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

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Induced hypothermia reduces circulating mitochondrial DNA in cardiac arrest patients

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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 In the 36°C group (n=6), blood was withdrawn at the same time intervals.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 analyst 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: ATCACATGGCTAGGCGGAG), NADH1 (forward: ATACCCATGGCCAACCTCCT, reverse: GGCGCTTTGCGTAGTTGTAT), 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>
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<th>36°C group (n=6)</th>
<th>33°C group (n=10)</th>
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<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>
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<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>
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</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
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


Reference List