<th>% of reference EE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Burn patients</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gore et al [15]</td>
<td>182</td>
<td>72 ± 9.8</td>
</tr>
<tr>
<td>Khorram- Sefat et al [8]</td>
<td>172</td>
<td>51 ± 20</td>
</tr>
<tr>
<td>Royall et al [16]</td>
<td>139</td>
<td>36.7 ± 18.8</td>
</tr>
<tr>
<td>Stucky et al [38]</td>
<td>137</td>
<td>40.85 ± 36.35</td>
</tr>
</tbody>
</table>

Data are given in mean ± SD
Clinical conditions influencing EE

The use of sedative medication generally lowers EE [20, 21]. However, one study showed a positive correlation between sedative dose and EE [22]. Different types of sedative medication did not result in differences in EE levels when measurements were corrected for body temperature [21].

An increase in EE caused by fever was found in sedated head injured patients [21] and in septic patients [23]. Conversely, induced hypothermia to 33°C resulted in a significantly lower EE in stroke patients [18]. However not all studies report such a decrease, as Badjatia et al. found no significant differences in EE between the hypothermic (33.5-35.5 ºC) and the normothermic (36.5-37.0 ºC) stroke patients [17]. In this study, shivering as a result of treatment with hypothermia was clearly shown to increase EE [17].

The severity of illness may influence EE. EE was related to either the APACHE II or III for sepsis [24, 25], ISS for trauma [26, 27] or %BSA for burn patients (table 5). Not all studies mentioned severity scores. In general however, patients with higher severity scores were more hypermetabolic, which was most distinct in patients with burns [8, 16]. An association between severity of illness and EE was also noted in septic trauma patients [22]. One study, however, reported a negative correlation between APACHE III scores and resting EE in sepsis [23].

Discussion

EE measurements were highest in burn patients, with all studies reporting substantially higher values than reference EE values. Also, EE values of burn patients were higher than EE values in most studies describing sepsis or trauma patients. A probable explanation is that burned patients are highly hypermetabolic and catabolic [28]. In both the sepsis and trauma patient populations we found increased EE values compared to the EE levels in healthy adults. Patients suffering from sepsis do not have consistently higher EE values than trauma patients. As expected, EE measurements in the CVA group were lowest, with two out of three studies (50 % of the patient population) not reporting increased EE compared to healthy adults [18, 19]. This may be due to the fact that in these patients an inflammatory state is not as apparent as in sepsis, trauma or burn patients. Taken together, there are profound differences in EE between specific patient populations, with burn patients having the highest EE values. A large variation was also observed within subgroups of patients, as well as a large variety between different studies.
The use of sedation was generally found to decrease EE in critically ill patients, without apparent differences between types of sedative medication [20, 21]. As expected, fever increases the levels of EE [21, 23]. The use of induced hypothermia seems to decrease EE [18]. One study found no significant difference in EE between normothermia and hypothermia, which was attributed by the authors to the heterogeneity of the patient population [17]. The positive correlation between body temperature and EE can be explained by thermogenesis. Of note, we found that EE was positively correlated to the severity of illness in the majority of the reviewed studies, suggesting that within a specific clinical condition, also disease severity should be taken into account when estimating the caloric need. However, it should be considered that variation was large [8, 16, 22].

Given the differences in EE between patient populations as well as the variance within patient groups, we feel it would be best to measure the EE in each individual patient when assessing the amount of nutrition. When predictive equations are used instead of indirect calorimetry, factors can be added correcting for the patients’ type of lesion and for clinical conditions such as the use of sedation, severity of illness and body temperature. However, various recommendations on estimating EE in patients have been made. According to some, predicting the individual EE by using an equation is not possible, because of the variation in EE in critically ill patients and the quantity of factors influencing EE [10]. Therefore, it was proposed to use the same predictive equations for all patients without adding factors. On the other hand, others hold that factors attributing to differences in energy expenditure between critically ill patients should be better understood to allow more accurate estimation of the caloric needs of individual patients [21]. In burned patients, it was recommend to use equations that do not give higher predictions of EE than 1.5-1.6 times basal EE to avoid overfeeding [8]. Taken together, there is no consensus on what the correcting factors should be. The summary of our findings point towards a patient-tailored approach, taking into account the clinical condition as well as disease severity.

Besides patient care, our findings may also have implications for future research. In studies comparing the impact of hyper- versus hypo-caloric nutrition on outcome, it should be considered that EE differs between patient populations. The use of predictive equations in such studies is inappropriate in predicting the actual EE and thus the caloric demand of individual patients. However, some of these studies claim a relation between predictive equations and outcome [6, 11]. When the ‘less than goal’ and ‘near goal’
amount of calories is based on those predictions and are related to outcome, results may be confounded. Therefore indirect calorimetry seems the best way to estimate the nutrition status of a patient and provide tailored care to possibly improve outcome.

There are several limitations to this review. The amount of data on measured EE or VO₂ is limited. Thereby, patient numbers are small. The most important limitation is that statistical analyses of data was limited as individual patient data were not available, rendering this study a descriptive review. Also, the ratio between male and female was not always given. Most collected measurements were performed during the first week. Thereby, variations in EE during the course of illness were not detected (23). As individual energy expenditure can fluctuate significantly from day to day [22], this may influence the reliability of the comparison of EE measurements in the current study. However, EE is largely stable in the course of the first week [18, 19, 23]. As we did not perform consecutive measurements, conclusions of this study only pertain to the first week following admission. Another limitation is that several factors such as age, body weight, types of nutrition, presence of shock, administration of medications such as insulin or inotropics and the use of mechanical ventilation were not considered. Patients and circumstances were quite heterogeneous and EE measurements were not corrected for that. However, we corrected for sex, which importantly determines EE [7]. There is a great need of collecting more data to improve the limited knowledge, before adapting this in daily ICU care. Future research should ideally include the influence of mechanical ventilation and inotropic drugs on EE values.

Nonetheless, this study attempts to give an indication of differences in EE between four distinct groups of patients. Despite the limitations of this study, results may have implications for estimating energy expenditure in clinical practice and for research goals.

**Conclusion**

Energy expenditure differs between and within patient populations. The use of sedatives, body temperature and severity of illness have an impact on EE values. The use of the same formula to calculate caloric need for each patient may not be appropriate.
Reference list

Energy expenditure in different patient populations on the Intensive Care: “One size does not fit all”


Mild hypothermia reduces ventilator induced lung injury, irrespective of reducing respiratory rate

Hamid Aslami; Maria T. Kuipers; Charlotte J.P. Beurskens; Joris J.T.H. Roelofs; Marcus J. Schultz; Nicole P. Juffermans

Translational Research, February 2012;159:110-117
Abstract

In the era of lung-protective mechanical ventilation using limited tidal volumes, higher respiratory rates are applied to maintain adequate minute volume ventilation. However, higher respiratory rates may contribute to ventilator induced lung injury (VILI). Induced hypothermia reduces carbon dioxide production and may allow for lower respiratory rates during mechanical ventilation. We hypothesized that hypothermia protects from VILI and investigated whether reducing respiratory rates further enhance lung protection in an in vivo model of VILI. During four hours of mechanical ventilation, VILI was induced by tidal volumes of 18 ml/kg in rats, with respiratory rates set at 15 or 10 breaths/minute in combination with hypothermia (32°C) or normothermia (37°C). Hypothermia was induced by external cooling. A physiological model was established. VILI was characterized by increased pulmonary neutrophil influx, protein leak, wet weights, histopathology score and cytokine levels compared to lung protective mechanical ventilation. Hypothermia decreased neutrophil influx, pulmonary and systemic interleukin–6 levels, histopathology score and tended to decrease pulmonary protein leak. Reducing respiratory rate in combination with hypothermia did not further reduce parameters of lung injury. In conclusion, hypothermia protected from lung injury in a physiological VILI model by reducing inflammation. Mild lowering of the respiratory rate did not further enhance protection.
Introduction

Mechanical ventilation can initiate as well as exacerbate lung injury, referred to as ventilator induced lung injury (VILI)\(^1\), thereby worsening other organ functions and contributing to mortality.\(^1\) The mechanisms of VILI involve mechanical processes, including overstretching and repetitive opening and closing of alveoli\(^2\). Also, a pro-inflammatory state is apparent, including cytokine production\(^3\), influx of neutrophils\(^4\) and a procoagulant state\(^5\). It is thought that these processes interact.

The use of limited tidal volumes of 6 ml/kg reduces morbidity and mortality in patients with acute lung injury (ALI)\(^6\);\(^7\). This “protective” mechanical ventilation strategy is adopted in guidelines worldwide\(^8\). To maintain adequate minute volume ventilation during lung protective ventilation, higher respiratory rates are applied, which may amplify lung injury by repetitive alveolar collapse\(^9\). In an experimental model, lower respiratory rates was shown to protect against VILI\(^10\). However, use of low respiratory rates combined with low tidal volume ventilation is limited by development of respiratory acidosis. Although (mild) respiratory acidosis may be protective in ALI\(^11\);\(^12\), severe acidosis is usually avoided, because of detrimental effects on immune function,\(^13\) right ventricular function\(^14\) and oxygenation\(^15\). New strategies are warranted, as is emphasized by recent reports on management of ICU patients, in which ventilation with deviated tidal volumes of 8–9 ml/kg was applied because of hypoxemia and acidosis\(^16\).

Induced hypothermia is applied in the intensive care patient to ameliorate hypoxia-induced brain damage following a cardiac arrest\(^17\). Also, hypothermia was found to be protective in several models of lung injury, including VILI\(^18\);\(^20\). Besides a reduction in inflammation, induced hypothermia reduces metabolism and thereby carbon dioxide production, which may allow for a lower respiratory rate to maintain adequate minute ventilation. In a VILI model, reducing respiratory rates in conjunction with hypothermia indeed augmented protection\(^21\). However, the presence of severe alkalosis, occurring already after one hour of mechanical ventilation, hampers extrapolation of these results. In the present study, we examined the effect of mild hypothermia in a model of VILI with a normal acid-base balance. We hypothesized that applying lower respiratory rates during hypothermia further reduces lung injury. Results may contribute to the question whether we should target the inflammatory response or minute volume ventilation in future studies of VILI.
Methods

The study was approved by the animal care and use committee of the Academical Medical Centre, Amsterdam, the Netherlands. Animal procedures were carried out in compliance with Institutional Standards for Humane Care and Use of Animal Laboratory Animals.

Anesthesia and instrumentation

The experimental protocol is as described earlier. Male Sprague Dawley rats (n=8 per group, Harlan, The Hague, The Netherlands) weighing 350-400 g received an intraperitoneal injection of anesthesia mix (0.15 ml 100g⁻¹ body weight) containing 90 mg kg⁻¹ ketamine (Eurovet Animal Health B.V., Bladel, The Netherlands), 0.5 mg kg⁻¹ medetomidine (Pfizer Animal Health B.V., Capelle a/d IJsel, the Netherlands) and 0.05 mg kg⁻¹ atropine (Pharmachemie, Haarlem, the Netherlands). Anesthesia was maintained by infusion of 50 mg kg⁻¹ ketamine at 0.5ml 100¹ hr⁻¹ via the tail vein. Normal saline was administered at 2 ml 100g⁻¹ hr⁻¹. Tracheotomy was performed, after which a metal cannula was inserted in the trachea. The metal cannula was connected to a ventilator (Servo 300, Siemens, Sweden). Hemodynamic monitoring was done by inserting a polyethylene catheter into the carotid artery (Braun, Melsungen, Germany) which was connected to a monitor (Siemens SC900, Danvers, USA). Arterial blood gas analysis was performed hourly (Rapidlab 865 blood gas analyzer, Bayern, Mijdrecht, the Netherlands), uncorrected for body temperature (alphastat method), which is considered a safe approach in critical ill patients as corrected blood gases can lead to hypercapnia, cerebral vasodilatation and increased intracranial pressure (23). Rectal temperature was monitored continuously (ama–digit ad 15th, Amarell, Kreuzwertheim, Germany).

Mechanical ventilation settings and induction of hypothermia

At baseline, all the animals were pressure controlled ventilated with 12 cmH₂O positive inspiratory pressure (PIP) and 5 cmH₂O positive end expiratory pressure (PEEP) with respiratory rate set at 35 breaths/min (lung protective (LP) mechanical ventilation). The PIP, PEEP and respiratory rate were changed to 23 cmH₂O, 0 cmH₂O and 15 breaths/min respectively in the VILI normal rate group and the respiratory rate was reduced to 10 breaths/min in the VILI low rate group without changing the PIP and PEEP levels. Tidal volumes were measured using a pneumotachometer (HSE, Harvard apparatus, Manheim, Germany) specific for rats. The pneumotachometer was calibrated using a 1 mL syringe according to the manufacturer’s instruction. Tidal volumes were recorded using respiration
software (HSE-BDAS basic data acquisition, Harvard apparatus, Manheim, Germany). The tidal volumes during LP mechanical ventilation were \( \sim 8.5 \text{ ml kg}^{-1} \) and in both VILI groups, the tidal volumes were \( \sim 18 \text{ ml kg}^{-1} \). The inspired oxygen fraction was kept at 60 % and the inspiration to expiration ratio at 1:2 in the experimental groups. Hypothermia was induced by placing icepacks on the abdomen, until rectal temperature reached 32°C in both VILI and LP group. In the normothermia VILI and LP groups, temperature was kept at 37°C with a heating pad.

**Bronchoalveolar lavage and assays**

After 4 hours of mechanical ventilation, blood was withdrawn from the carotid artery, followed by *en block* removal of the lungs. The right lung was ligated, followed by bronchoalveolar lavage (BAL) of the left lung (3 x 2 ml NaCl), of which 3-4 ml was retrieved. Cell counts were determined using a hematocytometer (Z2 Coulter Particle Counter, Beckman Coulter Corporation; Hialeah, Florida, USA) in BAL–fluids (BALF). Differential counts were done on cytospin preparations stained with Giemsa stain (Dade Behring AG, Dudingen, Switzerland). In BALF supernatant, protein levels (Oz Biosciences, Marseille, France), as well as interleukin (IL)–6, CINC3, tumor necrosis factor (TNF)-α and IL–10 (ELISA, R&D Systems; Abingdon, United Kingdom) were determined according to instructions from the manufacturers.

**Histopathology**

The right lung top was Hematoxylin–Eosin stained and analyzed by a pathologist who was blinded for group identity. Interstitial inflammation, endothelialitis, bronchitis, edema and pleuritis were scored on a scale of 0 – 4: 0 for normal lungs, 1 for <25% lung involvement, 2 for 25 – 50% involvement, 3 for 50–75% involvement and 4 for >75% lung involvement. Total histology score is the sum score of all parameters. The remaining right lobes were used to determine wet weight.

**Statistical analysis**

Data are presented as mean ± SD in the table or as mean ± SEM in the figures. Intergroup differences were analyzed by analysis of variance and Bonferroni’s post–hoc test, or by a Kruskal–Wallis test with Mann–Whitney *U* test according to the data distribution. A *p* value of < 0.05 was considered significant. Statistical analyses were carried out using SPSS version 16 (SPSS inc., Illinois, USA).
Results

A physiological model of VILI

Mean arterial pressure decreased in the experimental groups over time, but did not differ between groups and never dropped below 65 mmHg (Figure 1A + B). Hypothermia resulted in a decreased heart rate. Arterial blood gases remained within physiological limits in all groups (Table). Respiratory rates needed to be reduced in VILI groups, due to application of higher PIP (Table). Lowering the respiratory rate with 33% in the low rate group, did not result in acid-base disturbances (Table).

Table 1: Arterial blood gas analysis in a physiological rat model of lung protective (LP) and lung injurious mechanical ventilation.

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>LP</th>
<th>Ventilator induced lung injury</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal rate</td>
<td>Low rate</td>
</tr>
<tr>
<td></td>
<td>37°C</td>
<td>32°C</td>
</tr>
<tr>
<td>pH</td>
<td>T = 0</td>
<td>7.47±0.05</td>
</tr>
<tr>
<td></td>
<td>T = 4</td>
<td>7.40±0.09</td>
</tr>
<tr>
<td>pCO₂ (kPa)</td>
<td>T = 0</td>
<td>4.1±0.8</td>
</tr>
<tr>
<td></td>
<td>T = 4</td>
<td>5.3±2.0</td>
</tr>
<tr>
<td>pO₂ (kPa)</td>
<td>T = 0</td>
<td>39±2</td>
</tr>
<tr>
<td></td>
<td>T = 4</td>
<td>41±4</td>
</tr>
<tr>
<td>HCO₃⁻ (mmol/L)</td>
<td>T = 0</td>
<td>24±11</td>
</tr>
<tr>
<td></td>
<td>T = 4</td>
<td>24±14</td>
</tr>
<tr>
<td>Base Excess</td>
<td>T = 0</td>
<td>-0.6±2.3</td>
</tr>
<tr>
<td></td>
<td>T = 4</td>
<td>-0.5±1.0</td>
</tr>
<tr>
<td>Respiratory rate (breath/min)</td>
<td>T = 0</td>
<td>35±0</td>
</tr>
<tr>
<td></td>
<td>T = 4</td>
<td>33±4</td>
</tr>
</tbody>
</table>

Data shown as mean ± SD. *: VILI 37 °C vs. LP 37 °C, †: VILI 37 °C vs. VILI 32°C normal rate, #: VILI 32°C low rate vs. VILI 32°C normal rate and ‡: VILI 37°C normal rate vs. VILI 37°C low rate. Symbols indicate: p<0.05.

Local and systemic inflammation in VILI

VILI was associated with an increase in levels of protein in the BALF and lung wet weight (Figure 2) compared to LP ventilation (p<0.05 for all), indicating pulmonary leakage. There was an influx of neutrophils to the lung (Figure 2C,) and pulmonary levels of IL–6 were increased (Figure 3A, p<0.05 for all). Increase in inflammatory parameters in the lung
was accompanied by an increase in lung histopathology score compared to LP ventilation (Figure 4 + 5, p<0.05). Injurious mechanical ventilation led to an increase in systemic levels of IL–6 (Figure 3B, p<0.05). IL–10, CINC3 and TNF-α were not detectable in plasma or BALF. Increased inflammation in VILI was accompanied by a reduction in arterial pO₂ compared to LP ventilated groups (Table, p<0.05).

Figure 1: The effect of hypothermia on mean arterial pressure (MAP) (A+B), heart rate (C+D) and body temperature (E+F) during lung protective (LP) and lung injurious mechanical ventilation creating ventilator induced lung injury (VILI). Straight line and dotted lines represents normothermia and hypothermia respectively. The combination of straight line with closed squire and open diamond with dotted line represents VILI normothermia normal rate and VILI hypothermia low rate respectively. Closed square with dotted line represents VILI hypothermia normal rate (in B, D, and F). Mean ± SEM, *: vs. normothermia control, p<0.05.

The effect of hypothermia on pulmonary inflammation in VILI

Hypothermia reversed the rise in pulmonary inflammatory parameters induced by the ventilator in both VILI groups compared to normothermic controls, including lung wet weight, pulmonary neutrophil influx, IL–6 concentrations and lung histopathology score (Figure 2, 3, 4 + 5 p<0.05 for all). Hypothermia tended to reduce protein concentration in BALF. Also, hypothermia prevented a decrease in arterial pO₂ (Table, p<0.05).
Mild hypothermia reduces ventilator induced lung injury, irrespective of reducing respiratory rate.

Figure 2: The effect of hypothermia on levels of total protein (A), wet weight (B) and neutrophil influx (C) in bronchoalveolar lavage fluid (BALF) during lung protective (LP) mechanical ventilation and lung injurious ventilation creating ventilator induced lung injury (VILI). Mean ± SEM, *: p<0.05.

Figure 3: The effect of hypothermia on levels of IL-6 in bronchoalveolar lavage fluid (BALF) (A) and plasma (B) during lung protective (LP) mechanical ventilation and lung injurious ventilation creating ventilator induced lung injury (VILI). Mean ± SEM, *: p<0.05.

Figure 4: The effect of hypothermia on histopathology score of animals during lung protective (LP) mechanical ventilation and lung injurious ventilation creating ventilator induced lung injury (VILI) with the VILI groups the respiratory rate set at 15 breaths/min (normal rate) or 10 breaths/min (low rate). Mean ± SEM. *: p<0.05.
The effect of lower respiratory rates against VILI

Reducing respiratory rate in combination with hypothermia did not enhance the protective effect of hypothermia on parameters of pulmonary inflammation (Figure 2+3), nor on oxygenation (Table) in VILI. Also, reducing respiratory rate during normothermia had no effect on oxygenation and pulmonary and systemic inflammatory parameters in VILI.

Discussion

In an in vivo VILI model with normal acid-base balance, hypothermia reduced lung inflammation caused by the mechanical ventilator. Application of lower respiratory rates did not enhance the protective effect of hypothermia on lung injury.

The present study describes a physiological model of VILI. In previous VILI models, ventilation with large tidal volumes resulted in severe alkalosis or shock with acidosis, limiting the time of mechanical ventilation in the experiments to less than 2 hours. As pH status affects endpoints of VILI, extrapolation of results of experimental studies investigating the effect of hypothermia in VILI is hampered. In our model, arterial blood gas analysis remained within physiological limits, also after 4 hours of mechanical ventilation.
High positive inspiratory pressure caused alveolar-endothelial membrane disruption, with leakage of large proteins leading to permeability edema and gas exchange disturbances, in line with previous reports\textsuperscript{18,19}. Induction of hypothermia reduced permeability edema and improved oxygenation, demonstrated by lower pulmonary protein concentration, lower wet weight and higher arterial pO\textsubscript{2}, respectively, with a concomitant decrease in pro-inflammatory mediators. In line with this, hypothermia reduced neutrophil influx into the lung in our model\textsuperscript{19,20}, together with a decrease in IL–6 levels in BALF. Other cytokine levels were not increased in VILI. This is in line with some reports\textsuperscript{28,29} but not with all\textsuperscript{20,21}. There are several explanations for discrepant results. We used relatively low positive inspiratory pressures to create lung injury, in contrast to some previous models\textsuperscript{3,4,18}, as well an \textit{in vivo} design. Also, difference in duration of VILI as well in duration of hypothermia may contribute to differential results. An elevation of pulmonary levels of TNF-\alpha early in the course of inflammation may have returned to low levels at the end of the experiment\textsuperscript{(20)}. A short course of hypothermia may be more effective in reducing pro-inflammatory cytokine levels\textsuperscript{19} and to achieve a favorable change in the balance between pro- and anti-inflammatory cytokines by induction of IL–10\textsuperscript{30} than extended courses of hypothermia\textsuperscript{19}. In addition, the depth of hypothermia may account for differences in outcome. Whereas mild hypothermia is protective in ALI, deep hypothermia has been found to worsen lung injury.

Mechanical ventilation can also perpetuate systemic inflammation\textsuperscript{33}. High plasma cytokines associated with mechanical ventilation was found to contribute to multiple organ failure\textsuperscript{33}. Of note, the protective effect of mechanical ventilation with low tidal volumes\textsuperscript{7} was associated with lower systemic IL-6 levels compared to the application of conventional tidal volumes.

In the era of lung protective mechanical ventilation, higher respiratory rates are applied to meet adequate minute volume for ventilation\textsuperscript{7}. However, repetitive opening and closing of alveoli may be one of the mechanisms of VILI. Indeed, in vitro, high respiratory rates were found to induce injury to alveolar cells\textsuperscript{35}. The combination of high respiratory rates with high tidal volumes aggravates lung injury\textsuperscript{35–37}. Thereby, reducing respiratory rates may enhance lung protection. In this study, a mild reduction in respiratory rate did not further enhance hypothermia–mediated protection, nor did it alter markers of VILI during normothermia. Although a larger reduction in respiratory rate was previously found to offer protection\textsuperscript{21,37}, gross abnormalities in acid base balance or an \textit{ex vivo} setting in these models hamper clinical applicability. We provide the first \textit{in vivo} data, which do not
support interventions targeted at reducing respiratory rates in mechanically ventilated patients. However, a limitation of our findings is that the reduction in respiratory rate was only mild. This does not exclude that a larger reduction in respiratory rate may be advantageous. As pointed out, the development of severe acid-base imbalances is a limitation to such applications. Also, we used healthy animals rather than animals with pre-existing lung injury.

Conclusion

Hypothermia protected from lung injury in a physiological VILI model. Mildly lowering the respiratory rates did not further enhance lung protection. Whether high respiratory rates can be applied safely in patients with lung injury, cannot be dissected from our results. Targeting inflammation however, is a promising approach to reduce lung injury in mechanically ventilated patients.
Mild hypothermia reduces ventilator-induced lung injury, irrespective of reducing respiratory rate.


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Induced hypothermia is protective in a rat model of pneumococcal pneumonia, associated with increased ATP availability and turn-over

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Critical Care Medicine, March 2012; 40:919-926
Abstract

Objective: To determine the effect of induced hypothermia on bacterial growth, lung injury and mitochondrial function in a rat model of pneumococcal pneumosepsis.

Design: Animal study

Setting: University research laboratory

Subjects: Male Sprague-Dawley rats

Interventions: Subjects were inoculated intratracheally with *Streptococcus pneumoniae*, controls received saline. After development of pneumonia, mechanical ventilation was started with or without induced mild hypothermia (32°C). Bacterial growth and inflammatory markers were determined in bronchoalveolar lavage fluid (BALF), blood, and organs. Oxidative phosphorylation and ATP contents were measured in mitochondria isolated from liver and soleus muscle.

Measurements and main results: Inoculation with *S. pneumoniae* resulted in severe pneumonia, with bacterial dissemination, distal organ injury and blunted peripheral oxygen consumption upon mechanical ventilation. Hypothermia did not affect bacterial growth in BALF and in homogenized lungs compared to normothermic controls, but was associated with reduced bacterial dissemination to the spleen, with a trend towards reduced bacterial load in blood and liver. Hypothermia reduced lung injury, exemplified by reductions in pulmonary cell influx and BALF protein levels compared to controls. Hypothermia reduced BALF levels of interleukin (IL)-1β, tended to reduce BALF CINC-3 levels, but no effect was observed on BALF TNF-α and IL-6 levels. Induced hypothermia restored the fall in oxygen consumption and ATP levels in the liver, while ATP/ADP ratios remained low. In muscle, induced hypothermia also reversed low oxygen consumption due to pneumonia, but with an increase in ATP levels, while ATP/ADP ratios were low.

Conclusion: Hypothermia did not adversely affect bacterial growth, but rather reduced bacterial dissemination in a rat model of pneumococcal pneumosepsis. Furthermore, hypothermia reduced lung injury, associated with restored ATP availability and turn-over. These findings suggest that hypothermia may reduce organ injury by preventing sepsis-related mitochondrial dysfunction.
Induced hypothermia is protective in a rat model of pneumococcal pneumonia, associated with increased ATP availability and turnover.

Introduction

Sepsis triggers an uncontrolled and excessive systemic inflammatory response, which may develop into multiple organ dysfunction [1]. The mechanisms by which organ failure occurs are unknown. Prolonged shock with shunting in the microcirculation may contribute to inadequate tissue perfusion, thereby reducing metabolic function [2-4]. However, metabolic dysfunction has been found to persist despite adequate oxygen delivery, suggesting that the predominant defect may be a decreased use of oxygen in mitochondria.

During severe infection, the inflammatory response can result in direct damage to mitochondrial DNA, lipids and respiratory complexes, thereby inhibiting oxidative phosphorylation [5] and diminishing adenosine triphosphate (ATP) availability in organs [6]. When ATP levels drop below a certain threshold, apoptotic cell death pathways are triggered [3]. Energetic failure directly impacts outcome in sepsis, as skeletal muscle ATP concentrations were found to be depleted in patients with septic shock, together with structural changes in the mitochondria, associated with enhanced mortality [7]. Alternatively, it was proposed that a decrease in mitochondrial activity with subsequent reduced cellular metabolism may be a functional response to excessive inflammation, protecting the cell from dying during energetic failure. Thereby, reducing metabolic demand and thus ATP requirements could be protective in case of an excessive inflammation response.

Induced mild hypothermia is applied in patients with cardiac arrest to prevent ischemia-reperfusion injury, resulting in a better neurologic outcome and reduced mortality [8]. The underlying mechanisms of the protective effect of hypothermia include a slower metabolism, as well as prevention of mitochondrial dysfunction [9]. Induced mild hypothermia may also exert its protective effect by inhibition of leukocyte migration, phagocytosis and pro-inflammatory cytokine production [10, 11]. A protective effect of mild hypothermia was found in murine models of sterile acute lung injury [12-15]. In patients with severe acute lung injury, hypothermia applied as a last resort was found to reduce mortality [16]. The downside of hypothermia however, may be an increased risk of infections [17, 18], due to an impaired host response [19, 20]. In this study, we investigated the effect of induced mild hypothermia in a model of pneumococcal pneumosepsis. Pneumococcal pneumonia is the most common
pathogen causing pneumonia with organ failure, requiring ICU admission [21, 22]. We hypothesized that hypothermia would reduce ATP requirements during pneumosepsis by reducing inflammation, thereby reducing organ injury. Also, we investigated the effect of hypothermia on bacterial dissemination to distant organs.

**Materials & Methods**

This study was approved by the animal care and use committee of the Academical Medical Center, Amsterdam, The Netherlands. Animal procedures were carried out in compliance with Institutional Standards for Human Care and Use of Animal Laboratory Animals.

*Induction of pneumococcal pneumonia*

Male Sprague-Dawley rats (Harlan, The Hague, The Netherlands) weighing 350-400 grams were inoculated intratracheally with ~8 x 10^6 colony forming units (cfu's) of aerosolized *Streptococcus pneumoniae* (ATCC 6303; Rockville, MD, USA) using a trans-oral miniature nebulizer under light anesthesia with isoflurane 3%. Controls received saline. Supplemental fluid (10 ml/kg Ringers Lactate) was given intraperitoneally 16 hours after inoculation.

*Anesthesia, instrumentation and induction of hypothermia*

After 40 hours, anesthesia was induced by an intraperitoneal injection of an anesthesia mix (200μL/100g body weight) containing 90 mg/kg ketamine (Eurovet Animal Health B.V., Bladel, the Netherlands), 0.5 mg/kg medetomidine (Pfizer Animal Health B.V., Capelle a/d IJsel, the Netherlands) and 0.05 mg/kg atropine (Pharmachemie, Haarlem, the Netherlands). Anesthesia was maintained by infusion of 90 mg/kg ketamine at 0.5 ml/100g/hr through a venflon cannula, which was inserted in the tail vein. Tracheotomy was performed, a metal cannula was inserted in the trachea, tied down in situ and connected to a ventilator (Servo 900C, Siemens, Väsby, Sweden). Rats were pressure controlled ventilated with 13 cmH₂O over 5 cmH₂O positive end-expiratory pressure, an fraction of inspired oxygen (FiO₂) of 50%, an I:E ratio of 1:2 and a respiratory rate of 35 to 50 breaths per minute in respectively healthy or infected rats. Hemodynamic monitoring was done by inserting a polyethylene catheter into the right carotid artery (Braun, Melsungen, Germany) which was connected to a monitor (Siemens SC900, Danvers, USA). Arterial blood gas analysis was performed hourly (Rapidlab 865 blood gas analyzer, Bayern, Mijdrecht, the Netherlands, alphastat).
Induced hypothermia is protective in a rat model of pneumococcal pneumonia, associated with increased ATP availability and turnover.

After baseline measurements, hypothermia (32°C) was induced in infected and non-infected animals using icepacks on the abdomen (n=8 per group). In infected and non-infected controls, normothermia (37°C) was maintained by a thermo mattress. Temperature was monitored rectally (ama–digit ad 15th, Amarell, Kreuzwertheim, Germany).

**Bronchoalveolar lavage and measurements**

After 4 hours of mechanical ventilation, rats were bled. The lungs were removed en block; the right lung was ligated, followed by bronchoalveolar lavage (BAL) of the left lung (3 x 2.5 ml NaCl). The right lung was used to determine wet weight, as was the right kidney. Cell counts were determined using a hematocytometer (Z2 Coulter Particle Counter, Beckman Coulter Corporation; Hialeah, Florida, USA) in BAL–fluid (BALF). Differential counts were done on cytospin preparations stained with Giemsa stain (Dade Behring AG, Dudingen, Switzerland). CINC-3, IL1β, IL–6, TNF-α and IL10 levels were measured by ELISA according to instructions from the manufacturer (R&D Systems; Abingdon, United Kingdom), as were protein levels in BALF and urine (Oz Biosciences, Marseille, France). Creatinine was measured in blood samples using standard techniques. Glomerular filtration rate was calculated by dividing the product of urine creatinine level and urine volume by the plasma creatinine level. The left lung was fixed in 4% buffered formaldehyde and subsequently embedded in paraffin. Hematoxylin and eosin-stained lung sections were analyzed by a pathologist who was blinded to group identity. To score lung inflammation and damage, the entire lung surface was analyzed with respect to the following variables: interstitial inflammation, endothelialitis, bronchitis, edema, pleuritis, and thrombus formation, as described previously [23]. Each variable was graded on a scale of 0 to 4 (0, absent; 1, mild; 2, moderate; 3, severe; 4, very severe). The total histopathology score was expressed as the sum of the scores for all variables.

**Bacterial growth**

Undiluted blood (100 μL) and serial 10-fold dilution of BALF, homogenized lung, liver and spleen were plated on blood agar plates and incubated overnight at 37°C with 5% CO2. The number of cfu’s was counted the next day.
Induced hypothermia is protective in a rat model of pneumococcal pneumonia, associated with increased ATP availability and turnover.

Mitochondrial isolation

Mitochondria were isolated from the liver and right calf muscle (gastrocnemius and soleus muscle) by a differential centrifugation technique. Approximately ¼ of the liver or gastrocnemius muscle was excised and submerged in isolation buffer (200 mmol L-1 mannitol, 50 mmol L-1 sucrose, 5 mmol L-1 KH2PO4, 5 mmol L-1 3-(n-morpholino) propanesulfonic acid (MOPS), 1 mmol L-1 Ethylene glycol–bis (2-aminoethyl) ether–N,N,N′,N′-tetraacetic acid (EGTA), 0.1% bovine serum albumin (BSA), pH 7.15 adjusted with KOH) and minced into small pieces. In muscle samples, protease (Sigma-Aldrich, Steinheim, Germany) was added. The suspension was washed twice, homogenized in isolation buffer and centrifuged at 3220 g for 10 min. The pellet was resuspended in isolation buffer and centrifuged at 800 g for 10 minutes. The supernatant was centrifuged at 3220 g for 10 min. The final pellet was suspended in isolation buffer and kept on ice. All isolation steps were conducted at 4°C. Protein content was determined by the Bradford method (Bio–Rad, München, Germany).

Mitochondrial Oxygen consumption

Mitochondria (2 mg protein/ml) were suspended in respiration buffer (130 mmol/L KCl, 5 mmol/L K2HPO4, 20 mmol/L MOPS, 2.5 mmol/L EGTA, 1 μmol/L Na4P2O7, 0.1% BSA, pH 7.15 adjusted with KOH). Mitochondrial respiration was initiated by administration of complex 1 enhancers glutamate and malate (both 20mM, Aldrich, Steinheim, Germany) in a respiration chamber (System S 200A, Strathkelvin Instruments, Glasgow, Scotland). Exactly 60 seconds later, state 3 respiration was initiated by 200 μmol/L adenosine–diphosphate (ADP) injected into the respiration chamber. Respiration rates were recorded polarographically at 37°C under state 3 conditions and after complete phosphorylation of ADP to adenosine–triphosphate (ATP) (state 4 respiration). The respiratory control ratio (RCR, state 3 / state 4) was calculated as a parameter of mitochondrial coupling between respiration and oxidative phosphorylation.

ATP measurements

Samples of liver and the left calf muscle were taken using a freeze-clamp, snap-frozen and stored at -80°C. For nucleotide extraction, samples were grinded in liquid nitrogen using a ceramic mortar. Before the nitrogen liquid had evaporated, the semi-viscous tissue powder was added to 200 μl 0.4 M perchloric acid (HClO4), stirred, placed on ice for 10 minutes and centrifuged at 10000 g for 10 minutes, at 4°C. The supernatant was saved.
Induced hypothermia is protective in a rat model of pneumococcal pneumonia, associated with increased ATP availability and turnover.

The pellet containing total protein was dissolved in 1000 μl of 0.2 M NaOH and the protein content was measured with a copper-reduction method using bicinchoninic acid assay (Pierce, Rockford, USA).

Nucleotide profiles were determined by ion-exchange, using Whatman Partisphere SAX 4.6 x 125 mm column (5 μm particles) in combination with a Whatman 10 x 2.5 mm AX guard column (Clifton, NJ, USA). The buffers used were 9 mM NH4H2PO4, pH 3.5 (buffer A) and 325 mM NH4H2PO4, 500 mM KCl, pH 4.4 (buffer B). Nucleotides were eluted with a gradient from 100% buffer A to 90% buffer B in a total run time of 60 min, at a flow-rate of 1ml/min. The concentration of ATP and ADP were corrected for protein concentrations.

**Statistical analysis**

Data are presented as mean ± SD or median [IQR] according to distribution. To test groups Student’s t-test or analysis of variance (ANOVA) and Bonferroni’s post–hoc test will be used. If continuous data is not normally distributed a Kruskal–Wallis test will be used, followed by a Mann-Whitney U test. Categorical variables will be compared with the Chi-square test. A p value of < 0.05 was considered significant. Statistical analyses were carried out using GraphPad Prism version 5 (GraphPad Software inc., La Jolla, CA).

**Results**

*Inoculation with S. pneumoniae resulted in severe pneumonia and inflammation*

Pneumonia was demonstrated by an increased respiration rate, macroscopic lung infiltrates and bacterial growth in lungs and BALF (figure 1). Of the infected animals, 25% had positive blood cultures at the start of mechanical ventilation. All animals survived mechanical ventilation of 4 hours. Induced mild hypothermia resulted in a drop in heart rate in infected and healthy animals. Mean arterial pressure remained between 100 and 130 mmHg throughout the experiment and did not differ between groups. Acid-base balance remained within normal limits in all groups (table). Pneumonia resulted in a decrease in arterial oxygen tension.

Pneumonia induced pulmonary vascular leakage, indicated by an increase in BALF protein levels and lung wet weight compared to non-infected controls (figure 2), together with an increase in pulmonary cell influx and a trend for increased neutrophil influx. Pneumonia was associated with increased levels of IL-1β, IL-6 and TNFα in BALF; levels of CINC-3 were also higher with pneumonia as compared to controls, although differences did not
Induced hypothermia is protective in a rat model of pneumococcal pneumonia, associated with increased ATP availability and turnover.

Figure 1: Bacterial growth in rats infected with Streptococcus pneumoniae with induced hypothermia (P32°C) or normothermia (P). Dots represent the number of colony forming units (CFUs) in bronchoalveolar lavage fluid (BALF) (A), in lung (B), spleen (C) and liver tissue (D). Horizontal bars are means; (A) and (B) are log scales. *: P<0.05.

Table 1: Arterial blood analysis at baseline and after 4 hrs of mechanical ventilation in both hypothermia (32°C) and in normothermia (37°C), in animals infected with Streptococcus pneumoniae and healthy controls.

<table>
<thead>
<tr>
<th></th>
<th>Healthy 37°C</th>
<th>Healthy 32°C</th>
<th>Pneumonia 37°C</th>
<th>Pneumonia 32°C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time (hr)</strong></td>
<td>T = 0</td>
<td>T = 4</td>
<td>T = 0</td>
<td>T = 4</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>7.37±0.07</td>
<td>7.36±0.06</td>
<td>7.30±0.07</td>
<td>7.30±0.06</td>
</tr>
<tr>
<td><strong>pCO₂, kPa</strong></td>
<td>5.6±1.0</td>
<td>5.0±0.9</td>
<td>5.2±0.5</td>
<td>4.9±0.9</td>
</tr>
<tr>
<td><strong>pO₂, kPa</strong></td>
<td>30.6±1.9</td>
<td>30.6±4.0&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>28.8±4.6</td>
<td>30.6±2.7</td>
</tr>
<tr>
<td><strong>HCO₃, mmol/L</strong></td>
<td>38.5±2.5</td>
<td>24.3±5.0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>32.1±6.1</td>
<td>24.3±5.0&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

H; healthy, P; pneumonia.
<sup>a</sup> H(37°C) vs. H(32°C); <sup>b</sup> P(37°C) vs. P(32°C); <sup>c</sup> H(37°C) vs. P(37°C). Data are mean ± SD.
Induced hypothermia is protective in a rat model of pneumococcal pneumonia, associated with increased ATP availability and turnover.

Pneumonia resulted also in significantly increased lung injury scores (figure 4). In plasma, levels of IL-6 were increased compared to controls (figure 5). Pneumonia increased protein levels in urine and kidney wet weight. Plasma creatinine was not significantly affected (figure 6).

**Effects of induced mild hypothermia on bacterial growth**

Induced mild hypothermia was not associated with a change in bacterial growth from BALF or lung tissue when compared to normothermic controls (figure 1). Mild hypothermia was associated with fewer rats with positive blood cultures compared to normothermic rats (50 vs. 38%), albeit not reaching statistical significance. Mild hypothermia reduced bacterial dissemination to the spleen and tended to reduce dissemination to the liver compared to normothermic controls (figure 1).
Induced hypothermia is protective in a rat model of pneumococcal pneumonia, associated with increased ATP availability and turnover.

**Figure 3:** CINC-3 (A), Interleukine (IL)-1β (B), IL-6 (C) and tumour necrosis factor (TNF)-α (D) levels in bronchoalveolar lavage fluid (BALF) of rats infected with Streptococcus pneumoniae (P) and healthy controls (H) with induced hypothermia (32°C) or normothermia (37°C). Data are mean ± SD. *: P<0.05; **: P<0.01; ***: P<0.0001.

**Figure 4:** Lung Injury Score in the lung and histopathology pictures of rats infected with Streptococcus pneumoniae (P) and healthy controls (H) with induced hypothermia (32°C) or normothermia (37°C). Horizontal bars are means. **: P<0.01.

**Figure 5:** Interleukine (IL)-1β (A) and IL-6 (B) levels in plasma of rats infected with Streptococcus pneumoniae (P) and healthy controls (H) with induced hypothermia (32°C) or normothermia (37°C). Data are mean ± SD. *: P<0.05.
Induced hypothermia is protective in a rat model of pneumococcal pneumonia, associated with increased ATP availability and turn-over

Effects of induced mild hypothermia on organ injury

Hypothermia reduced BALF protein levels and cell count (figure 2) and tended to reduce the amount of neutrophils during pneumonia (figure 2). No differences were seen in lung wet weights between groups. Hypothermia reduced BALF IL-1β levels, but not levels of IL-6, CINC-3 and TNF-α. Induced hypothermia showed a trend to lower urine protein levels, but this was not statistically significant. Kidney wet weight was not affected by hypothermia. Plasma creatinine level was reduced by hypothermia (figure 6), with unchanged glomerular filtration rates compared to normothermic controls.

Effects of induced mild hypothermia on mitochondrial respiration and ATP availability during pneumonia

During pneumonia, liver mitochondrial oxygen consumption, as measured by state 3 respiration, was markedly decreased compared to healthy controls, whereas state 4 respiration and respiration control index (RCI) were unaltered (figure 7). The decrease in oxygen consumption was accompanied by a decrease in the amount of ATP in the liver, while ATP/ADP ratio remained low (figure 8). Induced mild hypothermia reversed the fall in oxygen consumption of liver mitochondria observed during pneumosepsis, without an effect on state 4 or RCI (figure 7), with a concomitant reversal of the fall in ATP content in liver, while ATP/ADP ratio remained low during induced hypothermia (figure 8).

Figure 6: Levels of protein in urine (A), kidney wet weight (B), plasma creatinine (C) and glomerular filtration rate (D) of rats infected with Streptococcus pneumoniae (P) and healthy controls (H) with induced hypothermia (32°C) or normothermia (37°C). Data are mean ± SD. *: P<0.05.
Induced hypothermia is protective in a rat model of pneumococcal pneumonia, associated with increased ATP availability and turnover.

In muscle, pneumosepsis decreased mitochondrial state 3 respiration, comparable to the effect on liver mitochondria, but this was accompanied by an increase in ATP levels and ATP/ADP ratio compared to healthy controls, suggesting increased ATP availability, but no conversion to ADP. This may not have been due to uncoupling of mitochondria, as pneumonia did not affect RCI. Induced mild hypothermia further increased muscle ATP levels in all groups. In pneumosepsis, high ATP levels were accompanied by a decrease in ATP/ADP ratio, suggesting increased ATP availability and ATP conversion to ADP. This was not observed in muscle from healthy controls.

**Discussion**

In this model of pneumococcal pneumosepsis in rats, induced mild hypothermia reduced bacterial dissemination and parameters of lung injury, possibly by preserving mitochondrial oxygen consumption, thereby improving ATP availability and maintaining ATP conversion to ADP. Hypothermia had a modest effect on kidney function.

We used an infectious model to study effects of hypothermia, which is relevant for the ICU, as hypothermia is induced for prolonged time periods in mechanically ventilated patients.
survivors of a cardiac arrest. These critically ill patients frequently have infectious complications, e.g. pneumonia due to aspiration or due to exogenous infections [24]. The effect of hypothermia on host response to infections is not known. It is generally feared that hypothermia may increase the risk of acquiring or aggravating infections, due to an impairment of the immune system [17]. Although most studies do not show an increase in infections [8, 11, 25], prolonged hypothermia (>24h) was found to be associated with an increase in inflammatory parameters [17, 26] as well as with an increase in infections [11, 17, 27]. We found however that hypothermia does not increase local bacterial growth. Induced mild hypothermia even reduced bacterial dissemination.
Induced hypothermia is protective in a rat model of pneumococcal pneumonia, associated with increased ATP availability and turnover to the spleen. Although statistical significance was not noted for dissemination to other organs, the consistency of findings may suggest a trend for local bacterial containment.

The mechanism behind these results could be prevention of translocation via the pulmonary capillaries due to a decrease in endothelial cell damage or pulmonary vascular permeability. Alternatively, bacterial replication may be reduced during hypothermia. Our results suggest that short term hypothermia does not aggravate severe infection. It can not be excluded however, that bacterial growth may show a rebound after discontinuation of hypothermia as only a short period of hypothermia was studied. Thereby, results should be interpreted within the limits of our short-term model.

Hypothermia reduced pulmonary inflammation during pneumonia, which was associated with less cell influx and reduced protein leakage into the bronchoalveolar compartment. These findings are in line with studies of the effect of induced hypothermia in sterile models of acute lung injury due to smoke inhalation or endotoxemia, in which it was found that hypothermia reduced neutrophil-influx, delayed the pro-inflammatory response and may reduce neutrophil-mediated endothelial damage and prevent leakage [12-15]. Of note, hypothermia also reduced cytokine levels in healthy animals, suggesting that mechanical ventilation may have contributed to an inflammatory reaction. In our study, induced hypothermia may reduce interstitial kidney tissue damage, as our results show a trend towards less protein leakage. Thereby, glomerular filtration rate may have improved [28], consistent with a reduction in plasma creatinine levels. Of note, not all parameters of organ injury were affected. Thereby, results suggest, but do not prove, that hypothermia protects against sepsis-induced organ failure. Unfortunately, survival experiments which may have underlined a protective effect of hypothermia were not feasible in this lethal model. Whether prolonged courses of hypothermia would confer protection more clearly remains to be established, as is the optimal cooling temperature by which protection is established with minimal adverse effects.

The mechanism of the observed effects of hypothermia may be a change in mitochondrial function. Most studies report a drop in mitochondrial oxygen consumption during sepsis [3, 6, 29], consistent with our findings [2, 3]. There are two contrasting propositions about decreased mitochondrial function. The first hypothesis is that mitochondrial ‘shutdown’ may be an adaptive response. In this view, hypothermia may result in decreased ATP demands, thereby preventing the levels of ATP from falling below a crucial threshold. In
favour of this view, hypothermia reduced pCO₂ levels in our study, with lower ATP/ADP ratios, which may imply a lower rate of ATP turnover. The second hypothesis suggests that decreased respiration and low energetic status is due to inflammatory damage to the mitochondria. In our study, hypothermia increased oxygen consumption, with concomitant increased ATP levels, while ATP/ADP ratio was maintained or even decreased, indicative of a higher conversion of ATP into ADP, whereas uncoupling of respiration to ATP production may be less likely [30].

We suggest that hypothermia is not associated with reduced ATP demand in this study, but may have limited energetic failure by increasing ATP availability, which does not support the theory of an adaptive response but rather may be the result of less mitochondrial damage. Of note, the centrifugation technique to isolate mitochondria may have favoured isolation of intact mitochondria while excluding damaged mitochondria, which may have contributed to lower ATP ratios in pneumonia. However, slow centrifugation speed can not account for the differences between hypo- and normothermia in the diseased animals.

Mitochondria from a central (liver) and distant (muscle) organ showed differential results. In muscle, we found an increase in ATP levels during pneumonia. This is in contrast with a previous study in sepsis patients in which a decrease in ATP in skeletal muscle was observed [7]. A conceivable explanation might be a generally very high concentration of ATP in rat muscle cells during inflammation [31]. Hypothermia resulted in high ATP levels in the muscle, in contrast to the effect on liver mitochondria. This might suggest that mitochondrial function is differentially regulated in various organs and even in different muscles. Of note, ATP measurements in this study do not distinguish between extracellular ATP from damaged mitochondria and cytosolic ATP. However, during infection, the effect of hypothermia was the same in liver as in muscle mitochondria, resulting in an increase in ATP and low ATP/ADP ratio.

**Conclusion**

Induced mild hypothermia reduced bacterial dissemination in a rat model of pneumococcal pneumosepsis and reduced lung injury, with a concomitant reversal of a fall in ATP levels and preserved mitochondrial oxidative phosphorylation. Results are encouraging in further exploring the possibilities of induced hypothermia as a strategy to control sepsis-related organ injury.
Induced hypothermia is protective in a rat model of pneumococcal pneumonia, associated with increased ATP availability and turnover.

Reference list

Induced hypothermia is protective in a rat model of pneumococcal pneumonia, associated with increased ATP availability and turnover.


Induced hypothermia reduces circulating mitochondrial DNA in cardiac arrest patients

Hamid Aslami; Charlotte J.P. Beurskens; Anita M. Tuip – de Boer; Nicole P. Juffermans

Submitted
Abstract

**Objective:** to determine the effect of hypothermia on circulating mitochondrial (mt) DNA in patients after an out-of-hospital cardiac arrest.

**Design:** Predefined post-hoc analysis of patients included in a multicenter randomized trial on the effect of temperature control management on outcome (the Target Temperature Management trial).

**Setting:** medical–surgical intensive care unit of a teaching hospital in Amsterdam, the Netherlands.

**Subjects:** Patients after an out–of–hospital cardiac arrest, with a Glasgow coma scale <8. Exclusion criteria were pregnancy, more than 4 hours between return of circulation and screening, cardiogenic shock and spontaneous hypothermia on admission.

**Intervention:** temperature control management (body temperature of 33°C or 36°C) during 24 hours.

**Measurements and main results:** Blood was drawn for mtDNA measurements at baseline, 24 hours later and when body temperature reached 36°C in the 33°C group (n=10). In the 36°C group (n=6), blood was withdrawn at the same time intervals. Healthy volunteers served as controls (n=2). Circulating levels of mitochondrial subunits COX3, NADH1, NADH2 and cytochrome–b were elevated in patients with cardiac arrest at all time points compared to healthy controls. While 33°C resulted in a significant decrease in mtDNA levels, no alteration was observed in the 36°C group.

**Conclusion:** Treatment with a target temperature of 33°C reduced circulating mtDNA levels in cardiac arrest patients. Release of circulating mtDNA from damaged mitochondria may be a mechanism of the beneficial effect of induced hypothermia inflammatory response following cardiac arrest observed in experimental models.
Introduction

Induced hypothermia is thought to limit tissue injury following ischemia-reperfusion and is applied in operative procedures in which the aorta needs to be clamped (2). The mechanisms of action are not fully known, but may include an effect on mitochondria. When oxygen delivery to tissue is compromised, apoptotic pathways are activated, leading to necrosis. Mitochondrial (mt) DNA released from necrotic tissue was found to drive the systemic inflammatory response syndrome (SIRS) in patients with tissue damage due to traumatic injury (3). In cardiac arrest patients, mtDNA is possible predictor of adverse outcome (4,5) and is thought to represent a marker for hypoxic tissue damage.

Notably, in animal models of ischemia-reperfusion injury, hypothermia was recently found to reduce the production of reactive oxygen species, with a concomitant improvement in mitochondrial function in the heart (6) and in the brain (7). These findings suggest a central role for mitochondria in the pathophysiology of organ damage after cardiac arrest and may point towards the mechanism of action of the beneficial effect of hypothermia in ischemia-reperfusion injury.

In the present study, we investigated the effect of induced hypothermia on circulating cell-free mtDNA in a post hoc single center analysis of a subgroup of patients randomized to a trial controlling for body temperature (8).

Methods

Included patients were part of the Target Temperature Management (TTM) trial, which is an open-label multicenter trial in which patients were randomized to 33°C or 36°C following an out-of-hospital cardiac arrest, assessor and analyist blinded (8). Analysis was planned prior to disclosure of results of the TTM trial. Eligible patients were > 18 years of age, had suffered an out-of-hospital witnessed cardiac arrest due to a myocardial cause, had regained spontaneous circulation (ROSC) and were admitted to the medical-surgical intensive care unit of a university hospital in Amsterdam, the Netherlands, with a Glasgow coma score ≤ 8. Exclusion criteria were pregnancy, more than 4 hours between ROSC and screening, cardiogenic shock and spontaneous hypothermia of < 30°C on admission. For sampling, patients were consecutively included between October 2011 and October 2012 and randomized using a web-based tool. Hypothermia was induced by intravenous infusion of ice cold Ringers lactate (4°C, 100 ml/min) and with a cold mattress.
Hypothermia was maintained during 24 hours, after which the patients were actively rewarmed. Controls were kept at 36°C. Temperature was measured using a bladder thermometer. Sedation was maintained using propofol and opiates. Shivering was treated with neuromuscular blocking agents. Other treatment consisted of either thrombosis prophylaxis or anticoagulant medication as deemed appropriate. Norepinephrine was infused to maintain a minimum mean arterial pressure of 65 mmHg. All patients received selective oropharyngeal decontamination. Blood from an arterial catheter was drawn at baseline, 24 hours later and when body temperature had returned to 36°C in the 33°C group. In the 36°C group, blood was withdrawn at the same time interval. Blood was also drawn from healthy volunteers (n=2) of 30–34 years of age. The National Intensive Care Evaluation (NICE) minimal dataset prospectively collects data to calculate the Acute Physiology and Chronic Health Evaluation (APACHE II) score. Other data were collected from the electronic patient data monitoring system. Blood was centrifuged at 600 g, during 10 minutes at 4°C and supernatant was stored at –80 for further analysis.

DNA isolation and PCR

Total DNA was isolated in 200µL plasma with Qiamp DNA kit (Qiagen, Venlo, Netherlands). DNA levels were measured by spectrophotometer and stored at –80. The expression of COX3 (forward: ATGACCCACCAATCACATGC, reverse: ATCACATGGCTAGGCCGGAG), NADH1 (forward: ATACCCATGGCCAACCTCCT, reverse: GGGCCTTTGCGTAGTTGTAT), NADH2 (forward: CTCACATGACAAAAACTAGCCCCCA, reverse: TCCACCTCAACTGCTGCTATGA) and cytochrome–B (forward: ATGACCCCAATACGCAAAAT, reverse: CGAAGTTTCATCATGCGGAG) were analyzed by reverse–transcription–polymerase chain reactions (RT–PCRs) using lightCycler®SYBR green I master mix (Roche, Mijdrecht, the Netherlands) and measured in a LightCycler 480 (Roche) apparatus using the following conditions: 5 min 95° C hot–start, followed by 40 cycles of amplification (95 °C for 10 seconds, 60 °C for 5 seconds, 72 °C for 15 seconds). Standard curves were constructed on serial dilutions of a concentrated complementary DNA sample for quantifications and presented as arbitrary unit (AU).

Statistical analysis

Data are expressed as mean with SD depending on data distribution. Measurements within the groups were analysed using paired t–test and between hypothermia and normothermia using a t–test or a Mann Whitney–u test depending on data distribution. A p value of < 0.05 was considered statistically significant (Graphpad Prism 5, CA, USA).
**Results**

In total 16 patients were included in this study, of whom 10 were enrolled in the 33°C group while in the remaining 6 patients, body temperature was kept at 36°C. There were no differences in demographic data between groups (table), nor in hemodynamic parameters, APACHE score, cumulative norepinephrine infusion and time to ROSC. Cumulative fluid balance tended to be higher in the 36°C group. At ICU discharge, no difference in mortality was observed between the groups. None of the included patients received blood transfusions before and during the study.

*Table 1: Characteristics of patients following cardiac arrest*

<table>
<thead>
<tr>
<th></th>
<th>36°C group (n=6)</th>
<th>33°C group (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>58 ± 12</td>
<td>65 ± 10</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>77 ± 6</td>
<td>89 ± 20</td>
</tr>
<tr>
<td>Sex</td>
<td>83% male</td>
<td>80% male</td>
</tr>
<tr>
<td>APACHE II score</td>
<td>23.8 ± 7.0</td>
<td>19.3 ± 8.9</td>
</tr>
<tr>
<td>Cumulative dose of norepinephrine (g/1st day)</td>
<td>7.6 ± 4.4</td>
<td>8.4 ± 7.2</td>
</tr>
<tr>
<td>Time to ROSC (minutes)</td>
<td>13.8 ± 4</td>
<td>13.5 ± 3</td>
</tr>
<tr>
<td>Fluid balance (liters/1st day)</td>
<td>3.2 ± 0.9</td>
<td>2.4 ± 1.8</td>
</tr>
<tr>
<td>PaO2/FiO2 ratio on admission</td>
<td>268 ± 71</td>
<td>245 ± 63</td>
</tr>
<tr>
<td>Alive on ICU discharge</td>
<td>67%</td>
<td>70%</td>
</tr>
</tbody>
</table>

Data are mean ± SD or percentages

Circulating levels of mtDNA were increased in patients after cardiac arrest compared to healthy controls (figure 1). There were no baseline differences between the 33°C and 36°C patients. After 24 hours, treatment with 33°C resulted in a relative reduction in levels of COX3 and NADH1, NADH2 compared to baseline (figure 2, p< 0.05), whereas
Induced hypothermia reduces circulating mitochondrial DNA in cardiac arrest patients. Levels in the 36°C group did not change. Levels of cytochrome–B were not decreased at 24 hours compared to baseline in both groups. At the end of the protocol, when 33°C patients had regained normal body temperature and in the 36°C patients, cytochrome–B levels were lower compared to baseline in both groups, with significantly lower levels in the patients treated with 33°C compared to patients in whom body temperature was kept at 36°C.

Discussion

In this small study, hypothermia reduced circulating mtDNA fragments in patients with out of hospital cardiac arrest. In these patients, SIRS with organ failure can develop in up to 30%, which can be clinically indistinguishable from bacterial sepsis (9). Mitochondria are thought to be descendants of bacteria, engulfed and locked up by host cells. Thereby, when outside the cell, parts of the mitochondrial molecules which resemble bacterial molecular patterns, including mtDNA, may trigger an immune response by binding to toll like receptors (3).

Elevated circulating levels of mtDNA have been found before in cardiac arrest patients, which correlated with outcome (4;5). Here, we confirm that mtDNA levels are increased in cardiac arrest patients. We expand these findings by showing that induced hypothermia reduces levels of circulating mtDNA. The TTM study, in which patients are randomized to a controlled temperature management of 33°C or 36°C (8), provides the unique study design to evaluate the effects of hypothermia on host response. We noted a gradual decline in levels of all markers of mtDNA following cardiac arrest, in particular of NADH2.
and cytochrome b. However, the differences between groups at 24 hours suggest that induced hypothermia exerts effects outside the scope of time. We propose that reduction of mtDNA levels may be a mechanism of the observed beneficial effects of induced hypothermia in inhibiting the inflammatory response as found before (2;6). Results also demonstrate the crucial role of mitochondria in the pathogenesis of hypoxic tissue injury. Of note, the TTM trial shows that hypothermia does not alter neurological outcome between the two groups. Thereby, the level of mtDNA is unlikely to be an important determinant of neurological outcome following cardiac arrest.

We did not find differences in baseline levels of possible confounders. However, numbers are small, therefore we cannot exclude the possibility of confounders.

In conclusion, in relatively small study hypothermia reduced circulating levels of mtDNA in patients with an out–of–hospital cardiac arrest. Prevention of mitochondrial damage and release of mtDNA may play a role in the beneficial effect of hypothermia during ischemia-reperfusion injury.
Induced hypothermia reduces circulating mitochondrial DNA in cardiac arrest patients.


Induced hypothermia improves ventilation in mechanically ventilated patients

Charlotte J. Beurskens; Janneke Horn; Margreeth B. Vroom; Marcus J. Schultz; Nicole P. Juffermans

Submitted
Abstract

Objective: Previous retrospective studies suggest a reduction in minute ventilation needed for \( \mathrm{CO}_2 \) elimination, when induced hypothermia (32-34°C) is applied in the Intensive Care Units (ICU). We hypothesized that induced hypothermia allows for improved gas exchange while maintaining protective ventilation settings in mechanically ventilated ICU patients.

Design: prospective observational cohort study

Setting: a mixed surgical- medical ICU of a university hospital

Patients: 56 patients admitted to the ICU and treated with induced hypothermia (32-34°C) for 24 hours and observed for 48 hours during protective mechanical ventilation

Interventions: Arterial blood gas were drawn three times, at the start and end of the hypothermic phase and after reaching normo-temperature. At the same time points respiratory data were collected from the electronic patient files. Dead space ventilation was calculated as \((\mathrm{PaCO}_2 - \mathrm{etCO}_2)/\mathrm{PaCO}_2\).

Measurements and main results: During hypothermia, median minute volume, tidal volume and respiratory rate did not change, while levels of \( \mathrm{PaCO}_2 \) levels and exhaled \( \mathrm{CO}_2 \) (etCO\(_2\)) decreased. Dead space ventilation, static compliance and \( P/F \) ratio also remained unchanged during hypothermia. Applied PEEP levels and plateau pressures could be decreased. After rewarming, \( P/F \) ratio and dead space ventilation decreased compared to end of hypothermic period, at unchanged minute volume, PEEP and \( \mathrm{PaCO}_2 \) levels. Static compliance, plateau pressures, etCO\(_2\) levels increased after rewarming.

Conclusions: Induced hypothermia improved ventilation in mechanically ventilated patients while maintaining lung protective ventilation settings and allowed for lower driving pressures and PEEP levels, providing a rationale to study whether induced hypothermia is a therapeutic option in patients in whom protective ventilation is hampered by severe respiratory acidosis.
Introduction

Induced hypothermia (32-34°C) is applied as a therapeutic intervention in Intensive Care Units (ICU) and in the operating room. The neurologic benefits are well described [1, 2], but clinical data on the effect of hypothermia on lung mechanics and gas exchange during mechanical ventilation are scarce. Retrospective data in ICU patients suggest a beneficial effect of hypothermia on gas exchange, with a decline of the partial CO₂ tension (PaCO₂), at unchanged minute ventilation [3], which may possibly be due to lowered CO₂ production.

Thereby, hypothermia may allow for reducing intensity of mechanical ventilation, with a reduction in applied driving pressures or tidal volumes needed for adequate ventilation. As both high pressure levels and high tidal volumes are associated with mortality during mechanical ventilation [4, 5], induced therapeutic hypothermia may be beneficial when protective mechanical ventilation is hampered by the need for application of potentially injurious ventilation settings to avoid severe acidosis. Effects of hypothermia on lung mechanics have not been described. In this study, we investigated the effect of induced hypothermia on lung mechanics and gas exchange in patients admitted after cardiac arrest. We hypothesized that induced hypothermia (32-34°C) allows for improved gas exchange while maintaining protective ventilation settings in mechanically ventilated ICU patients.

Methods

This prospective observational cohort study was approved by the local medical ethics committee of the Academic Medical Center, University of Amsterdam, the Netherlands and conducted in concordance with the principles of the declaration of Helsinki and good clinical practice. From January 2011 until October 2012, 56 cardiac arrest patients were included. All patients were admitted to a mixed surgical-medical intensive care unit (ICU) of a tertiary referral center in Amsterdam, the Netherlands after cardiopulmonary resuscitation and treated with induced hypothermia (32-34°C) for 24 hours. Patients were prospectively followed for 48 hours during which they were ventilated with tidal volumes of 6 ml/kg in a pressure controlled mode, which was switched to pressure support ventilation mode after stop of sedation. Ventilation was targeted to maintain normopH (7.35-7.45). Exclusion criteria were the presence of pulmonary fibrosis or inability to complete 24 hours of hypothermia according to treating physician. Other standard of care included administration of vasopressor therapy to target a blood pressure of at least
65 mmHg. Patients received either anticoagulant medication as deemed appropriate or thrombosis prophylaxis. Patients were not fed during the hypothermic phase. In all patients an arterial blood gas was taken (alpha stat) and ventilation data were collected from the electronic patient files at the start (T=1) and end (T=2) of the hypothermic phase and after reaching normotemperature (T=3). Dead space ventilation was calculated as (PaCO₂ - etCO₂)/PaCO₂. Static compliance was calculated as tidal volume (mL) divided by plateau pressure minus peak end expiratory pressure (PEEP). When plateau pressures were not scored, peak pressures were used. Other data were collected from the patient data monitoring system or the Dutch National Intensive Care Evaluation [6]. Data were analyzed by a Friedman and Wilcoxon signed rank test, since the data are not normally distributed. Data are expressed as median ± range. P<0.05 was considered significant.

Table 1: Baseline characteristics of patients admitted at ICU after cardiac arrest.

<table>
<thead>
<tr>
<th></th>
<th>n=56</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (Male/Female)</td>
<td>41/15</td>
</tr>
<tr>
<td>Age (years)</td>
<td>62 ± 14</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>177 ± 9</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>84 ± 19</td>
</tr>
<tr>
<td>Maximal leukocyte count</td>
<td>16.1 ± 8.1</td>
</tr>
<tr>
<td>SAPS II score</td>
<td>51 ± 16</td>
</tr>
<tr>
<td>APACHE III score</td>
<td>76 ± 33</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Previous medical history</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Chronic cardiovascular insufficiency</td>
<td>7 (13%)</td>
</tr>
<tr>
<td>Arrhythmia</td>
<td>20 (36%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>9 (16%)</td>
</tr>
<tr>
<td>Chronic respiratory failure</td>
<td>4 (7%)</td>
</tr>
</tbody>
</table>

| Lowest mean blood pressure during first 24 hours of ICU admittance (mmHg) | 59 ± 9 |
| Number of patients requiring vasoactive medication during first 24 hours of ICU admittance | 48 (86%) |
| Number of patients with confirmed infection during first 24 hours of ICU admittance | 7 (13%) |

Data expressed as mean ± SD or percentages
Results

Characteristics of patients are shown in table 1. A substantial part of patients required vasopressor therapy at the start of induced hypothermia. During hypothermia, median minute volume remained unchanged compared to baseline (10.5 ± 17.3 to 11.0 ± 20.1 L; P=0.9, figure 1), with unchanged tidal volume (5.5 ± 9.6 to 5.4 ± 9.8 mL/kg; P=0.23) and respiratory rate (24 ± 29 to 25 ± 28 breath/min; P=0.5), while levels of PaCO₂ levels (5.5 ± 5.8 to 4.8 ± 4.0 kPa; P=0.001) and exhaled CO₂ (etCO₂) (3.8 ± 4.1 to 3.6 ± 4.6 kPa; P=0.09) decreased. Dead space ventilation did not change during hypothermia compared to baseline (28.3 ± 53.6 to 28.8 ± 63.43 kPa; P=0.53). Applied PEEP levels could be decreased (7 ± 15 to 5 ± 13 cmH₂O; P=0.0001, figure 2), while P/F ratio remained unchanged (280 ± 754 to 268 ± 370 mmHg; P=0.6). Plateau pressures could be decreased (21 ± 31 to 21 ± 24 cmH₂O; P=0.039), whereas static compliance remained the same during hypothermia (33 ± 69 to 29 ± 89 cmH₂O; P=0.67).

After rewarming, P/F ratio decreased compared to end of hypothermic period (268 ± 370 to 207 ± 340 mmHg; P=0.0001) at unchanged PEEP levels (5 ± 13 to 5 ± 12 cmH₂O; P=0.4).
Induced hypothermia improves ventilation in mechanically ventilated patients

Static compliance increased after rewarming (29 ± 89 to 46 ± 126 cmH₂O; P=0.0001), as well as plateau pressures (21 ± 24 to 19 ± 28 cmH₂O; P=0.004). After rewarming, some patients were weaned from ventilation, resulting in less data points for at T=2. Levels of PaCO₂ remained unchanged compared to end of hypothermic period (4.8 ± 4.0 to 4.8 ± 3.3 kPa; P=0.71), while etCO₂ levels increased (3.6 ± 4.3 to 4.4 ± 3.3 kPa; P=0.0001). Dead space ventilation decreased (28.8 ± 63.4 to 12.9 ± 40.3 kPa; P=0.0001), with an associated increase in tidal volume (5.4 ± 9.8 to 6.3 ± 12.8 mL/kg; P=0.0001; data not shown) and decreased respiratory rate (25 ± 28 to 23 ± 33 breath/min; P=0.038; data not shown). Minute volume remained unchanged (11.0 ± 20.1 to 12.0 ± 22.8 L; P=0.06).

**Discussion**

Therapeutic induced hypothermia for 24 hours improved ventilation in mechanically ventilated patients while maintaining lung protective ventilation settings and allowing for lower PEEP levels and peak pressures. Induced hypothermia reduced PaCO₂ levels, at unchanged minute ventilation. As hemodynamic parameters did not change over time, suggesting pulmonary perfusion remained unchanged, reduced CO₂ levels may be due

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*Figure 2: Respiratory parameters in patients with induced hypothermia (32-34°C) for 24 hours at the start (T=1) and end (T=2) of the hypothermic phase and after reaching normotemperature (T=3). (A) PEEP (cmH₂O); (B) P/F ratio (mmHg); (C) plateau pressures (cmH₂O) and (D) static compliance. Data are expressed by median and range. *P<0.05; ***P<0.0001.*
to reduced CO₂ production during hypothermia. This improvement in ventilation during hypothermia is in line with retrospective data [3]. Thereby, driving pressures needed to generate a given minute volume ventilation were slightly lowered during hypothermia. The reduction in both PEEP levels and plateau pressures during hypothermia may be beneficial, since driving pressures are independently associated with mortality in patients with severe respiratory failure such as in ARDS [7-9]. Dead space ventilation did not vary during hypothermia, but did show a decrease after rewarming. This is mostly likely caused by the switch from controlled ventilation to spontaneous breathing [10]. Oxygenation, expressed as P/F ratio, declined after rewarming, which may be due to the fact that blood gases were measured alpha stat. Alternatively, the switch to spontaneous ventilation may have resulted in decreased oxygenation.

A limitation to our results is that it is not possible to differentiate between effects occurring over time following a cardiac arrest and induced hypothermia. Cardiopulmonary resuscitation after cardiac arrest results in an inflammatory state [11], which may have affected a change in ventilatory parameters over time. Nevertheless, ventilation settings changed after discontinuation of hypothermia, suggesting a causal effect. Furthermore, all patients were sedated, which also lower metabolism and CO₂ production. Thereby, it is not possible to dissect whether sedation or induced hypothermia contributed most to observed results. Whether induced hypothermia is beneficial by decreasing driving pressures and improving ventilation in patients in whom severe respiratory acidosis occurs as a result of respiratory failure remains to be determined.

Conclusions

Hypothermia improves ventilation in mechanically ventilated patients and allows for lower driving pressures and PEEP levels while maintaining lung protective ventilation settings. Results provide a rationale to study whether induced hypothermia is a therapeutic option in patients in whom protective ventilation is hampered by severe respiratory acidosis.
Reference list


Cardiac arrest patients have an impaired immune response, which is not influenced by induced hypothermia

Charlotte J. Beurskens; Janneke Horn; Anita M. Tuip – de Boer; Marcus J. Schultz; Ester M.M. van Leeuwen; Margreeth B. Vroom; Nicole P. Juffermans

Submitted
Abstract

**Purpose:** To study the effect of induced hypothermia on immune response after cardiac arrest.

**Methods:** Prospective observational cohort study in a mixed surgical-medical intensive care unit (ICU). Patients admitted at the ICU after surviving cardiac arrest were included and during 24 hours body temperature was strictly regulated at 33°C or 36°C. Blood was drawn at three time points: after reaching target temperature, at the end of the target temperature protocol and after rewarming to 37°C. Plasma cytokine levels and response of blood leucocytes to stimulation with Toll-like receptor (TLR) ligands lipopolysaccharide (LPS) from Gram-negative bacteria and lipoteichoic acid (LTA) from Gram-positive bacteria were measured. Also, monocyte HLA-DR expression was determined.

**Results:** Compared to healthy controls, cardiac arrest patients kept at 36°C had increased plasma cytokines levels, which was not apparent in patients kept at 33°C. Immune response to TLR ligands in patients after cardiac arrest was generally reduced, associated with lower HLA-DR expression. Patients kept at 33°C had preserved ability of immune cells to respond to LPS and LTA compared to patients kept at 36°C. These differences disappeared over time. HLA-DR expression did not differ between 33°C and 36°C.

**Conclusions:** Patients after cardiac arrest have a modest systemic inflammatory response compared to healthy controls, associated with lower HLA-DR expression and attenuated immune response to Gram-negative and Gram-positive antigens, the latter indicative of an impaired immune response to bacteria. Patients with a body temperature of 33°C did not differ from patients with a body temperature of 36°C, suggesting induced hypothermia does not affect immune response.
Introduction

Induced hypothermia is applied clinically to reduce ischemia-reperfusion injury during operative procedures and following cardiac arrest [1-3]. In cardiopulmonary surgery, hypothermia is associated with improved neurological outcome [4, 5]. Also, avoiding hyperthermia by controlling body temperature is associated with a favourable neurologic function in survivors of cardiac arrest [6], as well as with earlier shock reversal in septic shock patients [7]. The underlying mechanism of mitigating harmful effects of ischemia-reperfusion by controlling body temperature is proposed to be inhibition of an exaggerated systemic inflammatory response syndrome (SIRS). In a pig model of cardiac arrest, hypothermia reduced expression of pro-inflammatory cytokines within the brain [8]. Also in other models of hyper-inflammatory conditions, hypothermia reduced organ failure associated with a decrease of the inflammatory response [9-15]. Thereby, hypothermia may mitigate harm caused by an ‘overshoot’ of a systemic inflammatory response.

On the other hand, an adequate adaptive host immune response to pathogens and infection is crucial [16] and fever is considered an important factor for optimal antimicrobial host defence [17]. Hypothermia inhibits immune response [18], with delayed generation of pro-inflammatory cytokines by monocytes [19] and reduction of neutrophil and monocyte migration [20, 21]. The consequence of inhibiting host immune response by induced hypothermia, may be a higher infection risk. In particular, patients shortly after a cardiac arrest suffer from a dysregulated production of cytokines and may therefore be susceptible to nosocomial infection [22]. Two randomized trials in cardiac arrest patients reported no increased overall infection rates associated with hypothermia [1, 23], although a trend towards more infection could be noted [23]. In addition, prolonged hypothermia (for more than 48 hours) did not increase risk of infection in patients with brain injury, annotated all patients received selective decontamination of the digestive tract [24]. However, a recent systematic review in patients enrolled in randomized controlled clinical trials of therapeutic hypothermia for any indication, showed an association of hypothermia with increased prevalence of pneumonia and sepsis, although overall infection rate was not affected [25].

Patient studies describing the effect of induced hypothermia specifically on the innate immune response are scarce and studies lack an adequate control group [26]. Recently, the Temperature Target Management (TTM) trial was concluded, in which cardiac arrest
patients were randomized between maintaining body temperature at 33°C or at 36°C [23]. In this predefined sub study of the TTM trial, we investigated the effect of induced hypothermia on the innate immune response to toll like receptors (TLRs) ligands. In humans, TLRs are critical in the first host immune response to pathogens by mediating cytokine secretion [27]. In addition, we measured human leukocyte antigen-DR (HLA-DR) surface expression on monocytes, as low HLA-DR expression is associated with secondary infection and mortality in critically ill patients [28, 29] and hypothermia was shown to reduce HLA-DR expression in vitro [30]. We hypothesized that SIRS is reduced in patients with a target temperature of 33°C compared to patients with a target temperature of 36°C, but that hypothermia does not affect the immune response to pathogens.

Methods

Patient inclusion

The study was approved by the local medical ethics committee of the Academic Medical Center, University of Amsterdam, the Netherlands and conducted in concordance with the principles of the declaration of Helsinki and good clinical practice. From January 2011 until October 2012, adult patients admitted to the mixed surgical-medical intensive care unit (ICU) of a tertiary referral center in Amsterdam, the Netherlands after out-of-hospital cardiac arrest with a Glasgow Coma Score <8 and treated with therapeutic hypothermia (33°C) for 24 hours, were included in our study after their relatives gave informed consent. From March 2011, our center started enrollment in the TTM trial, patients who enrolled in the TTM trial were only included for our sub study after additional informed consent was obtained from the relatives. Exclusion criteria were pregnancy, out-of-hospital cardiac arrest of presumed non-cardiac cause, in-hospital cardiac arrest, known bleeding diathesis, suspected or confirmed acute intracranial bleeding, suspected or confirmed acute stroke, temperature on admission <30°C, un-witnessed asystole, persistent cardiogenic shock, known limitations in therapy, known disease making 180 day survival unlikely, known pre-arrest cerebral performance category 3 or 4, >240 minutes from return of spontaneous circulation (ROSC) to randomisation [23]. Patients included prior to the TTM trial (n=8) and during the TTM trial (n=12, 3 in the 33°C group and 9 in the 36°C group) did not differ in patients characteristics, Acute Physiology and Chronic Health Evaluation (APACHE) III, Simplified Acute Physiology Score (SAPS) II score, time to ROSC and cause of cardiac arrest (data not shown). Healthy volunteers were recruited and included after informed consent for a single blood donation.
Cardiac arrest patients have an impaired immune response, which is not influenced by induced hypothermia.

Study procedure

Patients included in our study received standard post-resuscitation care according to the current best practice or the post-resuscitation protocol of the TTM trial [23], including 24 hours of target temperature management to achieve a core body temperature of either 33°C or 36°C with the use of ice-cold saline (maximum 1 L) and a cooling device (Blanket roll, Cincinatti Sub Zero, Cincinatti). Temperature was measured using a bladder catheter. All patients were sedated with propofol, mechanically ventilated in a pressure controlled mode and selective digestive tract decontamination was administered. Patients received either anticoagulant therapy as deemed appropriate or thrombosis prophylaxis. Patients were not fed. Blood was drawn at three time points: after reaching target temperature (33°C or 36°C; T=1), at the end of the target temperature protocol (T=2) and after reaching 37°C (T=3). Healthy volunteers donated only once.

Measurements & Data collection

Data from the patient data monitoring system were collected, including previous medical history, age, gender, weight, length, maximal leukocyte count, APACHE III, SAPS II score, as registered in the Dutch National Intensive Care Evaluation [31]. Serum levels of Interleukin (IL)–1β, IL–1RA, IL–8, IL–10, Macrophage Inflammatory Proteins (MIP)-1, Monocyte Chemotactic Protein (MCP)–1 and soluble CD40 ligand were determined by Luminex, according to instructions of the manufacturer (Merck Millipore Chemicals BV; Amsterdam; the Netherlands). Serum levels of IL–6 and Tumor Necrosis Factor (TNF)-α were determined by ELISA, according to instructions of the manufacturer (R&D Systems; Abingdon, United Kingdom).

Whole blood stimulation

The response of blood leucocytes to stimulation with TLR ligands was determined in a whole blood stimulation system. Immediately after drawing, blood was diluted 1:1 with RPMI and stimulated with LPS (100 ng/ml; Sigma Aldrich, Steinheim, Germany) or LTA (10 μg/ml; Invivogen, San Diego, USA) as bacterial antigens of respectively Gram-negative and Gram-positive bacteria. After 2 or 24 hours stimulation in an 37°C incubator with 5% CO₂, whole blood was centrifuged at 600g for 10 minutes at 4°C. Supernatant was stored at -80°C and levels of Interleukin (IL)–6 and Tumor Necrosis Factor (TNF)-α were determined by ELISA, according to instructions of the manufacturer (R&D Systems).
Cardiac arrest patients have an impaired immune response, which is not influenced by induced hypothermia.

**HLA-DR expression**

HLA-DR expression on monocytes was analysed by fluorescence activated cell sorter after labelling by incubation with HLA-DR-FITC and CD14-PE monoclonal antibodies (Becton Dickinson, Erembogem, Belgium). Erythrocytes were lysed with lysis buffer (Becton Dickinson BV, Breda, the Netherlands) and the debris was washed away. The remaining leukocytes were fixated with 1% paraformaldehyde. The final flowcytometric analysis was done, using a flowcytometry (Becton Dickinson BV, Breda, the Netherlands). HLA-DR expression was measured on CD14-bright monocytes which have mainly anti-inflammatory properties [32] and CD14-dull monocytes which are more pro-inflammatory, since they selectively induce production of cytokines in response to viruses and immune complexes containing nucleic acids [33]. To compare patient samples measured at different time points, a standard series of quantum beads which are labelled with a known quantity of FITC fluorescent label (Quantum FITC-5 MESF (premix), Bangs laboratories, Fishers, USA) was analysed simultaneously with each measurement. Therefore, HLA-DR expression could be expressed in Molecules of Equivalent Soluble Fluorochrome (MESF) units.

**Statistical analysis**

Data are expressed by mean ± SD in the table and as median ± range in the figures, depending on distribution of the data. Differences between 33°C and 36°C groups were compared using an unpaired T-test or a Mann-Whitney U test, depending on distribution of the data. The effect of temperature over time was compared using a repeated measurement ANOVA or a Friedman test with either a Bonferroni’s or Dunn’s multiple comparison test correction. Differences between healthy volunteers and patients with a target temperature of either 33°C or 36°C over time were compared using a one–way ANOVA or Kruskal Wallis test, with either a Bonferroni’s or Dunn’s multiple comparison test, depending on distribution of the data. Statistical significance was set at P<0.05.

**Results**

**Patient characteristics**

Patients in 33°C and 36°C groups did not differ in previous medical history or disease severity (Table 1). After reaching target temperature at T=1, body temperature was 33.2 ± 0.7°C in patients with a target temperature of 33°C and 35.6 ± 0.9°C in patients with a target temperature of 36°C (p=0.002). At T=1, plasma samples could not be collected from 4 patients. One patient with a target temperature of 33°C died before the last time
Cardiac arrest patients have an impaired immune response, which is not influenced by induced hypothermia.

A confirmed infection within 24 hours after ICU admittance occurred in 2 patients, 1 patient in each group (Table 1).

Table 1: Baseline characteristics of 11 patients with a target temperature of 33°C compared 9 patients with a target temperature of 36°C, after reaching target temperature. Data expressed as mean ± SD.

<table>
<thead>
<tr>
<th></th>
<th>33°C</th>
<th>36°C</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (Male/Female)</td>
<td>8/3</td>
<td>8/1</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>64 ± 15</td>
<td>61 ± 15</td>
<td>0.70</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>173 ± 12</td>
<td>178 ± 7</td>
<td>0.27</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>81 ± 24</td>
<td>81 ± 13</td>
<td>0.95</td>
</tr>
<tr>
<td>Maximal leukocyte count</td>
<td>15.8 ± 4.9</td>
<td>16.1 ± 4.0</td>
<td>0.92</td>
</tr>
<tr>
<td>SAPS II score</td>
<td>54 ± 18</td>
<td>59 ± 18</td>
<td>0.58</td>
</tr>
<tr>
<td>APACHE III score</td>
<td>80 ± 44</td>
<td>88 ± 34</td>
<td>0.71</td>
</tr>
</tbody>
</table>

The effect of hypothermia on the SIRS reaction

Baseline inflammatory response was measured in plasma cytokines levels (figure 1). Levels of IL–1RA, IL–8, IL–10 and MCP–1 showed an increase in the patient group with a target temperature of 36°C compared to healthy controls, which was not apparent in the 33°C group. Levels of IL–1RA, but not of other cytokines, decreased after 24 hours of temperature management protocol (T=1 vs. T=2; figure 1) in the group with a target temperature of 36°C. Levels of IL–1RA, IL–8, IL–10 and MCP–1 were higher in the 36°C group compared to the 33°C group shortly after reaching target temperature, but these
Cardiac arrest patients have an impaired immune response, which is not influenced by induced hypothermia. Differences disappeared during temperature management over time (T=1; figure 1). Plasma levels of IL-1β, MIP-1, soluble CD40 ligand and TNF-α levels were not increased in patients compared to healthy controls, nor were there differences between the 33°C group and the 36°C group.

Figure 1: Cytokine levels in plasma of healthy controls, cardiac arrest patients with a target temperature of 33°C and cardiac arrest patients with a target temperature of 36°C, after reaching target temperature (T=1), at the end of the target temperature protocol (T=2) and after reaching 37°C (T=3). Data expressed as median with range. *: P < 0.05; **: P < 0.01.
Cardiac arrest patients have an impaired immune response, which is not influenced by induced hypothermia.

Figure 2: Cytokine production after whole blood stimulation with LPS or LTA, in plasma of healthy controls, cardiac arrest patients with a target temperature of 33°C and cardiac arrest patients with a target temperature of 36°C, after reaching target temperature (T=1), at the end of the target temperature protocol (T=2) and after reaching 37°C (T=3). Healthy volunteers are marked by white bars; cardiac arrest patients with a target temperature of 33°C are marked by grey bars, cardiac arrest patients with a target temperature of 36°C are marked by black bars. Every measurement is preceded by a blanc control stimulation. Data expressed as median with range. *: P < 0.05; **: P < 0.01.

The effect of hypothermia on the immune response to TLR ligands

Results of whole blood stimulated for 2 hours were not different from 24 hours stimulation. Therefore, only the results of 24 hours stimulation are shown (figure 2).

Response to LPS: The immune response to stimulation with Gram-negative antigen LPS resulted in lower levels of IL-6 and TNF-α in patients of the 36°C group compared to healthy controls, which increased towards the end of the temperature management protocol in the group with a target temperature of 36°C (figure 2, upper panel; T=1 vs. T=3). This increase was not observed in the 33°C group. TNF-α levels were decreased in the 36°C group compared to the 33°C group at baseline. However no significant differences between the groups appeared during the 24 hours of temperature management and after rewarming.

Response to LTA: Production of IL-6 did not differ between patient groups and healthy controls, nor were there differences over time between the 33°C and 36°C groups (figure 2, lower panel). The TNF production in response to stimulation with the Gram-positive antigen LTA was decreased in patients with a target temperature of 36°C compared to
Cardiac arrest patients have an impaired immune response, which is not influenced by induced hypothermia.

Healthy controls at the start of the temperature management and increased again after regaining normothermia (T=1 vs. T=3; figure 2). TNF-α level was also lower in the 36°C group compared to the 33°C group at T=1, but this difference between groups disappeared during temperature management over time.

The effect of hypothermia on HLA-DR expression

HLA-DR expression on CD14-bright monocytes was reduced in patients compared to healthy controls at all the time points for both the 33°C and 36°C group (figure 3). HLA-DR expression on both bright and dull monocytes further decreased over time (T=1 vs. T=3; figure 3). HLA-DR expression showed no differences between the 33°C and 36°C groups.

Discussion

Patients after cardiac arrest demonstrate a systemic inflammatory response syndrome compared to healthy controls, which was most noted for those who were kept at 36°C and blunted for those who were cooled to 33°C. This SIRS reaction was associated with lower HLA-DR expression and an attenuated immune response to Gram-negative and Gram-positive antigens compared to healthy controls. Between patients with a targeted
Cardiac arrest patients have an impaired immune response, which is not influenced by induced hypothermia.

An increased systemic inflammatory response following cardiac arrest compared to healthy controls has been shown before [22]. The inflammatory response in cardiac arrest patients treated with induced hypothermia, was previously studied by Bisschops et al, showing a temporary increase of IL–6 levels during hypothermia and increased levels of IL–8 and MCP–1 compared to baseline [26], with levels largely comparable to our patients. Anti-inflammatory cytokines IL–1RA and IL–10 were unaltered compared to baseline [26]. However, in this study, effects of body temperature could not be dissected from effects of ischemia-reperfusion injury following cardiac arrest. Our study expands on these findings by including a normothermic control group over time. We found that initial cytokine levels were reduced in patients with a target temperature of 33°C compared to 36°C, although differences were small. This decrease in inflammatory response is in line with multiple experimental studies demonstrating an inhibitory effect of hypothermia on cytokine levels [10-13, 19]. However, at the end of the 24 hours of the temperature management protocol, there were no differences between both groups in plasma cytokine levels. Thereby, any effect of hypothermia on systemic inflammatory response seems temporary.

The systemic inflammatory response in patients was accompanied by an initial decreased ability of immune cells to respond to TLR ligands LPS and LTA, although not all cytokines were affected. The ability to generate an immune response can also be measured by HLA-DR expression. Of interest, we found a clear reduction in the expression of HLA-DR following cardiac arrest, compared to healthy controls. A blunted host immune response to TLR ligands and a decreased HLA-DR expression have been shown before in septic shock patients [29, 34], which is thought to contribute to an increased risk of nosocomial infection. To the best of our knowledge, this attenuated immune response to bacterial antigens after cardiac arrest has not been reported before. Decreased HLA-DR expression together with a blunted response to TLR ligands suggests that also cardiac arrest patients have an impaired immune response to pathogens. In line with this, nosocomial infection occurs in up to 50-65% of cardiac arrest patients [35-37]. Thereby, cardiac arrest patients should be closely monitored for the development of nosocomial infection. Whether prophylactic antibiotics are warranted in this patient group remains to be determined.
To investigate effects of induced hypothermia on immune response in cardiac arrest patients, our study provided an appropriate experimental setting in a more or less homogenous patient population. We found that the decreased ability of immune cells to produce TNF-α in response to TLR ligands was more prominent in patients with a target temperature of 36°C compared to 33°C. Thereby, hypothermia does not seem to have an inhibitory effect on immune response to TLR ligands compared to normothermia. Also, we found no differences in HLA-DR expression between the 33°C and 36°C groups at any time points. These results are in line with the TTM trial which found no significant differences in infection rate between the 33°C and 36°C groups in a large cohort of cardiac arrest patients [23]. In contrast, a previous study in cardiac arrest patients found that induced hypothermia was an independent risk factor for infection [35]. However, that study lacked a normothermic control group. Also, a meta-analysis suggests that hypothermia increases risk of infection, but in a very heterogeneous patient population [25]. Taken together, our results suggest that having had a cardiac arrest may render the patient susceptible to infection by a decreased immune response, but this risk of infection is not further increased by induced hypothermia.

Limitations of our study are small sample size. However, larger sample sizes with these kinds of investigations including whole blood stimulations are intricate, since they are labour-intensive and expensive. However, our sample size is comparable to previous studies [24, 26]. Lastly, the inclusion period was extensive, but our standard of care protocol did not undergo relevant changes during this time period.

The relevance of our findings is that lowering body temperature may be done safely without compromising the immune response in patient groups who are thought to benefit from regulating body temperature. This may be especially interesting for those patients who suffer from an increased inflammatory response. Currently, a large trial on hypothermia in sepsis patients is ongoing (Trial number: NCT01455116).

**Conclusion**

Patients after cardiac arrest have a modest systemic inflammatory response compared to healthy controls. Cardiac arrest was associated with lower HLA-DR expression and attenuated immune response to Gram-negative and Gram-positive antigens, indicative of an impaired immune response. A body temperature of 33°C did not influence immune reaction compared to 36°C, suggesting that induced hypothermia in itself does not affect immune response.
Cardiac arrest patients have an impaired immune response, which is not influenced by induced hypothermia.


Cardiac arrest patients have an impaired immune response, which is not influenced by induced hypothermia after cardiac arrest as a "sepsis-like" syndrome. Circulation 106: 562-568


24. Kamps M, Bisschops L, van der Hoeven JG, Hoedemaekers CW. Hypothermia does not increase the risk of infection: a case control study. Crit Care 15: R48


Part II

Heliox ventilation
The potential of heliox as a therapy for acute respiratory distress syndrome in adults and children: a descriptive review

Charlotte J.P. Beurskens; Roelie M. Wösten-van Asperen; Benedikt Preckel; Nicole P. Juffermans

Submitted
Abstract

Introduction: In neonatal respiratory distress syndrome (RDS) and acute respiratory distress syndrome (ARDS) mechanical ventilation is often necessary to prevent hypoxia and hypercapnia. Mechanical ventilation can however induce or aggravate the lung injury caused by the respiratory distress. Helium, in a gas mixture with oxygen (heliox), has a low density, and can reduce the flow in narrow airways and allow for lower driving pressures. Also diffusion capacities of CO₂ can improve with heliox ventilation. We reviewed preclinical and clinical studies of the use of heliox ventilation in ARDS like syndromes.

Methods: A systematic search was executed in the PubMed and EMBASE database, with search terms referring to ARDS or an ARDS like condition and the intervention.

Results: 576 Papers were retrieved of which the majority were excluded, resulting in 20 papers of which 6 articles described animal models (3 paediatric; 3 adult animal models) and 14 were clinical studies, of which 12 described paediatric patient populations and 2 adult patient populations. In both paediatric and adult animal models, heliox improved gas exchange while allowing for less invasive ventilation in a wide variety of models using different ventilation modes. Clinical studies show a reduction in work of breathing during heliox ventilation, with a concomitant increase in pH and decrease in PaCO₂ levels, Compared to oxygen ventilation.

Conclusion: Although the evidence so far is limited, there may be a rationale for heliox ventilation in ARDS as an intervention to improve ventilation and reduce work of breathing.
Introduction

Acute respiratory distress syndrome (ARDS) is a common entity in critically ill patients, with a staggering mortality of 20% among paediatric patients and 60% among the elderly. Common features of ARDS are hypoxia and hypercapnia with a concomitant increased work of breathing, the latter due to both obstructed airways with increased airway resistance as well as to an increased need for CO₂ removal. These processes occurring during ARDS frequently warrant mechanical ventilation.

Pathophysiology of neonatal respiratory distress syndrome (RDS) includes a surfactant dysfunction. In both ARDS and neonatal RDS, mechanical ventilation can induce or aggravate pulmonary damage. Overstretching of alveoli by application of high tidal volumes or high driving pressures and by repetitive opening and closing of the alveoli can all lead to ventilator-induced lung injury and a pro-inflammatory state. Mechanical and inflammatory processes most likely interact: a mechanically stressed lung may produce an inflammatory reaction. Conversely, inflammation renders the lung susceptible to mechanical stress.

It is well recognized that limited tidal volume ventilation of 6 ml/kg is beneficial in both neonatal RDS and ARDS. Use of even lower tidal volumes was found to confer additional protection in ARDS and neonatal RDS. In addition to limited tidal volumes, also airway pressures are linearly associated with mortality. Application of relatively low plateau pressures (26–27 cm H₂O) can already generate an inflammatory response in the lung. Despite recognition that intensity of mechanical ventilation influences outcome of ARDS, application of limited tidal volume ventilation and low driving pressures can often not be achieved. Thereby, adjunctive therapies which allow for less invasive ventilation may be beneficial in ARDS.

Helium is an inert gas with a lower density than air, thus flow of helium through an airway is less turbulent, leading to lower resistance. As a result, during heliox ventilation, lower driving pressures are necessary to distribute oxygen to the distal alveoli to improve oxygenation. Also, diffusion capacities of CO₂ are increased, further resulting in improved gas exchange. Another potential benefit of helium is that it may have anti-inflammatory properties. Helium has been used to reduce the work of breathing during exacerbations of asthma and COPD, mostly in the paediatric population. Also in
ARDS, most data on helium ventilation are derived from the paediatric population, which may be related to an increased airway resistance in neonates and children compared to adults.

In light of the increasing awareness of the necessity to limit intensity of mechanical ventilation, we have summarized results from preclinical and clinical studies, which have explored the potential therapeutic use of heliox in ARDS in this descriptive review.

Methods

We performed a systematic search in the PubMed and EMBASE database to identify all publications of studies focusing on the effect of Heliox in ARDS. The databases were searched until January 2014.

The search included search terms referring to the condition (“lung injury”; “acute lung injury”; “acute respiratory distress syndrome”; “ards, human”; “lung injury”; “ards”; “respiratory distress syndrome”; “respiratory failure”; “hypoxia”; “mechanical ventilation”; “artificial respiration”) as well as to the intervention (“Helium”; “Heliox”).

For preclinical studies, parameters described for animal models of ARDS were used to select articles. In the clinical studies we accepted patient populations who were described as having acute respiratory failure with the need for respiratory support. Since the search gathered articles dating back to 1989, it was not always possible to use consensus criteria for ARDS. Titles were limited to English language. Retrieved papers were screened on relevance by reading of the abstract. The reference lists of selected articles were screened for additional relevant papers.

Results

Of 576 papers retrieved from Medline or EMBASE, 556 articles were excluded based on no use of helium during mechanical ventilation, no original data, or no ARDS/ARDS like condition, leaving 20 papers included and described in detail in this review (Figure 1).

The effect of heliox on lung mechanics and inflammation in animal models of ARDS

We found 3 articles describing the effect of heliox on gas exchange in paediatric animal models and 3 studies using adult animal models.
Neonatal RDS animal models
In a study in neonatal piglets with ARDS induced by saline lavage, animals were ventilated with 40% or 60% helium balanced with oxygen, in a high frequency oscillatory ventilation (HFOV) mode with fixed mean airway pressure, oscillation amplitude and frequency. The ventilation settings were targeted to reach PaCO$_2$ levels of 55-80 mmHg and a PaO$_2$ level above 100 mmHg. Heliox resulted in decreased PaCO$_2$ levels, combined with a modest improved oxygenation, together with an increased tidal volume delivery, as measured by pneumotachometer. As increased tidal volume delivery is unwanted in
neonatal RDS, the effect of heliox on gas exchange was investigated while keeping the tidal volume constant. Swine with saline-lavage induced ARDS were ventilated with 40% helium or 40% nitrogen while tidal volume was kept constant by adjusting the oscillation amplitude. At a constant tidal volume, helium did not alter oxygenation. However, the oscillation amplitude did decrease significantly during heliox ventilation, which relates to a decrease in peak inspiratory pressure (PIP).

Similar results were found during continuous positive airway pressure (CPAP) ventilation, using a neonatal pig model in which ARDS was induced by oleic acid. Heliox significantly improved gas exchange, reduced the need for oxygen and decreased PaCO$_2$ levels compared to animals ventilated with nitrogen. Besides improved gas exchange, heliox ventilation significantly decreased respiratory rate, tidal volume, minute ventilation and airway resistance, while respiratory compliance increased. Also, heliox improved the amount of aerated lung as measured by histomorphometrical analyses, showing a significantly larger percentage of the gas exchange area relative to the parenchymal area. Interestingly, in this model also lung inflammation was investigated. In lung tissue of animals ventilated for 4 hours, levels of IL-8 and myeloperoxidase (MPO), both indicators of neutrophil activation, were lower in animals ventilated with heliox compared to animals ventilated with nitrogen. Thereby, heliox improved ventilation and reduced barotauma, resulting in an attenuation of lung inflammation.

In conclusion, in paediatric animal models of ARDS ventilated in a HFOV mode, heliox improved gas exchange while allowing for less invasive ventilation, with a concomitant reduction in lung inflammation.

**ARDS animal models**

In an adult rabbit model of ARDS, lung injury was induced by oleic acid instillation. Animals were ventilated with low bias flow oscillation with a CO$_2$ scrubber, which is a modified HFOV system to reduce gas utilization. Animals were ventilated in cycles of 20 minutes, with a variable helium concentration (40-50-60-70%) balanced with oxygen. All heliox ventilation cycles were preceded by 20 minutes of ventilation with 40% oxygen and 60% nitrogen. After each cycle, a blood gas was drawn. All ventilator settings remained unaltered during the experiment. Ventilation with helium increased CO$_2$ clearance compared to nitrogen-ventilated animals, the magnitude of which correlated with the concentration of helium.
A study in an adult rat model of ARDS induced by saline lavage, focussed on the effect of helium on histopathological and immunohistochemical changes in lung tissue. Male rats were ventilated in a pressure-controlled mode for one hour with either heliox (50% helium; 50% oxygen) or 100% oxygen. After the intervention, rats continued to be ventilated for 2 hours with 50% oxygen before they were scarified and lung tissue was harvested. The severity of pathological features (infiltration of neutrophils, presence of oedema and haemorrhage and hyaline membrane formation) was graded. Heliox ventilation resulted in a reduction of all these features compared to the control group. Also MPO and inducible nitric oxide synthase in lung tissue, which are activated by respectively neutrophils or endothelial cells were reduced due to heliox ventilation. This study suggests a possible role for heliox in attenuating lung inflammation that seems to be unrelated to less invasive mechanical ventilation settings. Of note however, the control group in this study had hyperoxia, which is known to induce inflammation.

The effect of heliox on lung mechanics was studied in an adult rat model, where lung injury was provoked by injurious ventilator settings. With tidal volumes of 15 ml/kg rats were ventilated with either heliox (50% helium; 50% oxygen) or a standard gas mixture (50% oxygen; 50% air) for 4 hours, while adjusting respiratory rate to maintain normocapnia. Heliox ventilation significantly reduced minute volume ventilation, while maintaining a normal acid-base balance and adequate oxygenation. However, pulmonary protein levels and inflammatory cytokine levels were not affected by heliox ventilation.

Taken together, in animal experiments, heliox improved ventilation in ARDS models during pressure controlled ventilation and spontaneous breathing. The effects of heliox on inflammation yield contrasting results.

The effect of heliox on lung mechanics and gas exchange in clinical studies

Clinical studies on the effect of heliox in ARDS patients are limited. We found 14 articles, of which 12 were in the paediatric patient population and 2 in the adult patient population. All trials had small patient numbers (Table 1).

Neonatal clinical studies on RDS

An important and much referred study on the effect of helium on gas exchange was performed in premature newborn infants (weight <2000 g) with neonatal RDS in need of
The potential of heliox as a therapy for acute respiratory distress syndrome in adults and children: a descriptive review

Table 1: Overview of clinical studies, with patient numbers and main outcome

<table>
<thead>
<tr>
<th>Study (reference)</th>
<th>Number of patients</th>
<th>Study type</th>
<th>Patient population</th>
<th>Ventilation mode</th>
<th>Main outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elleau (30)</td>
<td>N=31</td>
<td>Randomized controlled trial</td>
<td>ARDS - Infant</td>
<td>Volume controlled</td>
<td>↓ Transcutaneous PO$_2$ / FiO$_2$ ratio</td>
</tr>
<tr>
<td>Colnaghi (32)</td>
<td>N=51</td>
<td>Randomized controlled trial</td>
<td>ARDS - Infant</td>
<td>Nasal CPAP</td>
<td>↑ PaO$_2$ / FiO$_2$ ratio; ↓ need for intubation</td>
</tr>
<tr>
<td>Winters (33)</td>
<td>N=5</td>
<td>Case report</td>
<td>ARDS - Infant</td>
<td>HFOV</td>
<td>↓ PaCO$_2$ levels</td>
</tr>
<tr>
<td>Migliori (34)</td>
<td>N=10</td>
<td>Observational intervention</td>
<td>ARDS - Infant</td>
<td>SIMV</td>
<td>↓ Peak Inspiratory pressures; ↓ Work of Breathing</td>
</tr>
<tr>
<td>Szczapa (35)</td>
<td>N=8</td>
<td>Observational intervention</td>
<td>ARDS - Infant</td>
<td>SIMV</td>
<td>↑ PaO$_2$ / FiO$_2$ ratio; ↓ PaCO$_2$ levels</td>
</tr>
<tr>
<td>Gross (36)</td>
<td>N=10</td>
<td>Observational intervention</td>
<td>Bronchiolitis - Infant</td>
<td>SIMV</td>
<td>= PaO$_2$ / FiO$_2$ ratio; = PaCO$_2$ levels</td>
</tr>
<tr>
<td>Kneyber (37)</td>
<td>N=13</td>
<td>Observational intervention</td>
<td>Bronchiolitis - Infant</td>
<td>Pressure controlled</td>
<td>↓ Airway resistance</td>
</tr>
<tr>
<td>Paret (38)</td>
<td>N=1</td>
<td>Case report</td>
<td>Bronchiolitis - Infant</td>
<td>Non Invasive Pressure controlled (Head hood)</td>
<td>↓ Need for intubation</td>
</tr>
<tr>
<td>Martinon-Torres (39)</td>
<td>N=15</td>
<td>Observational intervention</td>
<td>Bronchiolitis - Infant</td>
<td>CPAP</td>
<td>↓ Need for intubation; ↓ PaCO$_2$ levels</td>
</tr>
<tr>
<td>Liet (40)</td>
<td>N=39</td>
<td>Randomized controlled trial</td>
<td>Bronchiolitis - Infant</td>
<td>Non Invasive Pressure controlled (Head hood)</td>
<td>= Need for intubation</td>
</tr>
<tr>
<td>de Gammara (42)</td>
<td>N=8</td>
<td>Observational intervention</td>
<td>Bronchopulmonary dysplasia - Infant</td>
<td>Non Invasive Pressure controlled (Plexiglas chamber)</td>
<td>↓ Transcutaneous PO$_2$</td>
</tr>
<tr>
<td>Szczapa (43)</td>
<td>N=15</td>
<td>Observational intervention</td>
<td>Bronchopulmonary dysplasia - Infant</td>
<td>Volume controlled</td>
<td>↑ PaO$_2$ / FiO$_2$ ratio</td>
</tr>
<tr>
<td>Pizov (44)</td>
<td>N=7</td>
<td>Observational intervention</td>
<td>Respiratory failure - Adult</td>
<td>Tracheal insufflation</td>
<td>↓ PaCO$_2$ levels; ↓ Peak Inspiratory pressures</td>
</tr>
<tr>
<td>Kirby (45)</td>
<td>N=2</td>
<td>Case reports</td>
<td>ARDS - Adult</td>
<td>HFOV / BiPAP</td>
<td>↓ PaCO$_2$ levels</td>
</tr>
</tbody>
</table>

BiPAP: Bilevel Positive Airway Pressure; HFOV: High Frequency Oscillatory Ventilation; SIMV: Synchronized Intermittent Mandatory Ventilation; Nasal CPAP: Nasal Continuous Positive Airway Pressure; CPAP: Continuous Positive Airway Pressure
mechanical ventilation before 24 hours of life. In this double-blind randomized study, 31 infants were mechanically ventilated in a volume-controlled mode with either 78% heliox or nitox (78% nitrogen; 22% oxygen) for a maximum of 8 days, where after the ventilator was connected to the standard air-in-oxygen gas mixture again. Mechanical ventilation was targeted to maintain a transcutaneous PO2 (TcPO2) between 6-9 kPa and PaCO2 levels between 5-8 kPa. The heliox group showed an improved TcPO2/FiO2 ratio after 2 days of ventilation compared to the nitox group. After 4 days of ventilation, mean airway pressure and FiO2 could significantly be reduced in the heliox group compared to the nitox group. Complications due to neonatal RDS, including bronchopulmonary dysplasia and death, were significantly higher in the nitox group. Although the results suggest a beneficial effect of heliox on respiratory status, patient numbers in this study are small. Moreover this study was carried out before surfactant was introduced as therapeutic agent in infants with ARDS.

The effect of heliox in preterm born infants with neonatal RDS and ventilated with nasal continuous positive airway pressure (CPAP) was investigated in a randomized pilot study. The intervention group (N=27) received 80% heliox and the control group (N=24) received nasal CPAP with medical air. After 12 hours, heliox was replaced with medical air if nasal CPAP was still necessary. The main outcome was the requirement of mechanical ventilation within 7 days. Heliox significantly decreased the risk for intubation and need for surfactant therapy. A trend was seen towards improved gas exchange and shortened duration of nasal CPAP in favour of heliox ventilation.

The effect of heliox was studied in 10 preterm infants with neonatal RDS, who were ventilated long-term by synchronized intermittent mandatory ventilation (SIMV). Heliox (80% helium; 20% oxygen) replaced the air-in-oxygen gas mixture. Peak inspiratory pressures were adjusted to keep tidal volumes constant. Before, during and after 1 hour of heliox therapy, ventilatory parameters and pulmonary mechanics were measured. During heliox ventilation, peak inspiratory pressure, TcPCO2 and work of breathing were reduced, with a concomitant increase in TcPO2 and minute ventilation. Infants who showed a reduction in peak pressures of at least 20% were extubated. After extubation, they received bilevel positive airway pressure (BiPAP) with heliox for another 3 hours. Out of the 10 infants, 8 could be extubated. BiPAP did increase the need for FiO2, but only 1 infant needed re-intubation after 5 hours of ventilation with air-in-oxygen. Although the sample size is small and a control group is lacking, these results show the ability of heliox
to reduce work of breathing and the need for invasive pressure support ventilation, while gas exchange improves.

In a pilot study in 8 new-borns who were mechanically ventilated in a pressure-controlled SIMV mode because of respiratory failure due to meconium aspiration, 80% heliox was administered for 1 hour. There was a trend towards increased expiratory tidal volumes, minute ventilation and peak expiratory during heliox ventilation. Heliox significantly reduced the alveolar-arterial oxygen tension and increased PaO₂/FiO₂ ratio. Blood gas analysis showed a non-significant decrease in PaCO₂ levels, with a concomitant increase in pH values. These beneficial effects of heliox were reversed after ventilation was switched back to air-in-oxygen gas mixture.

These studies suggest that heliox improves gas exchange and allows for less invasive mechanical ventilation in the neonatal population. As most of the studies had outcomes focussed on lung mechanics, the effect of heliox on clinically relevant outcomes is not unequivocally proven, although a benefit towards prevention of intubation and less time on the ventilator was observed.

**Paediatric clinical studies on ARDS**

A summary of case reports reported the use of HFOV to administer heliox in 5 paediatric intensive care unit (ICU) patients with hypoxemic respiratory failure and respiratory acidosis, caused by a variety of underlying pathology. The concentration of helium in the gas mixture differed from 20 to 65% and exposure time varied from 2 to 6.5 hours. Despite this variation, in all patients the PaCO₂ levels dropped dramatically after the introduction of heliox compared to nitrogen-oxygen ventilation. Oxygenation remained adequate.

In paediatric patients, ARDS can also be triggered by bronchiolitis, which is caused by the respiratory syncytial virus (RSV). When bronchiolitis is severe, it can result in respiratory failure and the need for respiratory support. In mechanically ventilated children with bronchiolitis, the effect of stepwise increased percentages of helium in the ventilated gas mixture was investigated. Ten infants were ventilated in SIMV mode, with a nitrox gas mixture (50% nitrogen; 50% oxygen). During the study protocol, heliox was administered for 15 minutes with a concentration of respectively 50, 60 or 70% balanced with oxygen. Gas exchange was measured every 15 minutes, just before changing the gas mixture.
In this setting, heliox did not alter PaO\textsubscript{2}/FiO\textsubscript{2} ratio nor PaCO\textsubscript{2} levels compared to nitrox ventilation. These negative findings were ascribed to the relatively small CO\textsubscript{2} retention due to the used SIMV mode, the small sample size and to a possibly mild to moderate lung disease.

In another small study population, 13 infants with RSV infection were ventilated in a pressure controlled ventilation mode, with 60% heliox for two times 30 minutes \textsuperscript{42}, interspersed with nitrox ventilation (60% nitrogen; 40% oxygen). During ventilation with heliox, the airway resistance was reduced, without improving CO\textsubscript{2} elimination, PIP or end-expiratory lung volume. The effect on resistance reversed after switching back to nitrox ventilation. A case report on a 4-month old boy with RSV-induced bronchiolitis and respiratory failure described effects of ventilation with 80% heliox via an oxyhood at 5L/min \textsuperscript{43}. In this case, non-invasive heliox therapy avoided intubation and mechanical ventilation, as respiratory rate and PaCO\textsubscript{2} levels dropped dramatically. Heliox therapy was continued for 48 hours before the patient was weaned from the heliox gas mixture.

Non-invasive CPAP ventilation with 70% heliox has also been used long-term, for up to 14 days in infants with refractory acute bronchiolitis \textsuperscript{44}. In 15 paediatric patients admitted to the ICU, clinical condition, oxygenation and gas exchange were measured at baseline and during heliox therapy. Compared to baseline, heliox ventilation improved saturation, respiratory rate and PaCO\textsubscript{2} levels. These results were not confirmed in a randomized multicentre study in 39 infants investigating the effect of 78% heliox versus air-oxygen mix (78% nitrogen; 22% oxygen) through a non-invasive, inflatable head hood for at least 24 hours \textsuperscript{45}. Compared to air-oxygen, heliox did not generate any significant differences in the need for positive-pressure ventilation or gas exchange.

Taken together, effects of heliox in RSV-induced ARDS are less apparent than in ARDS due to other causes. A possible explanation could be that RSV represents a relatively mild version of ARDS in paediatric patients \textsuperscript{46}. 

\* \* \* 

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Paediatric clinical studies on bronchopulmonary dysplasia

Respiratory distress is also common in paediatric patients suffering from bronchopulmonary dysplasia (BPD), which may be considered a more chronic form of neonatal RDS. The effect of heliox ventilation was studied in 2 groups of 4 neonates with or without BPD. All infants were placed in a plexiglas chamber, which was filled successively with air-oxygen mix (78% nitrogen; 22% oxygen) and heliox (78% helium; 22% oxygen) for a maximum of 3 hours. Spontaneously breathing of heliox resulted in acute hypoxia, with significantly decreased TcPO$_2$ levels in the BPD group versus the control group, whereas TcPCO$_2$ was unaltered. These results limit the application of heliox in patients who are in need of higher oxygen demands.

In another study in 15 patients with respiratory failure due to BPD, 80% heliox was administered during mechanical ventilation for 1 hour. Compared to baseline, heliox increased tidal volume, dynamic compliance and peak expiratory flow rate. PaO$_2$/FiO$_2$ ratio improved during heliox ventilation, with a related decrease in the alveolar-arterial oxygen tension and oxygenation index. A non-significant decrease was seen in PaCO$_2$ levels, with an increase in pH. All these beneficial effects reversed after switching back to a normal gas mixture.

In conclusion, these clinical studies in paediatric patients suggest that heliox ventilation reduces work of breathing and the need for mechanical ventilation, with the clearest effect in ARDS and less effect in RSV-induced respiratory failure. Due to small samples sizes, results were often non-significant.

Adult clinical studies on ARDS

Clinical studies of the effect of heliox in adult patients with ARDS are scarce. To reduce hypercapnia, tracheal gas insufflation with 100% oxygen or 100% helium was investigated in 7 mechanically ventilated patients with respiratory failure due to various aetiologies. Tracheal insufflation was administered at 2, 4 and 6L/min for 15 minutes. Heliox decreased PaCO$_2$ levels with both gases. Compared to baseline, the maximum flow of helium resulted in a decrease in PIP. Overall the efficiency of tracheal insufflation, calculated by dividing the PaCO$_2$ change by the change in PIP, improved with the use of helium.
In a summary of 2 case reports of patients with bronchiolitis obliterans syndrome and acute respiratory failure following lung transplantation, 60% heliox was administered either via BiPAP or HFOV. Heliox ventilation increased pH and decreased PaCO₂ levels and therefore respiratory status was improved.

Safety of heliox ventilation

There is extensive experience with heliox in asthma and COPD patients. Complications have not been reported. The lower density of helium causes inaccurately high readings from flow meters calibrated for air and/or oxygen. Thereby, the flow transducer within the ventilator needs adjustment to correctly measure the flow.

The safety of heliox during mechanical ventilation in patients with acute respiratory failure is rarely described. In paediatric patients, the frequency of complications was described, without any appreciable effect of heliox ventilation. Another case report describes that heliox ventilation was safe during pregnancy.

Conclusion

In general, both preclinical and clinical studies showed improved ventilation and gas exchange with heliox ventilation. Neonatal RDS animal models mostly used HFOV, in which heliox improved gas exchange while allowing for less invasive ventilation, with an associated decrease in lung inflammation. In adult animal ARDS models, heliox improved ventilation during both pressure controlled ventilation and spontaneous breathing. However, the effects of heliox on inflammation show contrasting results, raising the question if heliox decreases inflammation by reducing the intensity of ventilation or whether it also has a direct anti-inflammatory effect.

Effects of heliox on intensity of mechanical ventilation are expected immediately following application of heliox ventilation. In line with this, all clinical studies on ARDS showed promising effects of heliox on short term outcomes, including minute volume ventilation, applied pressures and in most studies also gas exchange. In the paediatric patient studies, heliox ventilation was also found to influence relevant clinical outcomes, including reduced work of breathing and need for mechanical ventilation. In the adult clinical studies, heliox long-term outcomes have not been investigated.

Taken together, the summarized evidence in this review suggests a rationale for heliox ventilation in ARDS. However, data on clinical outcome is limited.
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The potential of heliox as a therapy for acute respiratory distress syndrome in adults and children: a descriptive review


The potential of heliox as a therapy for acute respiratory distress syndrome in adults and children: a descriptive review.


Heliox Allows for Lower Minute Volume Ventilation in an Animal Model of Ventilator-Induced Lung Injury

Charlotte J. Beurskens; Hamid Aslai; Friso M. de Beer; Margreeth B. Vroom; Benedikt Preckel; Janneke Horn; Nicole P. Juffermans

*PLoS One, October 2013; 8(10):e78159*
Abstract

*Background:* Helium is a noble gas with a low density, allowing for lower driving pressures and increased carbon dioxide (CO$_2$) diffusion. Since application of protective ventilation can be limited by the development of hypoxemia or acidosis, we hypothesized that therefore heliox facilitates ventilation in an animal model of ventilator–induced lung injury.

*Methods:* Sprague-Dawley rats (N=8 per group) were mechanically ventilated with heliox (50% oxygen; 50% helium). Controls received a standard gas mixture (50% oxygen; 50% air). VILI was induced by application of tidal volumes of 15 mL kg$^{-1}$; lung protective ventilated animals were ventilated with 6 mL kg$^{-1}$. Respiratory parameters were monitored with a pneumotach system. Respiratory rate was adjusted to maintain arterial pCO$_2$ within 4.5-5.5 kPa, according to hourly drawn arterial blood gases. After 4 hours, bronchoalveolar lavage fluid (BALF) was obtained. Data are mean (SD).

*Results:* VILI resulted in an increase in BALF protein compared to low tidal ventilation (629 (324) vs. 290 (181) μg mL$^{-1}$; p<0.05) and IL-6 levels (640 (8.7) vs. 206 (8.7) pg mL$^{-1}$; p<0.05), whereas cell counts did not differ between groups after this short course of mechanical ventilation. Ventilation with heliox resulted in a decrease in mean respiratory minute volume ventilation compared to control (123±0.6 vs. 146±8.9 mL min$^{-1}$; P<0.001), due to a decrease in respiratory rate (22 (0.4) vs. 25 (2.1) breaths per minute; p<0.05), while pCO$_2$ levels and tidal volumes remained unchanged, according to protocol. There was no effect of heliox on inspiratory pressure, while compliance was reduced. In this mild lung injury model, heliox did not exert anti-inflammatory effects.

*Conclusions:* Heliox allowed for a reduction in respiratory rate and respiratory minute volume during VILI, while maintaining normal acid-base balance. Use of heliox may be a useful approach when protective tidal volume ventilation is limited by the development of severe acidosis.
Introduction

In acute respiratory distress syndrome (ARDS), aerated lung volume is diminished [1]. Regression of healthy lung parts and increased airway resistance results in overdistention of non–injured alveoli, even during lung protective ventilation with limited tidal volume and plateau pressures [2], thereby contributing to ventilator–induced lung injury (VILI). The only proven strategy aimed at limiting lung injury is low tidal volume ventilation [3]. However in patients with severe ARDS, tidal volumes and pressures that exceed recommendations of the ARDS network are often applied [4]. Adjunctive therapies such as extracorporeal CO$_2$ removal have been shown to allow for a further decrease in tidal volume ventilation with additional protection in ARDS [5,6]. However, use of these devices come with a certain complication rate and require expertise. Thereby, other therapeutic strategies are needed that allow for a decrease in peak pressures and minute volume ventilation for adequate gas exchange, which may facilitate adherence to protective ventilation strategies.

Helium is an inert gas with a lower density than air [7], allowing for less turbulent flow through airways, leading to lower airway resistance. As a result, during mechanical ventilation with a helium/oxygen mixture (heliox), lower driving pressures are needed to distribute oxygen to the distal alveoli compared to ventilation with oxygen [8]. Also, diffusion of CO$_2$ is increased during heliox, which in addition might facilitate ventilation. The use of heliox is clinically applied in patients with high airway resistance due to exacerbations of chronic obstructive pulmonary disease (COPD) or status asthmaticus [9,10,11]. In paediatric animal models of ARDS [12,13,14], heliox ventilation was found to improve gas exchange during high–frequency oscillatory ventilation and attenuate lung inflammation [13]. However, data on the effect of heliox on ARDS during conventional mechanical ventilation are sparse and data are limited to paediatric models.

In this study we investigated the effects of heliox in an adult rat VILI model. We hypothesized that the use of heliox facilitates CO$_2$ elimination allowing for lower minute volume ventilation. Furthermore, the effect of heliox on the inflammatory response was investigated.
Material and Methods

The animal care and use committee of the Academic Medical Centre, University of Amsterdam, Netherlands approved this study. Animal procedures were carried out in compliance with Institutional Standards for Use of Animal Laboratory Animals. Male Sprague–Dawley rats (Harlan, The Hague, The Netherlands), weighing 350–400 grams were anaesthetized by intraperitoneal injection of 90 mg kg⁻¹ ketamine, 0.125 mg kg⁻¹ dexmedetomidine and 0.05 mg kg⁻¹ atropine. Anaesthesia was maintained by infusion of 10 mg mL⁻¹ ketamine at 2.7 mL per hour. A solution of saline and bicarbonate was administered at 2.5 ml per hour. A tracheotomy was performed and a metal cannula was inserted in the trachea. Two sutures were placed around the exposed part of the trachea into which the cannula was inserted and tied down thoroughly. The cannula was then connected to a ventilator (Servo 900C, Siemens, Sweden). The ventilators were calibrated for the heliox gas mixture according to the instruction of the manufacturer using a pressure reduction valve to allow the high-pressure of the heliox tank to be reduced to safe and usable pressures for ventilation (Linde Gas Therapeutics, Eindhoven, the Netherlands). Hemodynamic parameters were monitored by inserting a polyethylene catheter into the carotid artery connected to a monitor (Siemens SC900, Danvers, USA). Temperature was monitored rectally and maintained at 37ºC by a thermo mattress.

VILI was induced by application of tidal volumes of 15 mL kg⁻¹ and zero PEEP during 4 hours. Lung protective (LP) ventilation was maintained by applying 6 mL kg⁻¹ and 5 cmH₂O PEEP. FiO₂ was set at 50%, I:E ratio of 1:2 and adjustment of respiratory rate to maintain PaCO₂ within 4.5–5.5 kPa, according to hourly drawn arterial blood gases. The alveolar-arterial gradient was calculated as follows: A – a gradient = (Fraction of inspired oxygen (%) /100) × (P_{Atmosphere} – P_{H2O}) – (PaCO₂/0.8) – PaO₂. Dead space was calculated using the formula: Dead space = (PaCO₂- etCO₂)/ (PaCO₂)*100.

Rats were ventilated pressured controlled, with either heliox (technical gas 50% oxygen; 50% helium; blended by Linde Gas Therapeutics) or 50% oxygen in air gas mixture (n=8 per group). In total 32 animals were studied of which 16 received heliox (8 VILI; 8 LP ventilation) and 16 received 50 % oxygen in air gas mixture (8 VILI; 8 LP ventilation). Tidal volumes were strictly maintained using a pneumotachometer (Hugo Sachs Elektronik, Harvard apparatus, March–Hugstetten, Germany) specific for rats. The pneumotachometer is a transducer for airflow measurement, placed between the metal
The pneumotachometer was calibrated using a 1 mL syringe according to the manufacturer’s instruction. Tidal volumes were recorded using respiration software (HSE-BDAS basic data acquisition, Harvard apparatus, March–Hugstetten, Germany) and displayed on a computer screen throughout the whole experiment. Since small tidal volumes are delivered it is not possible to ventilate the animals in a volume-controlled setting. We set a pressure controlled ventilation mode and started with an inspiratory pressure of 10 cmH₂O. In case of a deviation of the set tidal volume, the inspiratory pressure was adjusted [15,16,17]. Compliance was calculated by dividing tidal volume per kilogram by inspiratory pressure.

After 4 hours of mechanical ventilation, rats were bled and plasma was centrifuged at 1800g for 10 minutes at 4ºC. Lungs were removed en block. After the right lung was ligated, bronchoalveolar lavage was done by flushing the left lung 3 times with 2.0 ml NaCl, yielding about 5 ml of lavage fluid. In the bronchoalveolar lavage fluid (BALF), cells were counted using a hematocytometer (Z2 Coulter Particle Counter, Beckman Coulter Corporation, Florida). After centrifugation of BALF (300g; 10 min.; 4ºC), protein levels were measured (Oz Biosciences, Marseille, France) and levels of Interleukin (IL)–1β, IL–6, cytokine–induced neutrophil chemo attractant (CINC)–3, and Tumor Necrosis Factor (TNF)–α were determined by ELISA, according to instructions of the manufacturer (R&D Systems; Abingdon, United Kingdom).

**Statistical analysis**

Data are expressed by mean and SEM. The effect of heliox versus oxygen within either the VILI or the LP group was compared using a one–way ANOVA or Kruskal Wallis test, with either a Bonferroni’s or Dunn’s multiple comparison test, depending on distribution of the data. Statistical significance was set at $P<0.05$.

**Results**

All animals survived the experimental protocol. Mean arterial pressure remained above 90 mmHg in all groups. Heliox did not affect blood pressure or heart rate compared to the oxygen-ventilated animals.
VILI model

Application of high tidal volumes resulted in VILI, characterized by an increase in mean pulmonary protein leakage and BALF levels of IL–6 and CINC–3 (figure 1). Pulmonary cell influx was not increased in VILI compared to controls, nor was the BALF level of IL1–β and TNF–α (data not shown). Applied respiratory rate needed to keep PaCO₂ within predefined limits (4.5-6 kPa) increased during mechanical ventilation in both VILI groups, yielding higher minute volume ventilation over time, while compliance decreased over time (figure 2).

Figure 1: Inflammatory parameters in an animal model of ventilator–induced lung injury (N=8 per group), treated with heliox ventilation. Heliox is marked by white bars and oxygen/air by grey bars. Data are MEAN ± SEM. *: P < 0.05; **: P < 0.01. (A) Protein levels; (B) IL–6 levels; (C) CINC–3 levels and (D) cell count in bronchoalveolar lavage fluid.

Effects of heliox

Ventilation with heliox resulted in a decrease in minute volume ventilation compared to oxygen ventilation, due to an adjustment in respiratory rate, while tidal volumes were kept at 6 mL kg⁻¹ according to protocol (figure 2). PaCO₂ levels and pH remained unchanged (table 1); according to the study protocol in which set arterial PaCO₂ levels were targeted. Heliox did not affect mean airway pressure, peak and inspiratory pressure or compliance (figure 2). A-a gradients showed no differences (figure 2). Dead space in VILI was reduced by heliox after 4 hours (figure 2). Heliox ventilation did not decrease any of the parameters of lung inflammation, including pulmonary oedema, BALF protein or cytokine levels (figure 1).
Heliox Allows for Lower Minute Volume Ventilation in an Animal Model of Ventilator-Induced Lung Injury

Table 1: Gas exchange and respiratory parameters at baseline and after 4 hours of injurious mechanical ventilation in an animal model of lung injury during and after treatment with heliox ventilation.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Time (hr)</th>
<th>Oxygen</th>
<th>Heliox</th>
<th>Oxygen</th>
<th>Heliox</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>T = 0</td>
<td>7.44 ± 0.04</td>
<td>7.41 ± 0.03</td>
<td>7.42 ± 0.08</td>
<td>7.43 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>T = 4</td>
<td>7.33 ± 0.04</td>
<td>7.36 ± 0.03</td>
<td>7.38 ± 0.10</td>
<td>7.40 ± 0.05</td>
</tr>
<tr>
<td>pCO2 (kPa)</td>
<td>T = 0</td>
<td>4.38 ± 0.7</td>
<td>4.86 ± 0.6</td>
<td>5.09 ± 1.0</td>
<td>4.98 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>T = 4</td>
<td>4.99 ± 0.4</td>
<td>5.81 ± 0.9</td>
<td>5.07 ± 1.1</td>
<td>4.76 ± 0.5</td>
</tr>
<tr>
<td>pO2 (kPa)</td>
<td>T = 0</td>
<td>34.2 ± 1.8</td>
<td>31.7 ± 1.5</td>
<td>32.7 ± 1.9</td>
<td>32.0 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>T = 4</td>
<td>31.7 ± 2.5</td>
<td>30.8 ± 2.8</td>
<td>32.5 ± 3.1</td>
<td>30.9 ± 2.2</td>
</tr>
<tr>
<td>etCO2 (mmHg)</td>
<td>T = 0</td>
<td>16.7 ± 2.1</td>
<td>17.6 ± 2.9</td>
<td>10.0 ± 0.6</td>
<td>11.2 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>T = 4</td>
<td>16.7 ± 3.6</td>
<td>18.2 ± 2.2</td>
<td>10.0 ± 1.3</td>
<td>12.3 ± 1.6</td>
</tr>
</tbody>
</table>

N=8 per group. Data are MEAN ± SD.

LP = lung protective VILI = ventilator induced lung injury

Figure 2: Respiratory parameters in an animal model of lung injury during treatment with heliox ventilation (N=8 per group). Data are MEAN ± SEM. VILI is marked by open triangles; LP ventilation is marked by filled circles. Heliox ventilation is marked by a disconnected line and oxygen/air by a continuous line. Comparisons are between heliox and oxygen within the VILI or the LP group. *: P < 0.05; **: P < 0.01; ***: P < 0.001.
(A) Minute volume ventilation (mL min⁻¹); (B) respiratory rate (breaths per min); (C) inspiratory pressure (cm H₂O); (D) mean airway pressure (cm H₂O); (E) tidal volume (mL kg⁻¹); (F) A-a gradient; (G) dead space (mmHg) and (H) compliance (mL cm H₂O⁻¹).
Discussion

In a rat VILI model, increased minute volume ventilation was needed to maintain adequate gas exchange. Heliox allowed for a reduction in minute volume ventilation, while CO₂ removal remained unaltered. These effects are in line with findings in pediatric ARDS models [12,13,14], as well as in pediatric and adult patients with respiratory failure due to exacerbation of COPD or asthma [9,10,18,19,20]. In ARDS models, heliox has mostly been studied as a driving gas during HFOV. In this setting, heliox improved CO₂ elimination, which was found to be due to an increase in tidal volume delivery, thereby hampering the use in clinical practice in ARDS patients [14,21]. In our study, ventilation improved at unchanged tidal volume delivery during conventional pressure controlled mechanical ventilation settings.

The theory that heliox ventilation may allow for lower peak inspiratory pressures in ARDS patients has been postulated before [11], as heliox establishes a more laminar flow through small airways compared to oxygen [7,8]. In our study however, heliox did not allow for a reduction in inspiratory pressures applied to achieve pre set tidal volumes. Thereby, compliance of the respiratory system also remained unchanged. This is in line with a study in children with acute bronchiolitis due to infection with respiratory syncytial virus, in which heliox decreased airway resistance at unchanged tidal volume delivery during pressure controlled ventilation, but did not allow for application of lower peak pressures [22]. Whether helium has therapeutic potential in ARDS, by increasing ventilation in order to increase adherence to protective ventilation strategies including low tidal volume ventilation and use of limited inspiratory pressures, remains to be determined.

Ventilation with heliox did not attenuate pulmonary inflammation in VILI. These results are in contrast with previous findings in models of ARDS in which an anti–inflammatory effect of heliox has been described [13,23]. There may be several explanations for these disparate results. In neonatal pigs, heliox was found to reduce tidal volumes with a concomitant decrease in inflammation [13]. In that study, it was not clear whether heliox exerted direct anti–inflammatory effects or that inflammation was reduced due to less mechanical stress [13,23,24]. In a rat model in which ARDS was induced by saline lavage during pressure controlled ventilation, use of 80% heliox decreased inflammation, already after 1 hour of ventilation [23]. In this study, tidal volumes were not measured.
Possibly, the higher concentration of heliox may have been beneficial. In our study, we choose to keep tidal volumes constant during the experiment while adjusting respiratory rate to maintain an adequate gas exchange, in order to dissect whether reduction of lung injury was due to heliox ventilation alone and not due to application of tidal volumes lower than 6 mL kg⁻¹. As tidal volume is a clear risk factor for ARDS [25,26] it is possible and even probable that further reducing tidal volumes during heliox ventilation would result in reduced lung injury.

An alternative explanation for the absence of an anti-inflammatory effect may be that our VILI model is mild. Although pulmonary protein leak and cytokine levels differed between VILI and LP controls, pulmonary edema and cell influx were unaltered. Of note, these tidal volumes are used in daily practice, so therefore the model is relevant to investigate the effect of gas mixtures on inflammatory and respiratory parameters [26]. Taken together, we conclude that in this relevant VILI model, heliox does not have anti-inflammatory properties. In line with this, heliox did not alter immune response of healthy volunteers stimulated ex vivo with various bacterial stimuli [27].

**Conclusion**

Heliox ventilation allowed for decreased minute volume ventilation in this rat VILI model. Whether helium has therapeutic potential in ARDS, by increasing adherence to protective ventilation strategies including lowering of tidal volume and use of limited inspiratory pressures, requires further investigation.
Reference list


Mechanical ventilation with heliox in an animal model of acute respiratory distress syndrome

Charlotte J. Beurskens; Hamid Aslami; Friso M. de Beer; Joris J.T.H. Roelofs; Margreeth B. Vroom; Nicole P. Juffermans

Intensive Care Medicine Experimental, February 2014; 2(8)
Abstract

*Background:* Heliox has a lower density and higher diffusion capacity compared to oxygen-in-air. We hypothesized that heliox ventilation allows for a reduction in minute volume ventilation and inspiratory pressures needed for adequate gas exchange in an animal model of acute respiratory distress syndrome.

*Methods:* After intratracheal instillation of lipopolysaccharide (1 mg/kg), adult rats were randomized to ventilation with either a gas mixture of helium/oxygen (50:50%) or oxygen/air (50:50%). They were mechanically ventilated according to the ARDSnet recommendations with tidal volumes of 6 ml/kg and monitored with a pneumotachometer. Bronchoalveolar lavage fluid was analyzed for markers of lung injury, and embedded lung sections were histologically scored for lung injury.

*Results:* Heliox limited the increase in driving pressures needed to achieve preset tidal volumes, with a concomitant decrease in loss of compliance. Heliox did neither allow for reduced minute volume ventilation in this model nor improve gas exchange. Also, heliox did not reduce lung injury.

*Conclusions:* Heliox modestly improved respiratory mechanics but did not improve lung injury in this rat model of acute respiratory distress syndrome.
Introduction

Obstructed airways with increased airway resistance and high inspiratory pressures needed for adequate gas exchange are common features in acute respiratory distress syndrome (ARDS) [1-3]. Limited tidal volume ventilation of 6 ml/kg is proven to be beneficial in ARDS [4]. However, application of limited tidal volume ventilation can often not be achieved in ARDS because of hypoxemia and acidosis [5, 6]. Also, application of relatively low plateau pressures and driving pressures can already be harmful [7, 8]. Thereby, mechanical ventilation may aggravate ARDS, the extent of which is affected by the intensity of ventilation. In ARDS, higher minute volumes are needed to compensate for increased oxygen demand and carbon dioxide production, increasing the risk of additional injury by mechanical ventilation. Therefore, in ARDS, adjunctive therapies which allow for less invasive ventilation are worth exploring.

Helium is an inert noble gas with a lower density compared to nitrogen. Thereby, mechanical ventilation using a gas mixture of oxygen and helium (heliox) can reduce turbulent gas flow and establish a more laminar flow [9]. This biochemical feature enables a reduction in the work of breathing in patients with obstructed airflow due to increased airway resistance during exacerbations of asthma and COPD [10]. Another important feature of heliox is the increased diffusion of CO₂ compared to air [9]. Consequently, heliox decreases inspiratory pressures required to establish a set gas flow and to enable gas exchange in distant alveoli [11, 12].

Use of heliox has been evaluated in paediatric animal models of ARDS induced by oleic acid or saline [13-15], showing that heliox ventilation improved gas exchange during high–frequency oscillatory ventilation. We recently showed that heliox improves CO₂ removal and decreases driving pressures in patients mechanically ventilated according to the recommended protective strategy, as well as in an animal model of lung injury inflicted by high tidal volume ventilation [16, 17].

In this study, we investigated the effects of heliox in an adult lung injury model. We hypothesized that the use of heliox reduces minute volume ventilation and inspiratory pressures with improved compliance. Furthermore, we hypothesized that the use of heliox facilitates CO₂ elimination during protective mechanical ventilation. Although without a clear mechanism known, heliox showed anti-inflammatory effects in ARDS
models in previous studies [14, 18], so the effect on the inflammatory response was also investigated.

Methods

Animal study design

The animal care and use committee of the Academic Medical Center, University of Amsterdam, Netherlands approved this study. Animal procedures were carried out in compliance with Institutional Standards for Use of Animal Laboratory Animals.

Induction of lung injury, anaesthesia, instrumentation and mechanical ventilation

Male Sprague-Dawley rats (Harlan, The Hague, The Netherlands), weighing 350-400 grams, were randomized to four experimental groups. Two groups were anesthetised using a trans–oral miniature nebulizer under light anesthesia (97% oxygen with 3% isoflurane) and intratracheally instilled with 1 mg/kg Escherichia Coli lipopolysaccharide (LPS) (L4131, 7.5 mg/kg, Sigma Aldrich, Steinheim, Germany). Control groups received no instillation. 2 Hours after LPS instillation, animals were anesthetised by intraperitoneal injection of 90 mg/kg ketamine (Nimatek®; Eurovet Animal Health BV, Bladel, NL), 0.125 mg/kg dexmedetomidine (Dexdomitor®; Orion Pharma, Espoo, Finland) and 0.05 mg/kg atropine (Atropine sulphate; Centrafarm B.V., Etten–Leur, the Netherlands). Via a tail vein venflon cannula, anaesthesia was maintained by infusion of 10 mg/ml ketamine at 2.7 ml per hour. A solution of saline and 4.2 mg/ml bicarbonate (Fresenius Kabi Nederland BV,’s Hertogenbosch, NL) was administered at 2.5 ml per hour.

A tracheotomy was performed and a metal cannula was inserted in the trachea. Two sutures were placed around the exposed part of the trachea into which the cannula was tied down thoroughly. The cannula was then connected to a ventilator (Servo 900C, Siemens, Sweden). The ventilators were calibrated for the heliox gas mixture according to the instruction of the manufacturer using a pressure reduction valve to allow the high-pressure of the heliox tank to be reduced to safe and usable pressures for ventilation (Linde Gas Therapeutics, Eindhoven, the Netherlands).

Hemodynamic parameters were monitored by inserting a polyethylene heparinised saline (1:1000) filled catheter into the right carotid artery (Braun, Melsungen, Germany) that was connected to a monitor (Siemens SC900, Danvers, USA). Temperature was monitored
Mechanical ventilation with heliox in an animal model of acute respiratory distress syndrome

Rats were ventilated in a pressure controlled mode for 4 hours, with either heliox (technical gas 50% oxygen; 50% helium; blended by Linde Gas Therapeutics) or 50% oxygen in air gas mixture. In total 32 animals were studied of which 16 received heliox (8 LPS; 8 Healthy controls) and 16 received 50% oxygen in air gas mixture (8 LPS; 8 Healthy controls).

Lung protective (LP) ventilation was maintained, according to a fixed protocol, by applying 6 ml/kg and 5 cmH₂O positive end-expiratory pressure (PEEP). FiO₂ was set at 50% with an inspiration to expiration ratio of 1:2 and adjustment of respiratory rate to maintain arterial PaCO₂ within 4.5-6.0 kPa, according to hourly drawn arterial blood gases (RAPIDlab 865 blood gas analyzer, Bayern, Mijdrecht, the Netherlands).

Tidal volumes were strictly maintained using a pneumotachometer (Hugo Sachs Elektronik, Harvard apparatus, March–Hugstetten, Germany) specific for rats. The pneumotachometer is a transducer for airflow measurement, placed between the metal cannula and the ventilator. For both heliox and oxygen ventilation the pneumotachometer was calibrated using a 1 mL syringe according to the manufacturer’s instruction. Tidal volumes were recorded using respiration software (HSE-BDAS basic data acquisition, Harvard apparatus, March–Hugstetten, Germany) and displayed on a computer screen throughout the whole experiment. We set a pressure controlled ventilation mode and started with an inspiratory pressure of 15 cmH₂O. Tidal volumes were targeted by adjusting the inspiratory pressure [17, 19-21]. The inspiratory pressures were recorded every hour. The driving pressure was calculated by inspiratory pressure minus PEEP. Compliance was calculated by dividing the tidal volume per kilogram by the driving pressure. Minute volume was calculated in millilitres per minute by multiplying respiratory rate with the measured tidal volume.

**Inflammation measurements**

After 4 hours of mechanical ventilation, rats were bled and plasma was centrifugated at 1800g for 10 minutes at 4°C. The lungs were removed en block and the right lung was ligated. A bronchoalveolar lavage was done by flushing the left lung with 3 times 2.0 ml NaCl, yielding approximately 5.5-6.0 ml of bronchoalveolar lavage fluid (BALF).
In BALF, cells were counted using a hemocytometer (Z2 Coulter Particle Counter, Beckman Coulter Corporation; Hialeah, Florida, USA). After centrifugation of BALF (300g; 10 min.; 4°C), protein levels were measured (Oz Biosciences, Marseille, France) and levels of Interleukin (IL)-1β, IL-6, cytokine-induced neutrophil chemoattractant (CINC)-3, and Tumor Necrosis Factor (TNF)-α were determined by ELISA in BALF and blood, according to instructions of the manufacturer (R&D Systems; Abingdon, United Kingdom).

The upper lobe of the right lung was fixed in 1% buffered formaldehyde and subsequently embedded in paraffin and afterwards cut into 5-μm-thick sections. Lung sections were fixed on glass slides and stained with hematoxylin and eosin. Lung sections were analyzed by a pathologist, who was blinded to group identity, with use of the total histology score. This score consists of several parameters, including interstitial inflammation, endothelialitis, edema, bronchitis, thrombus and pleuritis. All parameters were scored on a scale of 0–4: 0 for normal lungs, 1 for <25% lung involvement, 2 for 25–50% involvement, 3 for 50–75% involvement, and 4 for >75% lung involvement. The total histology score was calculated as the sum score of these parameters, with a maximum of 24. Furthermore, the pathologist was asked to choose one representative illustration per group.

**Statistical analysis**

To compare time points (T=0 vs. T=4) within the same subject, a paired T-test with Bonferroni correction was used if data were normally distributed, or Wilcoxon signed rank test in case of non-normal distribution. The effect of heliox versus oxygen-in-air at specific time points was compared using a one-way ANOVA or Kruskal Wallis test, with either a Bonferroni’s or Dunn’s multiple comparison test, depending on the distribution of the data. Statistical significance was considered to be at P <0.05 or at P<0.0125 after Bonferroni correction. Data are expressed as mean±SD.

**Results**

All animals survived our experimental protocol. In animals receiving LPS, mean arterial pressure dropped over time (162±19 to 128±18 mmHg), whereas mean arterial pressure remained constant in the healthy controls. Heliox did not affect blood pressure or heart rate compared to the oxygen-in-air ventilated animals.
**LPS induced acute lung injury**

The instillation of LPS intratracheally resulted in a sufficient model of acute lung injury, based on the guidelines described by the American Thoracic Society [22]. The inflammatory response, one of the main features that characterize acute lung injury in animal models, was evidenced by increased pulmonary cell influx in the BALF (Figure 1 A) and an increase in BALF cytokine levels of IL-1β, IL-6, TNF-α and CINC-3 (Figure 1 C-F). Protein levels in BALF were not significantly different, due to large variation (Figure 1B). Another important marker of lung injury in animals is the histological evidence of

**Figure 1:** Inflammatory parameters in an animal model of LPS-induced lung injury and healthy controls. The animals were ventilated with heliox or oxygen-in-air (N = 8 per group). (A) Cell count. (B) Protein levels. (C) IL-1β levels. (D) IL-6 levels. (E) TNF-α levels. (F) CINC-3 levels in BALF. Healthy animals are marked by open dots and LPS animals by black dots. Data are presented as mean ± SD. *P < 0.05; **P < 0.01; ***P < 0.001.

**Figure 2:** Total histology score in an animal model of LPS-induced lung injury and healthy controls. The animals were ventilated with heliox or oxygen-in-air (N = 8 per group). Healthy animals are marked by open dots and LPS animals by black dots. Data are mean ± SD. *P < 0.05; **P < 0.01.
tissue injury, which in our study measured is by the total histopathology and which also increased due to LPS instillation (Figure 2). The pictures, chosen by the pathologist, show a representative example of each intervention group, showing interstitial inflammation, endothelialitis, edema, bronchitis and pleuritis produced by the intratracheally LPS instillation.

Gas exchange at baseline and after 4 hours of lung protective mechanical ventilation with heliox or oxygen-in-air was also affected by LPS instillation. Baseline pH showed a significant decrease with a concomitant increase in PaCO₂ levels in LPS challenged animals compared to healthy controls (Table 1). These differences in gas exchange disappeared during the experiment, due to adjustments in respiratory rates. Applied respiratory rate needed to keep PaCO₂ within predefined limits (4.5-6.0 kPa) increased during mechanical ventilation in both LPS treated groups, yielding an increased percentage of change in minute volume ventilation versus the baseline measurement (Figure 3 A). Inspiratory pressures applied to target tidal volumes of 6 ml/kg were also adjusted during the experiment, resulting in higher inspiratory and driving pressures in LPS treated animals compared to healthy controls, with a significant increase in the animals ventilated with oxygen only (Figure 3 B/C). A concomitant decrease in percentage of change of compliance was seen in LPS treated animals compared to healthy controls, with again a significant increase in the animals ventilated with oxygen (Figure 3 D).

Table 1: Gas exchange in an animal model of LPS–induced lung; ventilated with heliox or oxygen-in-air. N = 8 per group. Data are presented as mean ± SD.

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Oxygen-in-air</th>
<th>Heliox</th>
<th>Oxygen-in-air</th>
<th>Heliox</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T = 0</td>
<td>7.44 ± 0.04</td>
<td>7.42 ± 0.03</td>
<td>7.35 ± 0.05&lt;sup&gt;A&lt;/sup&gt;</td>
<td>7.34 ± 0.08&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>T = 4</td>
<td>7.33 ± 0.04&lt;sup&gt;C&lt;/sup&gt;</td>
<td>7.36 ± 0.03&lt;sup&gt;D&lt;/sup&gt;</td>
<td>7.30 ± 0.06</td>
<td>7.30 ± 0.08</td>
</tr>
<tr>
<td>pCO₂ (kPa)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T = 0</td>
<td>4.38 ± 0.7</td>
<td>4.86 ± 0.6</td>
<td>5.78 ± 0.6&lt;sup&gt;A&lt;/sup&gt;</td>
<td>6.10 ± 1.4&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>T = 4</td>
<td>4.99 ± 0.4</td>
<td>5.81 ± 0.9</td>
<td>5.84 ± 0.77</td>
<td>5.75 ± 1.29</td>
</tr>
<tr>
<td>pO₂ (kPa)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T = 0</td>
<td>34.2 ± 1.8</td>
<td>31.7 ± 1.5</td>
<td>33.2 ± 1.7</td>
<td>32.3 ± 3.3</td>
</tr>
<tr>
<td>T = 4</td>
<td>31.7 ± 2.5</td>
<td>30.8 ± 2.8</td>
<td>28.5 ± 3.9</td>
<td>28.8 ± 3.4</td>
</tr>
</tbody>
</table>

<sup>A</sup> Oxygen-in-air LPS T = 0 vs. oxygen-in-air healthy T = 0, significant after Bonferroni’s multiple comparison test (P < 0.01); <sup>B</sup> Heliox LPS T = 0 vs. heliox healthy T = 0, significant after Bonferroni’s multiple comparison test (P < 0.01); <sup>C</sup> Oxygen-in-air healthy T = 0 vs. oxygen-in-air healthy T = 4, significant after Bonferroni correction (P < 0.0125); <sup>D</sup> Heliox healthy T = 0 vs. heliox healthy T = 4, significant after Bonferroni correction (P < 0.0125).
Effects of Heliox

Heliox did not result in a reduction of inflammatory parameters when compared to the oxygen-in-air ventilation. No effect was seen in the pulmonary neutrophil influx, protein or cytokine levels in BALF (Figure 1 A-F) or in the total histology score in the lung (Figure 2). Also, use of heliox did not alter gas exchange (Table 1). Comparing the change versus baseline, heliox did not influence minute volume ventilation needed for adequate gas exchange compared to oxygen-in-air ventilation (Figure 3 A). Heliox did however abrogate the increase in inspiratory and driving pressures compared to baseline needed to generate tidal volumes of 6 ml/kg in LPS treated animals compared to oxygen-in-air (Figure 3 B/C). Concomitantly, the relative decrease in compliance in LPS treated animals was abrogated with heliox ventilation versus oxygen-in-air ventilation (Figure 3 D).

Discussion

In this animal model of induced acute lung injury due to LPS instillation, heliox abrogated the increase in inspiratory and driving pressures needed to target pre-set tidal volumes over time. Furthermore, heliox diminished the decrease in compliance compared to
baseline in LPS treated animals. In contrast to our hypothesis, heliox did not allow for a reduction in minute volume ventilation during lung-protective mechanical ventilation. Moreover, heliox ventilation showed no effect on neither gas exchange or on lung inflammation. Thereby, our results are somewhat in contrast to studies investigating heliox ventilation in paediatric models as well as to findings in our own animal model of ventilator-induced lung injury, where heliox improved CO₂ removal [13-17].

There may be several explanations for the absence of a beneficial effect of heliox in our model. Within our study design, we strictly regulated tidal volumes at 6 ml/kg. This study design was chosen to reduce confounders, influencing our results. With this design, we believe any measured effect of heliox on lung injury could be ascribed to heliox only and not to a difference in tidal volumes. This distinction is important, because as a result of a more laminar flow pattern compared to oxygen-in-air ventilation, the mechanism by which heliox improves CO₂ removal might be by an increased tidal volume delivery. In line with this, it was shown that during high frequency oscillatory ventilation in an animal ARDS model, heliox did not alter gas exchange if tidal volume was kept constant [23]. If the findings would also be applicable during protective mechanical ventilation, based on the characteristics of heliox, we hypothesized a decrease in inspiratory pressures needed to generate a set tidal volume, as found before [12]. However, our effects on inspiratory and driving pressures, as well as the compliance were only modest. Since we cannot exclude that heliox may be beneficial by allowing for a further reduction of tidal volumes, which was shown before to increase protection in ARDS [24-26], the choice for strictly keeping tidal volumes of 6 ml/kg in these experiments limits the interpretation of our results.

An alternative explanation for differences with previous studies may be related to differences in ventilation modes. The previous studies that showed a beneficial effect of heliox were all performed in paediatric animal models, mostly during high frequency oscillatory ventilation [13-15]. This same mode was also used in infants, showing a decreased airway resistance during heliox ventilation [27].

Differences may also relate to the underlying disease state. Whereas airway obstruction is present in our model of acute lung injury, the severity of airway obstruction is far less compared to asthma, respiratory syncytial virus or other disease states in which hyper-reactivity of bronchi plays an important role [27-29]. In line with this, in a test lung, the effect of heliox on reducing the inspiratory effort was shown to be dependent on the
kind of obstruction and severity [30]. Our model may also have been a too mild model of lung injury, in which beneficial effects may be hard to tease out. However, our model is clinically relevant and represent the most important parameters of acute lung injury in animals [22].

The anti-inflammatory effects of heliox, previously reported in ARDS models [14, 18], could not be reproduced in our model. A clear mechanism on how heliox can affect lung injury markers and histology scoring is not known, however in healthy volunteers helium resulted in an attenuated expression of inflammatory cell surface markers on leukocytes and platelets in blood [31]. It is postulated that the influence of helium might be via cell-mediated immunity [32]. Furthermore, ventilation with heliox might lower shear stress and barotraumas and therefore have an anti-inflammatory effect [14]. However, our results indicate that there is no direct anti-inflammatory effect due to heliox ventilation.

**Conclusion**

Heliox modestly limited the increase in inspiratory and driving pressures applied to target pre-set tidal volumes, with a concomitant restraint of the reduced compliance between LPS treated animals and healthy controls. However, heliox ventilation did not allow for lower minute volume ventilation, nor did it have an effect on gas exchange, lung inflammation or lung damage in an animal model of acute lung injury.
Mechanical ventilation with heliox in an animal model of acute respiratory distress syndrome

Reference list


Helium ventilation for treatment of post cardiac arrest syndrome: a safety and feasibility study

Daniel Brevoord; Charlotte J. Beurskens; Walter M. van den Bergh; Wim K. Lagrand; Nicole P. Juffermans; Jan M. Binnekade; Benedikt Preckel; Janneke Horn

Submitted
Abstract

Background: The only treatment available for comatose patients after cardiac arrest is induced hypothermia. Helium reduces ischemic injury in animal models, and might ameliorate neurological injury in patients after cardiac arrest. As no studies exist on the use of helium in patients after cardiac arrest we investigated whether this is safe and feasible.

Methods: The study was an open-label single arm intervention study, in a mixed-bed academic intensive care unit. 25 patients with a presenting rhythm of ventricular fibrillation or pulseless tachycardia, return of spontaneous circulation within 30 minutes and treatment with hypothermia after circulatory arrest were included. Helium was administrated in a 1:1 mix with oxygen for 3 hours. A safety committee reviewed all ventilation problems, complications and mortalities. Outcome and mortality data were compared with matched historical control patients.

Results: Helium ventilation was started 4:59±0:52 (mean ± SD) hours after circulatory arrest. In one patient, helium ventilation was discontinued prematurely due to oxygenation problems. This was caused by pre-existing pulmonary oedema, and there was no relation with helium ventilation. Sixteen (64%) patients had a favourable neurological outcome.

Conclusions: We found that helium ventilation is feasible and can be used safely in patients treated with hypothermia after cardiac arrest. No adverse events related to helium ventilation occurred during the three hours of ventilation. There was no difference in outcome between helium treated patients and matched controls.
Introduction

Out of hospital cardiac arrest is a major cause of morbidity and mortality, afflicting 335 per million per year in the Netherlands with an overall mortality of 81%1. Half of the patients admitted to the intensive care unit (ICU) leave the hospital with an unfavourable neurological outcome3-3. Circulatory arrest and subsequent return of circulation leads to ischaemia reperfusion injury of the whole body and is particularly injurious to the brain and myocardium4. Brain injury is the major cause of mortality and morbidity after cardiac arrest5. Therefore, patients admitted after cardiac arrest should receive treatment aimed at reducing brain injury as part of the post-resuscitation care. The only effective treatment currently available is mild hypothermia6,7. Despite this therapy, outcome results are disappointing and therapies to further reduce ischaemia reperfusion injury after cardiac arrest are needed.

The noble gas helium reduced ischaemia reperfusion injury in both in vitro and in vivo animal models, thereby reducing e.g. myocardial infarct size8-14. Inhalation of helium before, during or following a period of cerebral ischaemia also reduced the size of cerebral infarction in rats8-14. Helium might be capable of reducing neurological and myocardial injury in patients after cardiac arrest, but no clinical studies in this field have been done. Clinically, helium is used to ventilate both adults and children with severe obstructive pulmonary disease and helium inhalation is generally considered to be safe15. Recently we demonstrated that helium induces preconditioning in healthy volunteers, thereby protecting against endothelial dysfunction after regional forearm ischaemia16. Prior to investigating the use of helium as a therapeutic agent in neurological damaged patients, we performed a safety and feasibility study, investigating whether helium ventilation can safely be used in patients admitted to the ICU after cardiac arrest.

Methods

This was an open-label single arm intervention study, performed in the mixed surgical-medical ICU of a university hospital. The study was approved by the local medical ethics committee of the Academic Medical Centre (protocol number NL 30466.018.09) and was conducted in concordance with the principles of the declaration of Helsinki and good clinical practice. The study was registered with the Dutch Trial Registry (www.trialregister.nl) under NTR2257. Patients were included after obtaining informed consent from their legal representative.
Inclusion criteria were admission after witnessed out of hospital cardiac arrest (OHCA), with the first registered rhythm being ventricular fibrillation (VF) or tachycardia (VT) and treatment with mild hypothermia. Return of spontaneous circulation (ROSC) had to occur within 30 minutes and helium ventilation had to be started within 6 hours after cardiac arrest. Exclusion criteria were oxygenation problems (necessitating a FiO₂ >50% and >10 mmHg positive end expiratory pressure [PEEP]), neurological deficits or severe disability before cardiac arrest, and comorbidities with a life expectancy of less than 6 months. The described ventilation settings were limits during the study-protocol as well.

As a control group, propensity-score matched patients were selected from the PROPAC II study database. For every helium treated patient three controls were selected who met the same inclusion and exclusion criteria, including presenting rhythm, and a propensity score was calculated by logistic regression analysis using age, gender and duration of circulatory arrest as variables.

**Study procedures**

After inclusion, helium ventilation was initiated as soon as possible. Helium was administered from a pressurised cylinder containing 1780 L heliox (Heliox21, BOC Ltd, UK), as a 50/50 helium/oxygen mixture, using a heliox compatible Servo-I ventilator (Maquet, Netherlands). Helium ventilation was done in pressure control mode, peak pressure was set to achieve a tidal volume of 6 ml/kg ideal body weight, with 5-10 mmHg of PEEP and the respiratory frequency was controlled to maintain a pCO₂ of 4.5-5.5 kPa and a pH of 7.35-7.45 (alpha-stat). A pO₂ of ≥10 kPa and a saturation of ≥95% were aimed for. After switching to helium, a setup period with repeated blood gas analysis was used to reach the target values for pCO₂ and pH. When these measurements were within the normal limits helium ventilation was continued during a 3-hour period. Since the objective of this study was to investigate the safety and feasibility, and not the effectiveness, of helium ventilation, helium ventilation was stopped if the cylinder was empty before the end of the 3-hour period.

Data collected were age, gender, Body Mass Index (BMI), simplified acute physiology score II (SAPS II), acute physiology and chronic health evaluation score II (APACHE II), pre-existent cardiovascular disease or malignancy, cause of arrest, time until first shock, time to ROSC, the use of coronary angiography and percutaneous coronary interventions and the need for hemodynamic support at admission.
Serum samples for analysis of creatine kinase (CK), creatine kinase muscle-brain (CK-MB) and troponin-T were drawn at admission and at 6, 12, 18, 24, and 48 hours. Serum samples for analysis of neuro specific enolase (NSE) levels were drawn 24 and 48 hours after admission. NSE serum samples were centrifuged and stored at -20 until analysis by immunoassay (kit for ELECSYS, Roche).

Outcome was assessed by telephone interview of the patient or caregiver 30 days after admission. The Glasgow Outcome Scale (GOS) was used; poor outcome was defined as death or vegetative state (GOS 1-2). For matched control patients, outcome after one month and NSE levels 48 hours after admission to the ICU were available from the PROPAC II database.

Primary objective of the study was to investigate the safety and feasibility of helium administration in patients after cardiac arrest. Safety endpoints were the inability to adequately ventilate the patient using helium within the predetermined limits (FiO₂ 50% and ≤10 mmHg PEEP), and death related to helium. To determine the probability of an adverse event being related to helium treatment all serious adverse events were evaluated by an independent safety committee, consisting of an intensive care physician, an anaesthesiologist and a neurologist.

Secondary objectives were to investigate the effect of helium ventilation on outcome (GOS), brain injury (NSE) and cardiac injury (CK, CK-MB, and troponin-T).

**Statistics**

There is no data on the effectiveness or the occurrence of adverse events of helium treatment in patients after OHCA. Therefore, a formal sample size calculation could not be performed. We expected a mortality rate of approximately 50%, and therefore chose to include 25 patients, to be able to detect an increase in adverse events related to helium. This is also a sample size which is used in similar studies.

SPSS 19 (IBM, Armonk, New York, USA) was used for statistical analysis unless stated otherwise. Continuous data are presented as mean with standard deviation when normally distributed, and otherwise as median and interquartile range, while categorical data are presented as numbers with proportions. For comparison of outcome between cases and controls the odds ratio with 95% confidence interval was calculated using a conditional logistic regression analysis, using STATA 10.0 (StataCorp, College Station, Texas, USA). Student’s t-test or Mann-Whitney U test was used for comparison of NSE values between cases and controls. Statistical significance was defined as p ≤ 0.05.
Results

Between April 2010 and October 2011, 106 patients admitted after OHCA were screened for eligibility, of which 64 patients were not eligible, 13 patients were eligible but were missed by the physician on call, in four patients study participation was refused by the legal representative, and finally 25 patients were included (fig. 1). Baseline characteristics of patients and matched controls are presented in table 1.

Figure 1: Flow schedule of patients

Helium ventilation was started 4:59±0:52 (mean ± SD) hours after arrest, and 21±13 (mean ± SD) minutes was used to reach target values for pCO₂ and pH. After that, helium ventilation was continued for a total of 3:10±39 (mean ± SD) hours. In six patients the treatment was stopped prematurely; in five patients the heliox cylinder was empty before completion of the 3 hour treatment protocol, due to high minute volumes needed and the duration of the adjustment period. In one patient, ventilation with helium was terminated prematurely. This patient had slight hypoxia at the time of inclusion due to pulmonary oedema following cardiac arrest, requiring 10 cmH₂O PEEP and a FiO₂ of 50%
to maintain an oxygen saturation (sO₂) of >90% and a PaO₂ of 8.4 kPa. Shortly after the initiation of helium ventilation, the sO₂ dropped to 84% and the PaO₂ to 7.1 kPa, and it was decided to discontinue the study protocol and switch back to a normal gas mixture. Only after increasing FiO₂ to 70% and PEEP to 12, oxygenation improved in this patient. These ventilation settings had to be maintained for several days. As the hypoxia was pre-existing and persisting, the safety committee concluded that the ventilation disorders were not caused by the short use of helium.

Table 1: Baseline characteristics of patients and matched controls.

<table>
<thead>
<tr>
<th></th>
<th>Patients (25)</th>
<th>Controls (75)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex</td>
<td>20 (80%)</td>
<td>61 (81%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>64.8 ± 12.1</td>
<td>61.8 ± 13.2</td>
</tr>
<tr>
<td>BMI* (kg/m²)</td>
<td>27.4 ± 4.8</td>
<td></td>
</tr>
<tr>
<td>SAPS II score†</td>
<td>53.6 ± 18.6</td>
<td></td>
</tr>
<tr>
<td>APACHE II score‡</td>
<td>20.0 ± 8.6</td>
<td></td>
</tr>
<tr>
<td>Comorbidity Cardiovascular disease</td>
<td>14 (56%)</td>
<td>41 (55%)</td>
</tr>
<tr>
<td>Malignancy</td>
<td>4 (16%)</td>
<td>7 (9%)</td>
</tr>
<tr>
<td>Cause of OHCA§ Acute Infarction</td>
<td>17 (68%)</td>
<td></td>
</tr>
<tr>
<td>Chronic Infarction</td>
<td>4 (16%)</td>
<td></td>
</tr>
<tr>
<td>Structural Heart Disease</td>
<td>3 (12%)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>1 (4%)</td>
<td></td>
</tr>
<tr>
<td>Time to 1st shock (min)</td>
<td>8 ± 7</td>
<td></td>
</tr>
<tr>
<td>Time to ROSC ll (min)</td>
<td>16 ± 7</td>
<td>16 ± 8</td>
</tr>
<tr>
<td>CAG **</td>
<td>20 (80%)</td>
<td></td>
</tr>
<tr>
<td>PCI ††</td>
<td>15 (60%)</td>
<td></td>
</tr>
<tr>
<td>IABP ‡‡ or Impella</td>
<td>9 (36%)</td>
<td></td>
</tr>
<tr>
<td>Inotropics or vasopressors</td>
<td>12 (48%)</td>
<td></td>
</tr>
</tbody>
</table>

Data are mean ± SD or percentages.
* Body Mass Index, † Simplified Acute Physiology Score II, ‡ Acute Physiology and Chronic Health Evaluation II, § Out-of-Hospital Cardiac Arrest, ll Return of spontaneous circulation, ** Coronary Angiography, †† Percutaneous Coronary Intervention, ‡‡ Intra-aortic Balloon Pump
Nine patients died within 30 days (36%); in all patients post-anoxic brain injury was the cause of death. None of these deaths were related to helium ventilation. At 30 days follow-up, the surviving 16 patients (64%) all had a favourable outcome, 13 patients (81%) resided at home, two patients (13%) in a rehabilitation centre and one patient was still hospitalized (6%).

In the propensity matched historic control group, 30-day mortality was 36% and 69% of the patients had a favourable outcome at one month follow-up. Compared to controls, the odds ratio (OR) for mortality was 0.87 (95% CI 0.31-2.4) in patients treated with helium; the OR for poor outcome was 1.3 (0.47-3.7).

Serum levels of CK, CK-MB, troponin-T and NSE of helium treated patients are presented in table 2. Helium treated patients had a mean NSE value of 44±51 µg/L at 24 hours, and 54±94 µg/L at 48 hours after arrest, compared to 33±55 µg/L for controls at 48 hours (p=0.22).

Table 2: Laboratory data of helium patients.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>6 hrs</th>
<th>12 hrs</th>
<th>18 hrs</th>
<th>24 hrs</th>
<th>48 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK* (U/L)</td>
<td>340 ± 314</td>
<td>1205 ± 961</td>
<td>1627 ± 1263</td>
<td>1963 ± 2115</td>
<td>1828 ± 1775</td>
<td>1556 ± 1749</td>
</tr>
<tr>
<td>CK-MB† (µg/L)</td>
<td>22 ± 27</td>
<td>128 ± 238</td>
<td>169 ± 241</td>
<td>180 ± 248</td>
<td>154 ± 223</td>
<td>64 ± 74</td>
</tr>
<tr>
<td>Troponin (µg/L)</td>
<td>0.3 ± 0.4</td>
<td>1.0 ± 0.76</td>
<td>2.2 ± 2.7</td>
<td>1.8 ± 2.6</td>
<td>1.6 ± 1.8</td>
<td>1.3 ± 1.4</td>
</tr>
<tr>
<td>NSE‡ (µg/L)</td>
<td></td>
<td>44 ± 51</td>
<td>54 ± 94</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are mean ± SD.

* Creatinine Kinase, † Creatinine Kinase Muscle-Brain, ‡ Neurospecific Enolase

Discussion

This is the first study focusing on organ protective effects of helium in patients after cardiac arrest. We found that helium ventilation is feasible and can be used safely in patients treated with hypothermia after OHCA. No adverse events related to the helium ventilation occurred during the three hours of ventilation with this noble gas.

These results might open the door to a new treatment of brain injury following cardiac arrest. Helium might reduce the reperfusion injury, but in this small study, we found no indication for that. However, the study was not powered to study the ability of helium to
reduce organ injury and larger studies are needed to investigate the potential therapeutic value of helium in organ protection following ischemia reperfusion.

Although the mortality rate is lower than values normally reported in the literature for ICU patients admitted after cardiac arrest, this is probably due to the patient selection. We included patients who had a witnessed arrest, presented with VF or VT, and had a resuscitation time of thirty minutes or less, all factors that have a positive effect on outcome. When compared to matched historical controls, the helium treated patients had similar mortality rate and clinical outcome. NSE levels, used as marker for brain injury, were also comparable between treated patients and controls.

Comparison of our results to studies with helium or other noble gasses in patients after cardiac arrest is not possible, as this has never before been studied. Only animal studies have been performed showing conflicting results regarding neuroprotective properties of helium. In an in vitro model of traumatic brain injury helium had a protective effect, and in an in vivo rat model using MCA occlusion helium inhalation reduced infarct size. More positive effects in a MCA occlusion model were reported, but this protective effect was only seen when the animals were allowed to cool down in a flow chamber. The authors suggested that the protection was mediated by the induction of hypothermia. Finally, two studies in neonatal rats in which one common carotid artery was temporally occluded, demonstrated neuroprotection by helium. Other studies did not find a beneficial effect of helium on cerebral injury. In an in vitro model using oxygen glucose deprivation to induce brain injury helium provided no beneficial effect. Another study using a model of MCA occlusion found that helium only provided protection when given directly at the time of reperfusion, and in an inspired fraction of 70%. Until today, the exact underlying mechanisms mediating possible organ protective effects of helium are still unclear.

Other noble gasses have also been used as neuroprotective agents. In a pig model of cardiac arrest, xenon reduced brain injury. The neuroprotective effects of xenon are currently being investigated in ongoing clinical trials (NCT00934700, NCT00879892, ISRCTN75602528).

It is known that the results of animal studies investigating neuroprotection in different animal models are difficult to translate to the human situation. Many neuroprotective drugs have been studied in stroke patients, based on positive animal experiments, but no
effective drug has ever been found for humans\textsuperscript{26,27}. A large difference with focal ischaemic stroke models is that in patients after OHCA the vasculature of the brain is intact and open. As soon as circulation is restored, neuroprotective agents can easily reach the brain cells and perform their actions.

We chose to start with a small study, which makes conclusions about possible effectiveness insignificant and might underestimate the side effects of helium ventilation. Especially longer periods of helium ventilation, which might be needed for an optimal treatment effect, could lead to more ventilation problems. This would be the logical topic to address in a subsequent study.

Second, the open-label use of helium inadvertently introduces a risk for bias, however by using endpoints that are not influenced by observer interpretation (mortality, vegetative state and laboratory assessments) the risk for observer bias was reduced.

Third, the setting of a single ICU of a university hospital limits extrapolation of the results. However, since the objective of the study was to investigate the safety and feasibility, we feel that these limitations are of minor concern at this stage. All patients were ventilated with 50% helium in order to give the same dosage. This also meant that all patients received 50% oxygen, regardless of their oxygenation status, which could lead to supranormal oxygen tensions in some patients. A high PaO\textsubscript{2} during or after cardiac arrest has been linked to an increase in mortality, and might influence a beneficial effect of helium\textsuperscript{28-30}.

To summarize, we demonstrated for the first time that helium ventilation for three hours is safe and feasible in patients after OHCA. This might open the route for further studies investigating the effectiveness of this new organ protective treatment modality.
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Heliox improves carbon dioxide removal during lung protective mechanical ventilation

Charlotte J. Beurskens; Daniel Brevoord; Wim K. Lagrand; Walter M. van den Bergh; Margreeth B. Vroom; Benedikt Preckel; Janneke Horn; Nicole P. Juffermans

Submitted
Abstract

Introduction: Helium is a noble gas with a low density. This allows lower driving pressures in mechanical ventilation and increased carbon dioxide (CO₂) diffusion. We hypothesized that heliox facilitates ventilation in adult patients during lung–protective mechanical ventilation using low tidal volumes.

Methods: Prospective observational cohort sub study of an open label single arm intervention study. Twenty four patients were included, who were admitted to the ICU after a cardiac arrest. Patients were mechanically ventilated for 3 hours with heliox (50% helium; 50% oxygen) during a fixed protective ventilation protocol (6 ml/kg), with prospective observation for changes in lung mechanics and gas exchange. Statistics by Bonferroni post correction with statistical significance set at P<0.02.

Results: During heliox ventilation, respiratory rate was decreased (25±4 vs. 23±5 L min⁻¹, P=0.01). Minute volume ventilation showed a trend to decrease compared to baseline (11.1±1.9 vs. 9.9±2.1 L min⁻¹, P=0.03), while reducing PaCO₂ levels (5.0±0.6 vs. 4.5±0.6 kPa, P=0.02) and peak pressures (21.1±3.3 vs. 19.8±3.2 cmH₂O, P=0.02).

Conclusions: Heliox improved CO₂ elimination while allowing reduced minute volume ventilation in adult patients without lung injury during protective mechanical ventilation.
Introduction

Helium is an inert gas with lower density than air (1), allowing for less turbulent flow through airways, leading to lower airway resistance. As a result, during mechanical ventilation with a helium/oxygen mixture (heliox), lower driving pressures are needed to distribute oxygen to the distal alveoli compared to ventilation with oxygen (2). Also, diffusion of carbon dioxide (CO₂) is increased during heliox, which in addition might facilitate ventilation. Due to these properties, there may be a rationale to use heliox in patients with severe pulmonary disease with respiratory failure in whom protective mechanical ventilation with low tidal volumes is not feasible due to the development of respiratory acidosis e.g. in acute respiratory distress syndrome (ARDS). Until date, use of heliox is clinically applied using high frequency ventilation in paediatric patients (3,4) and in patients with high airway resistance due to severe asthma, most often also in children (5,6). Clinical data on adult patients during conventional mechanical ventilation are limited.

The aim of this study was to investigate the effect of heliox on gas exchange as part of a safety and feasibility study on the potential of heliox ventilation to improve neurological outcome after cardiac arrest (7). We hypothesized that the use of heliox also allows for increased CO₂ elimination in adults during conventional mechanical ventilation with low tidal volumes.

Methods

The study was approved by the local medical ethics committee of the Academic Medical Center, University of Amsterdam, the Netherlands (protocol number NL 30466.018.09) and conducted in concordance with the principles of the declaration of Helsinki and good clinical practice. The study was registered with the Dutch Trial Registry (www.trialregister.nl) under NTR2257. From all patients or their legal surrogate written informed consent was obtained. It was a prospective observational cohort sub study of an open label single arm intervention study, performed in the mixed surgical-medical intensive care unit (ICU) of a tertiary referral center in Amsterdam, the Netherlands. From April 2010 to October 2011, patients admitted to the ICU after cardiopulmonary resuscitation (CPR) because of a witnessed out-of-hospital cardiac arrest, were included in the study after informed consent was given by their relatives. Inclusion criteria were return of spontaneous circulation within 30 minutes of arrest and coma on admission. Exclusion
Heliox improves carbon dioxide removal during lung protective mechanical ventilation

Criteria were hypoxemia with a need for ventilation with a FiO2 higher than 50% or more than 10 cmH2O positive end–expiratory pressure (PEEP), pregnancy, severe disability, a neurological disorder or co–morbidity with life expectancy of less than 6 months.

During heliox treatment patients were mechanically ventilated in a pressure controlled mode, using a Servo–I ventilator, which was adjusted and calibrated for heliox ventilation. Helium (Linde Gas Therapeutics, Eindhoven, the Netherlands) was mixed with oxygen to achieve a concentration of 50% helium and 50% oxygen. Respiratory settings were modified using a study protocol. Respiratory rate was adjusted to target pH of 7.35 – 7.45 and PaCO2 of 4.5 – 5.5 kPa, with an inspiration to expiration (I:E) ratio of 1:2, while maintaining a tidal volume of 6 ml/kg predicted body weight. No changes were made to I:E ratio, FiO2 and PEEP levels during heliox treatment. After 3 hours, heliox was switched back to oxygen in air and patients were ventilated according to our standard ICU protocol with tidal volumes of 6 ml/kg predicted body weight. All patients were treated with therapeutic hypothermia (32°C–34°C) as part of standard care in patients with decreased consciousness after CPR. Target temperature had been reached by the time heliox ventilation was initiated and was maintained during heliox ventilation. For sedation, propofol and opiates were used. Neuromuscular relaxants were given as a bolus, but only during shivering.

Respiratory parameters were measured over time, starting just prior to heliox ventilation (T=−1), within 15 minutes after start heliox (T=0), during heliox treatment (T=1 – T=3) and until 3 hours after heliox was switched back to oxygen in air (T=4 – T=6). Dynamic compliance was measured during heliox ventilation (T=1 – T=3), as this was a read–out at the Servo–I ventilator only. Resistance was calculated by dividing the pressure difference by air flow per minute and PaCO2 / end tidal CO2 gradient by dividing the difference between PaCO2 and end tidal CO2 by PaCO2. Arterial blood gas analysis was determined hourly (Alpha stat, RAPIDLab 1200, Siemens, Deerfield, USA).

**Statistical analysis**

Data are expressed by mean and SEM. Time points within the same subjects were compared using paired T–test or Wilcoxon signed rank test, depending on distribution of the data. A total of three comparisons were made between several time points (T=−1 vs. T=0; T=0 vs. T=3; T=3 vs T=6). Using Bonferroni post correction, statistical significance was set at P<0.02.
Results

106 patients were screened, of whom 29 were eligible. Of these, informed consent was refused in 4 cases. Of 25 included patients, heliox was discontinued within 15 minutes in one patient due to hypoxemia, requiring a PEEP level above 10 cm H₂O. This patient was excluded from further analyses. In the remaining 24 patients, of whom 83% was male with a mean age of 64.9±12.3 years, no acute infections were present at start of the study, 1 patient suffered from COPD, no other lung pathology was reported. During the study protocol, no changes in hemodynamics were observed.

Due to the switch of ventilation gas mixture from oxygen (T=−1) to heliox (T=0), respiratory settings needed adjustment according to the study protocol with limited tidal

Figure 1: Respiratory parameters during heliox ventilation for 3 hours (T=0 to T=3) and after switch to normal oxygen in air mixture (T3 to T6). Measurements started prior to heliox administration (T=−1). Data are MEAN ± SEM. (A) Minute volume ventilation (L min⁻¹); (B) respiratory rate (breaths min⁻¹); (C) peak pressure (cm H₂O); (D) PaCO₂ / end tidal CO₂ gradient (mmHg); (E) airway resistance (cm H₂O mL⁻¹ sec⁻¹) and (F) lung compliance (ml cm⁻¹ H₂O). *: P<0.02
volume ventilation. Minute volume ventilation slightly rose after switching from oxygen to heliox, but no significant difference was found between before and right after the start of heliox ventilation (figure 1). Thereafter, during heliox ventilation, respiratory rates were adjusted to targeted pH and PaCO₂ levels, in accordance with the study protocol. This resulted in a significant decrease in respiratory rate and tended to decrease minute volume ventilation (figure 1). Tidal volumes remained stable at 6 ml/kg according to study and standard ICU protocol and did not change over time (data not shown). Peak pressures could be decreased during heliox ventilation (figure 1). PaCO₂ / end tidal CO₂ gradient increased immediately after start of heliox ventilation, but showed no effect over time (figure 1). Airway resistance and dynamic compliance by the ventilator did not change during heliox ventilation (figure 1).

Switch of oxygen in air to heliox ventilation resulted in a rapid decrease in PaCO₂ levels, which increased again at discontinuation of heliox (figure 2). Also end tidal CO₂ decreased immediately after applying heliox ventilation and increased again after heliox discontinuation (figure 2). Both PaCO₂ levels and end tidal CO₂ showed no changes during the 3 hours of heliox ventilation (figure 2). In agreement with an increased CO₂ elimination, an increase in pH to 7.37 was seen shortly after the application of heliox.

Figure 2: Gas exchange during ventilation with heliox for 3 hours (T=0 to T=3). Measurements started just prior to heliox administration (T= –1) until 3 hours after heliox discontinuation (T=3 to T=6). Data are MEAN ± SEM. (A) PaCO₂ (kPa); (B) end tidal CO₂ measurements (kPa); (C) pH measured hourly and (D) PaO₂/FiO₂ ratio (mmHg). *: P < 0.02; **: P < 0.01; ***: P < 0.001.
Heliox improves carbon dioxide removal during lung protective mechanical ventilation (figure 2). In the course of heliox ventilation, pH tended to increase further. Oxygenation was not altered significantly after start of heliox or after switching back to oxygen (figure 2).

**Discussion**

In adult patients ventilated with protective mechanical ventilation strategy according to current ventilation guidelines (8), use of heliox improved ventilation, by allowing lower minute volume ventilation while PaCO₂ levels decreased.

The use of heliox ventilation has been mostly investigated in respiratory conditions such as upper–airway obstruction, asthma, bronchiolitis and croup. Results indicate that heliox improves gas exchange and reduces work of breathing (4-6). Most of the studies were performed in the pediatric population. In this study we focussed on adult patients. Cardiac arrest patients are obviously not the patients who are expected to benefit most from lowering minute volume ventilation, because these patients do not have obstructed airflow. Nevertheless this population was studied, since the feasibility study investigating neuroprotective properties of heliox (7), enabled us to investigate the response to long-term heliox ventilation in adult patients ventilated with pressure controlled ventilation modes and currently recommended protective settings. The reduction of respiratory rate and the decrease of peak pressures during heliox ventilation are promising results. However, it remains to be determined whether heliox is beneficial in patients with respiratory failure in whom protective ventilation is hampered by the development of respiratory acidosis.

Our study has several limitations. As this study was a secondary analysis of a safety and feasibility study on the use of heliox in cardiac arrest patients, the number of patients was not primarily powered to investigate the effects of heliox on ventilation. This may explain observed trends but absence of statistical significance. However, our data clearly show an increased CO₂ removal and improved ventilation, starting immediately after start of heliox ventilation. Long term effects could not be studied as heliox ventilation was limited to 3 hours. Another limitation may be that all patients received therapeutic hypothermia, which is known to decrease PaCO₂ levels (9). However, throughout the whole study period, temperatures were in the range of therapeutic induced hypothermia. Thereby, observed effects could not be due to hypothermia.
Furthermore, the capnography was not adjusted for heliox ventilation, which may have resulted in underestimation of end tidal CO₂ (10) and increase in PaCO₂ / end tidal CO₂ gradient after start of heliox. However, as PaCO₂ levels decreased and end tidal CO₂ remained stable during heliox ventilation, we believe increased CO₂ removal can be endorsed to the use of heliox.

**Conclusions**

Heliox ventilation improved CO₂ elimination and allowed for decreased minute volume ventilation, in patients ventilated in a pressure controlled mode according to the guidelines of the ARDS network (8). Results suggest that heliox may be a therapeutic possibility in patients in whom protective mechanical ventilation is hampered by the development of respiratory acidosis.
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Summary and general discussion

Charlotte J.P. Beurskens and Nicole P. Juffermans
Mechanical ventilation is applied on a daily basis in the intensive care unit and thus ventilator-induced lung injury (VILI) is a well-known problem in critically ill patients. Although a protective ventilation strategy, using low tidal volumes is adopted worldwide [1], VILI still is an important issue [2, 3], with a staggering mortality rate of 60% in patients suffering from acute respiratory distress syndrome (ARDS) [4, 5]. Therefore, new therapies are warranted to target VILI. We investigated induced hypothermia and heliox ventilation as potential therapeutic strategies to reduce the intensity of mechanical ventilation. The rationale behind the application of induced hypothermia and heliox ventilation is extensively discussed in chapter 1.

**Induced hypothermia**

Part I of this thesis explores the possibility of induced hypothermia as a strategy to reduce VILI.

First, we investigated the actual metabolic state in critically ill patients, by reviewing the literature on energy expenditure in specific patients population in chapter 2. We learned that energy expenditure differs greatly among critically ill patient populations, as well within patient groups. In general, higher disease severity scores were associated with a higher metabolic state and the use of induced hypothermia seemed to decrease energy expenditure. Thereby, results seem to confirm the concept that metabolism during severe critical illness is high and is responsive to induced hypothermia. Another important finding in chapter 2 was that the use of a ‘one size fits all’ formula to estimate caloric need in the critically ill might not be appropriate because of the large variation within the patient groups. Therefore, given the variety of energy expenditure, it is advisable to measure metabolic demands by calorimetry for each patient individually.

In chapter 3, a clinically relevant model of VILI was used, with normal acid base balances, to study effects of induced hypothermia. Hypothermia reduced lung injury, which was independent from an adjustment on ventilator settings. This suggested that hypothermia does not ameliorate atelectotrauma, but has a direct anti-inflammatory effect in VILI. The hypothesized results on the reduction on lung injury by adjusting ventilator settings were not confirmed. Therefore our second hypothesis that hypothermia may reduce the inflammatory response was endorsed. Whether a reduction in tidal volume reduces barotrauma remains to be established.

The effect of induced hypothermia on ventilator settings was further explored in a clinical trial in hypothermic patients in chapter 6. Induced hypothermia improved ventilation
and allowed for lower driving pressures and PEEP levels during mechanical ventilation, while maintaining lung protective ventilation settings. It could not be determined in this study whether hypothermia also reduced lung injury, as these patients did not have lung injury. However, with these results we show that hypothermia is an effective intervention to lower the intensity of mechanical ventilation and underline our rationale of applying induced hypothermia to reduce VILI.

In chapter 4 we studied the effect of induced hypothermia on mitochondrial function. During sepsis, mitochondrial function is severely impaired, which is thought to be due to inflammatory damage to the respiratory chain subunits. We found improvement of the mitochondrial function, reflected by high ATP availability and increased oxidative phosphorylation of ADP to ATP. The efficiency of the mitochondrial function was unaffected by induced hypothermia. All this suggests that mitochondrial damage inflicted by inflammation is mitigated by the application of induced hypothermia.

The hypothesis that mitochondria may be better preserved due to hypothermia is underlined by findings in chapter 5. In cardiac arrest patients, induced hypothermia decreased levels of circulating mitochondrial DNA in the hypothermic patients. Within this small study population (N=10 vs. N=6), no effects on outcome could be measured. However, given that mitochondrial DNA is a marker of tissue damage [43], reduction of circulating mitochondrial DNA may reflect less damage to the mitochondria due to an inhibition of the inflammatory response.

Taken together, these clinical results, combined with the experimental data, suggest induced hypothermia reduces the inflammatory response and therefore the mitochondrial damage. As ATP availability and ATP turnover increase, this undermines the theory of an adaptive response of mitochondrial ‘shutdown’, with reduced ATP demand and less oxygen consumption.

Concerns remain about the risk of infection, when using induced hypothermia. In the infectious model of Streptococcus pneumoniae pneumosepsis in chapter 4, hypothermia (32°C) reduced markers of lung injury, while ventilation settings were unaltered. Interestingly, hypothermia also decreased bacterial dissemination. These results point towards a preserved endothelial-epithelial barrier function by reduction of injury. It could, however, not be determined whether this beneficial effect remains after rewarming and ultimately affects outcome. Thereby, the safety of inducing hypothermia during fulminant infection remains a concern.
Chapter 7 describes the results of induced hypothermia on immune response to bacterial antigens. We found that patients after cardiac arrest have a systemic inflammatory response compared to healthy controls, associated with an attenuated immune response to bacterial antigens. These findings resemble what is termed ‘immune paralysis’ in sepsis patients. Apparently, the same phenomenon is present in patients who have survived a cardiac arrest and suffered a sterile inflammatory response. These findings may have relevance, as they suggest that cardiac arrest patients are prone to develop nosocomial infection. Another important finding of this study is that hypothermia did not alter immune response to bacterial antigens compared to normothermia. As host response to bacterial antigens is critical in combatting infections, we believe that induced hypothermia itself does not increase risk of infection.

Conclusion

We believe to have established that hypothermia reduces lung injury in both sterile (chapter 3) and infectious (chapter 4) experimental models of VILI and ARDS. The reduced lung injury is in line with extensive research in animal models of several hyper-inflammatory conditions [6-13]. In these models hypothermia showed a reduction in organ failure that was associated with a decrease of the pro-inflammatory cytokines levels [6-13]. We ascribed these findings to a protective effect on mitochondrial function. This finding warrants further exploration of mitochondria as a therapeutic target.

In our clinical studies, induced hypothermia also showed a reduction of the systemic inflammatory response (chapter 7), but without compromising the immune response to bacterial antigens. These results are in contrast with clinical data in cardiac arrest patients that show induced hypothermia was an independent risk factor for infection [14]. Also a recent systematic review of patients, treated with therapeutic hypothermia for any indication, showed an association of hypothermia with increased prevalence of pneumonia and sepsis, although the overall infection rate was not affected [15]. However, these studies were performed without a normothermic control group, as was done in our studies (chapter 5; 7). Taken together, our results suggest that although a critically ill patient is more prone to infection by a decreased immune response, this risk of infection is not further increased by induced hypothermia. As induced hypothermia has shown to improve ventilation (chapter 6), hypothermia could be a potential strategy to lower intensity of mechanical ventilation in patients in whom protective ventilation is not feasible.
Recently it was shown that induced hypothermia (33°C) did not further improve neurological outcome after cardiac arrest, compared to a target temperature of 36°C [16]. Therefore, it might be only thermoregulation and maintaining normothermia that is sufficient to avoid the ‘overshoot’ of the systemic inflammatory response.

With regards to VILI, induced hypothermia can reduce the baro- and biotrauma, but whether induced hypothermia or just maintaining normothermia is protective, needs to be determined in future research.

**Heliox ventilation**

Part II of this thesis describes the possibility of heliox ventilation as a strategy to reduce VILI.

In chapter 8, available data on the effect of application of helium ventilation in animal ARDS models and clinical studies of critically ill patients with ARDS or respiratory failure due to ARDS-like syndromes were reviewed. In both animal models and clinical studies, heliox improved gas exchange, while allowing for less invasive ventilator settings. Studies predominantly focussed on neonatal and paediatric patient populations. Also outcome parameters were usually short-termed and concentrate on lung mechanics. Therefore, although potentially promising in ARDS, the effect of heliox on clinically relevant outcomes is not unequivocally proven.

Until now, heliox is often applied in patients with respiratory failure due to severe asthma or COPD to avoid endotracheal intubation and mechanical ventilation. In these patient populations work of breathing and elevated airway resistance are of more importance, compared to the ARDS patient population. Therefore, we believe the effect of heliox is more outspoken in these patient populations. Nevertheless, ARDS patients do have increased airway resistance with inadequate gas exchange, resulting in the need of mechanical ventilation with larger tidal volumes and higher inspiratory pressures to prevent the development of respiratory acidosis.

Data on the effect of heliox in ARDS are limited to paediatric models. To determine whether heliox ventilation exerts effects in a specific cause of lung injury, two models of lung injury were used in this dissertation. In chapter 9 in a clinically relevant VILI model, heliox ventilation allowed for a reduction in minute volume ventilation, while maintaining normal acid-base balance. Heliox did not allow for a decrease in driving pressures, possibly because this model was too mild. In a more severe ARDS animal
model, induced by intratracheal instillation of lipopolysaccharide (chapter 10), heliox ventilation allowed for a decrease in driving pressures needed to achieve the pre-set tidal volumes. Taken together, these experimental data suggest that heliox can reduce the intensity of mechanical ventilation and maintain protective ventilation in ARDS.

In chapter 11 and 12, clinical data was gathered on the effect of heliox on patients during conventional mechanical ventilation. Heliox ventilation in critically ill patients did not result in negative side effects or desaturation (chapter 11). Also, the delivery of tidal volumes was done accurately as the used ventilator was adjusted and calibrated. This is relevant, as the reduced gas density of heliox, compared to oxygen-in-air, results in an underestimated flow and tidal volumes, when the ventilator is not calibrated. When taking this into account, helium ventilation can be safe and is feasible in patients on conventional mechanical ventilation.

In chapter 12, we looked in more detail to the effect of heliox on ventilation in cardiac arrest patients during conventional mechanical ventilation. Heliox ventilation improved CO₂ elimination and allowed for decreased minute volume ventilation and peak pressures. Although this patient population is not our patient group of interest, this study provides proof that heliox allows for a reduction in ventilator settings. Therefore, heliox ventilation may be a therapeutic possibility in patients in whom protective mechanical ventilation is hampered by the development of respiratory acidosis.

**Conclusion**

The results of heliox ventilation on VILI and ARDS are modest, suggesting the models used were not representative for ARDS. However, in both animal models, parameters described as clinical relevant for animal models of ARDS were present [17]. Since lung injury is caused by a variety of insults and patients with acute lung injury constitute of a very heterogeneous patient population [18, 19], we chose two different animal models. The model of VILI (chapter 9) induced by barotrauma is clinically relevant, because all patients who are mechanically ventilated are at risk of developing ventilator-associated lung injury [20]. The ARDS animal model (chapter 10) is a frequently used method to mimic indirect lung injury, resulting from a systemic inflammatory response syndrome after for example trauma or major surgery. Although in our clinical studies, patients did not have pre-existing lung injury (chapter 12), heliox ventilation still resulted in less invasive mechanical ventilation by reducing the minute volume ventilation and used
pressures. These results are of relevance, since most data on the use of heliox ventilation is done in paediatric animal models or patient populations (chapter 8).

Taken together, in both preclinical and clinical studies, heliox allows for a reduced intensity of mechanical ventilation. However, our effects of heliox ventilation are small compared to the effects of heliox in asthma and COPD. We believe this can be ascribed to the fact that in ARDS, airway resistance plays a less important role than in exacerbations of asthma or COPD. Also therefore, we feel that our data do not point towards large studies in the future to investigate heliox as a therapy in patients with severe ARDS. Such studies would require too large numbers of patients to yield an effect of heliox ventilation. However, since heliox ventilation is proven to be safe and feasible, we believe heliox could be applied as a rescue therapy in individual patients who are suffering from respiratory failure. In these patients, usually ventilated with injurious large tidal volumes and pressures to maintain an adequate gas exchange, heliox ventilation could reduce the intensity of mechanical ventilation.
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Dutch summary / Nederlandse samenvatting

Charlotte J.P. Beurskens
Op de intensive care wordt een groot deel van de patiënten kunstmatig beademd. Zij zijn hiertoe zelf niet meer toe in staat, meestal door een ernstige ziekte of na een operatie. Hoewel deze mechanische beademing noodzakelijk is en levensreddend kan zijn, is het in het afgelopen decennium ook duidelijk geworden dat beademing, ook schadelijk kan zijn. De schade die door mechanische beademing wordt veroorzaakt wordt ook wel VILI (Ventilator-Induced Lung Injury) genoemd.

Tegenwoordig zijn er richtlijnen die moeten voorkomen dat de longen teveel beschadigd worden tijdens beademing. Het is helaas niet altijd mogelijk deze richtlijnen te volgen bij alle patiënten die op de intensive care worden opgenomen. Met name bij de mensen die al beschadigde longen hebben, in de vorm van ARDS (Acute Respiratory Distress Syndrome), is het moeilijk om deze richtlijnen te volgen. Het is echter van groot belang dat bij alle patiënten op de intensive care de schade door beademing wordt teruggebracht. Met die reden hebben wij onderzocht of het koelen van patiënten tot 32 graden (hypothermie) dan wel het beademen van patiënten met helium en zuurstof (heliox) een oplossing zou kunnen bieden voor dit probleem. Dit proefschrift beschrijft het effect van zowel hypothermie als beademing met heliox als interventies met als doel de schade die mechanische beademing met zich meebrengt te beperken.

Voor beide strategieën wordt de rationale besproken in hoofdstuk 1. De rationale bij koeling komt er kort gezegd op neer dat het koelen van de patiënt mogelijk de schade door mechanische beademing beperkt, omdat het de ontstekingsreactie die ontstaat bij longschade zou kunnen verminderen. Er wordt echter gedacht dat koelen het risico op infectie kan vergroten door het dempen van de afweerreactie van het lichaam. Bovendien kan koeling ervoor zorgen dat het metabolisme van een patiënt minder snel werkt. Bij beademing met heliox geldt dat helium een edelgas is, met als bijzondere eigenschap dat het in vergelijking met zuurstof een kleinere dichtheid heeft. Dit betekent dat als je een patiënt beademt met helium en zuurstof, dit gasmengsel veel gemakkelijker de longen in stroomt en minder weerstand genereert dan wanneer een patiënt beademd wordt met stikstof en zuurstof. Doordat de weerstand kleiner is bij de beademing met heliox zou ook de schade door mechanische beademing kunnen afnemen.
Deel 1 – Hypothermie

In deel 1 beschrijven we de mogelijkheid om koeling in te zetten als strategie om longschade te verminderen. Het begint met hoofdstuk 2, waarin we de literatuur hebben nagezocht op studies die de verschillen in metabolisme beschrijven tussen verschillende groepen patiënten die op de intensive care worden opgenomen. Het blijkt dat de verschillen tussen deze groepen erg groot zijn. In alle groepen kwam wel naar voren dat bij ernstig zieke patiënten het metabolisme doorgaans sneller werkte en dat bij patiënten die gekoeld worden het metabolisme werd geremd.

In hoofdstuk 3 en 4 worden diermodellen beschreven waarin we longschade hebben gesimuleerd, door met schadelijk teugvolumina te beademnen of door de dieren een longinfectie te geven. In beide modellen werd aangetoond dat koeling de longschade verminderde. Naast het testen van onze hypothese in diermodellen, hebben we ook verschillende patiëntenstudies uitgevoerd. Deze worden beschreven in hoofdstuk 5, 6 en 7. In hoofdstuk 5 hebben we geprobeerd het mogelijke mechanisme van het gunstige effect van koeling uit te diepen. We hebben gekeken naar de mitochondriën. Mitochondriën reguleren de zogenaamde energiehuishouding van cellen en zijn vaak aangetast tijdens ziekte. In zowel ons diermodel van hoofdstuk 4 als bij de patiënten in hoofdstuk 5, lijkt het erop dat de energiehuishouding intact blijft tijdens ziekte, wanneer koeling wordt toegepast.

In hoofdstuk 6 hebben we het effect van koeling onderzocht in patiënten die beademend worden volgens de huidige richtlijnen van beademing op een beschermende manier. Het bleek dat koeling de intensiteit van de beademing verminderde en de richtlijnen gehandhaafd konden blijven in deze patiënten. Hoewel we niet de longschade zelf hebben onderzocht, tonen we hiermee wel aan dat koeling een mogelijkheid kan zijn om de intensiteit van beademing te verminderen in patiënten bij wie beschermende beademing met lage drukken niet mogelijk is vanwege zieke longen.

De eerder genoemde risico’s van koeling hebben we onderzocht in hoofdstuk 7. Daar hebben we bekeken of er een groter infectierisico is en of de afweer aangetast wordt tijdens koeling. We hebben patiënten vergeleken die gekoeld waren tot 33°C met patiënten die op 36°C gehouden werden. Deze studie toont aan dat: na een hartstilstand er een verminderde afweer is. Echter gezien het feit dat er geen verschil in afweer was tussen de twee groepen, vermindert koelen de afweer niet verder, wat doet vermoeden dat koeling op zichzelf niet het infectierisico vergroot. Dit is een belangrijke conclusie,
die ervoor zorgt dat koeling overwogen kan worden als strategie om longschade te verminderen.

Concluderend kunnen we zeggen dat koeling gunstige effecten heeft op de schade van de beademing en dat het gevaar op infectie lijkt mee te vallen. Wij denken dan ook dat koeling een strategie kan zijn om de intensiteit van beademing te verminderen en daarmee de longschade.

**Deel 2 – Heliox beademing**

In **deel 2** beschrijven we de mogelijkheid van heliox beademing als strategie om longschade te verminderen. Ook in dit deel beginnen we in **hoofdstuk 8** met een overzicht van de literatuur over heliox in patiënten met longschade. Het blijkt dat deze literatuur zich vooral richt op diermodellen met jonge dieren en kinderen met longschade. Hierdoor is het moeilijk om een conclusie te trekken over het effect van heliox beademing in volwassen patiënten. In dit hoofdstuk blijkt echter wel dat alle onderzoeken de rationale van heliox beademing als therapie bij longschade, op basis van de kleinere dichtheid ten opzichte van zuurstof, onderschrijven.

Om aan te tonen dat heliox beademing verlichting zou kunnen bieden bij longschade in volwassenen, hebben we twee diermodellen opgezet. In het diermodel in **hoofdstuk 9** wordt longschade door mechanische beademing aangebracht in de longen van volwassen dieren. In het diermodel in **hoofdstuk 10** worden de longen aangetast door een component van de celwand van bacteriën, met een steriele infectie tot gevolg. In zowel het in **hoofdstuk 9** als het in **10** beschreven onderzoek zorgt heliox beademing ervoor dat de longen met minder hoge drukken beademd konden worden. Hoewel het effect van heliox beademing in **hoofdstuk 9** en **10** is aangetoond, konden we geen verschil waarnemen in daadwerkelijke longschade. Dat de effecten van heliox niet heel groot zijn komt mogelijk omdat de weerstand in de longen in deze diermodellen niet groot genoeg is. Wanneer in diermodellen de weerstand in de luchtwegen groter is, heeft heliox naar verwachting meer profijt van zijn lagere dichtheid en is het te verwachten effect groter.

We hebben ook patiënten onderzocht op het effect van heliox beademing. **Hoofdstuk 11** beschrijven we een studie waarin we hebben onderzocht of heliox beademing überhaupt mogelijk is voor langere tijd in volwassen patiënten. Daar concluderen we dat het veilig is om patiënten die zijn opgenomen op de Intensive Care, drie uur lang te beademen met heliox.
Het effect van heliox op de beademing diepen we verder uit in hoofdstuk 12. De patiënten die we daar hebben onderzocht hadden geen zieke longen, waardoor de effecten van heliox niet heel erg groot zijn. Desalniettemin zien we wel dat heliox gemakkelijker de longen in stroomt en de intensiteit van de beademing wordt verminderd.

Concluderend denken wij dat beademing met heliox zeker potentie heeft, maar dat dit alleen werkt in longen die een sterk vergrote weerstand vertonen in de luchtwegen. Aangezien dit niet het geval is in de meeste patiënten die opgenomen worden op de intensive care, zien wij heliox beademing op dit moment meer als laatste redmiddel dan als een standaard strategie om longschade te voorkomen.

Daarmee is de algehele conclusie van dit proefschrift dat het effect van koeling van patiënten een mogelijkheid zou kunnen zijn om de longschade die mechanische beademing met zich meebrengt te verminderen en dat het beademen met heliox eerder zou kunnen fungeren als een uitzonderlijke therapie in patiënten met zeer ernstig zieke longen.
Appendices

Acknowledgements / Dankwoord
PhD Portfolio
Publications
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Acknowledgement / Dankwoord

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Charlotte
PhD Portfolio

Name          C.J.P. Beurskens
PhD period    February 2010 – February 2014
PhD supervisors Prof. M.B. Vroom, MD; PhD
               Prof. M.J. Schultz, MD; PhD
               N.P. Juffermans, MD; PhD

PhD training

Courses
2010          AMC World of Science
               Introduction to Medical Microbiology
               Laboratory Animals
               BROK (‘Basiscursus Regelgeving Klinisch Onderzoek’) Crash course
2011          Scientific Writing in English for Publication
               Oral presentation
               Practical Biostatistics
               Basic Laboratory safety
2012          Clinical Data Management
               Project management

Conferences & presentations
2011          ISICEM, Brussels
               Poster presentation “Induced hypothermia is protective in a rat model of pneumococcal pneumonia”
2011          ESICM, Berlin
               Oral presentation “Induced hypothermia increases ATP availability and turn-over in a rat model of pneumococcal pneumosepsis”
2012          ATS, San Francisco
               Poster presentation “Heliox Allows For A Reduction In Respiratory Minute Volume In A Rat Model Of Ventilator-Induced Lung Injury”
               Poster presentation “Heliox Ventilation Improves Ventilation In Patients With Induced Hypothermia”
2013  ESICM, Paris
Poster presentation “Mechanical ventilation with heliox in a rat model of Acute Respiratory Distress Syndrome”
Poster presentation “The effect of induced therapeutic hypothermia on lung mechanics in mechanically ventilated patients”

2014  ISICEM, Brussels
Poster presentation “Induced hypothermia does not affect the immune or inflammatory response”

Other activities
2010-2013  APROVE Board membership
2011  APROVE Symposium “Insane in the Brain”
2012  APROVE Symposium “Science and Scams”
2012-2013  AMC Graduate School Board membership
2012-2013  Editor booklet “Promoveren doe je zo”
Publications


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Curriculum Vitae

Charlotte Beurskens was born on the 12th of March 1985 in Heerlen. In 2003 she graduated from secondary school at the Bernardinus College in Heerlen. She started her medical training at the University of Amsterdam and obtained her medical degree in January 2010. During her study she was an active member of the medical faculty association and the student association Orionis. In 2007 she stayed 6 months in Istanbul, Turkey to perform research at the Institute for Experiment Medicine at the Genetic department of the University of Istanbul.

In February 2010 she started her PhD training at Laboratory of Experimental Intensive Care and Anesthesiology (L.E.I.C.A.) and the Department of Intensive Care in the Academic Medical Center in Amsterdam, under the supervision of Prof. Dr. M.B. Vroom, Prof. Dr. M.J. Schultz and Dr. N.P. Juffermans. After 4 years, this resulted in her dissertation titled “Potential therapeutic strategies aimed at reducing intensity of mechanical ventilation in ARDS”, which she will defend at the 20th of June 2014. During her PhD she acted as a member and chair of the board of APROVE, the PhD association of the AMC. Also she joined the AMC Graduate School Board and contributed as an editor and writer to the booklet “Promoveren doe je zo”, published for beginning PhD students.

In September 2014 she will be starting her anaesthesiology residentship at Academical Medical Center, University of Amsterdam, the Netherlands under the supervision of Prof. Dr. W.S. Schlack.