Effects of hypothermia on the liver in a swine model of cardiopulmonary resuscitation

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BACKGROUND: The study aimed to explore the effects of hypothermia state induced by 4 °C normal saline (NS) on liver biochemistry, enzymology and morphology after restoration of spontaneous circulation (ROSC) by cardiopulmonary resuscitation (CPR) in swine.

METHODS: After 4 minutes of ventricular fibrillation (VF), standard CPR was carried out. Then the survivors were divided into two groups: low temperature group and normal temperature group. The low temperature (LT) group (n=5) received continuously 4 °C NS at the speed of 1.33 mL/kg per minute for 22 minutes, then at the speed lowering to 10 mL/kg per hour. The normal temperature (NT) group (n=5) received NS with normal room temperature at the same speed of the LT group. Hemodynamic status and oxygen metabolism were monitored and the levels of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) were measured in blood samples obtained at baseline and at 10 minutes, 2 hours and 4 hours after ROSC. At 24 hours after ROSC, the animals were killed and the liver was removed to determine the Na⁺-K⁺-ATPase and Ca²⁺-ATPase enzyme activities and histological changes under a light or electron microscope.

RESULTS: Core temperature was decreased in the LT group (P<0.05), while HR, MAP and CPP were not significantly decreased (P>0.05) compared with the NT group (P>0.05). The oxygen extraction ratio was lower in the LT group than in the NT group (P<0.05). The serum levels of ALT, AST and LDH increased in both groups but not significantly in the LT group. The enzyme activity of liver ATP was much higher in the LT group (Na⁺-K⁺-ATPase enzyme: 8.64±3.32 U vs. 3.28±0.71 U; Ca²⁺-ATP enzyme: 10.92±2.12 U vs. 2.75±0.78 U, P<0.05). The LT group showed less cellular edema, inflammation and few damaged mitochondria as compared with the NT group.

CONCLUSION: These data suggested that infusing 4 °C NS continuously after ROSC could quickly lower the core body temperature, while maintaining a stable hemodynamic state and balancing oxygen metabolism, which protect the liver in terms of biochemistry, enzymology and histology after CPR.

KEY WORDS: Therapeutic hypothermia; Cardiac arrest; Liver; Hemodynamics

INTRODUCTION

Cardiac arrest (CA) is often followed by serious hepatic dysfunction and multiple organs dysfunction syndrome (MODS), which cause a low survival rate. Thus, it makes sense to protect the liver to avoid liver damage during cardiopulmonary resuscitation (CPR). It has recently been confirmed, through many clinical trials, that therapeutic hypothermia could protect brain function and improve neurological outcome. However, there is few studies on whether therapeutic hypothermia after CPR can protect the liver. Therefore, we used a swine CA model, and after resumption of spontaneous circulation (ROSC), induced the hypothermic state by continuous infusion of 4 °C normal saline (NS). We
assessed the changes in hemodynamic and oxygen metabolism, and compared the difference in hepatic function, enzyme activity and histology, in an attempt to provide a basis for the use of 4 °C NS after ROSC.

METHODS

Animal preparation

All trials conformed to the specific guidelines developed by the local government for animal experimentation. Twelve domestic swine of either sex, weighing 28±2 kg, were used in this study; their care was in accordance with our institutional guidelines. Before the experiment, the swines were fasted, but had free access to water for one day. Anesthesia was induced by ear vein injection of propofol (0.2 mL/kg), and maintained with intravenous infusion of 3% pentobarbital (30 mg/kg) at a rate of 8 mg/kg per hour. A cuffed 6.5 mm endotracheal tube was advanced into the trachea and the animals were mechanically ventilated with a volume-controlled ventilator (Sero 900c, Siemens, Germany), using a tidal volume of 15 mL/kg, respiratory frequency of 18 breaths/min, and room air. Respiratory frequency was adjusted to maintain end tidal Pco\textsubscript{2} (EtPc\textsubscript{2}) at 35–40 mmHg. A 7F central venous catheter (Arrow, USA) was advanced from the right external jugular vein into the right atrium to measure right arterial pressure (RAP). A 7F sheathing canal was inserted to the left internal jugular vein as a temporary pacemaker conductor. A Swan-Ganz catheter (Edwards, USA) was advanced from the left femoral vein into the pulmonary artery and connected to a vigilance II CCO monitor (Edwards, USA). A 5F catheter (Terumo, Japan) was inserted from the right femoral artery into the aortic arch to measure aortic pressure (AOP).

Experimental protocol

The temporary pacemaker conductor was inserted into the right ventricle through the right sheathing canal, and connected to an electrical stimulator (GY-600A, China) programmed in the S1S2 mode (300/200 ms), 40 V, 8:1 proportion and 10 ms step length, to provide continuous electrical stimulus until ventricular fibrillation (VF). VF was defined as an ECG showing waveforms corresponding to VF and a rapid decline in MAP towards zero. Ventilation was stopped while inducing VF. After 4 min of VF, manual CPR was carried out at a frequency of 100 compressions/min with mechanical ventilation at FiO\textsubscript{2} 100% and a compression-to-ventilation ratio of 30:2. After 2 minutes of CPR, if the spontaneous circulation was not restored, defibrillation was attempted once using a diphase 150 J. If spontaneous circulation was still not achieved, CPR was continued for further 2 minutes and defibrillation was attempted again. ROSC was defined as an unassisted pulse with a systolic arterial pressure exceeding 80 mmHg for more than 5 minutes. If spontaneous circulation was not restored within 15 minutes, we regarded the animal as dead.

The survivors with ROSC were randomly placed into two groups (n=5/group), low temperature (LT) group and normal temperature (NT) group. The LT group was continuously treated with 4 °C NS at 1.33 mL/kg per minute for 22 minutes, then slowed the speed to 10 mL/kg per hour. The NT group was continuously treated with NS with normal temperature as the same speed used in the LT group. The animals were monitored for 4 hours, and killed at 24 hours after ROSC by pentobarbital overdose.

Measurements

Hemodynamic parameters, including core temperature (T), heart rate (HR), cardiac output (CO), aortic systolic pressure (AOS), aortic diastolic pressure (AOD), right atrial systolic pressure (RAS) and right atrial diastolic pressure (RAD), were measured continuously and we recorded the values at baseline and at 10 minutes, 30 minutes, 1 hour, 2 hours, 3 hours and 4 hours after ROSC. Blood samples were obtained at baseline and 2 hours, 4 hours after ROSC to analyze blood gas, and the serum levels of ALT, AST and LDH.

Other parameters including coronary perfusion pressure (CPP), mean arterial pressure (MAP), oxygen supply (DO\textsubscript{2}), oxygen consumption (VO\textsubscript{2}), oxygen extraction ratio (ERO\textsubscript{2}), systemic vascular resistance (SVR) and pulmonary vascular resistance (PVR) were calculated using standard formulae.

After the animals were killed at 24 hours after ROSC, the liver was excised and tissue samples were immediately frozen at −70 °C until the measurement of the activities of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase and Ca\textsuperscript{2+}-ATPase enzyme. Enzyme activity was assessed by measuring the optical density of Pi decomposed from ATP by the tissue protein using an enzyme-linked immunosorbent assay. Na\textsuperscript{+}-K\textsuperscript{+}-ATPase and Ca\textsuperscript{2+}-ATPase activity was determined using standard formulae. The remaining tissue was preserved in 10% formaldehyde and 4% paraformaldehyde to observe pathological changes under a light microscope and changes in tissue ultramicrostructure under a transmission electron microscope (TEM) (Hitachi H-600, Japan). The
pathologic data were assessed by reviewers blinded to the experimental groups.

**Statistical analysis**
Measurement data were expressed as means±SD, and were analyzed by Student's t test with SPSS version 11.5. Values of \( P<0.05 \) were considered statistically significant.

**RESULTS**

**Hemodynamic status and oxygen metabolism**
Two of the twelve animals died during CPR, ten survived. Compared to the NT group, temperature in the LT group was obviously decreased after ROSC, which was particularly noticeable from 30 minutes to 4 hours after ROSC \( (P<0.05) \), while HR, MAP and CPP were not significantly decreased \( (P>0.05) \). CO was decreased after ROSC in the LT group \( (P<0.05) \), but just as similar as that at each time point in the NT group \( (P>0.05) \). The \( \text{DO}_2 \) decreased after ROSC in both groups, while \( \text{VO}_2 \) decreased more obviously in the LT group \( (P<0.01) \). The oxygen extraction ratio in the LT group was significantly lower than that in the NT group at 2 and 4 hours after ROSC \( (P<0.05) \) (Figure 1).

**ALT, AST and LDH**
After ROSC, the level of AST tended to increase in

![Graphs showing temperature, CO, CPP, SVR, DO<sub>2</sub>, VO<sub>2</sub>, and ERO<sub>2</sub> changes](image-url)
Table 1. Changes of AST, ALT and LDH in the LT and NT groups after CPR (mean±SD)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>n</th>
<th>Baseline</th>
<th>ROSC 10 min</th>
<th>2 h</th>
<th>4 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NT</td>
<td>5</td>
<td>66.00±8.60</td>
<td>116.25±15.65</td>
<td>141.75±8.14</td>
<td>175.00±14.31</td>
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<tr>
<td>LT</td>
<td>5</td>
<td>69.50±12.04</td>
<td>114.75±3.82</td>
<td>119.75±6.95</td>
<td>126.25±11.15</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NT</td>
<td>5</td>
<td>44.25±7.37</td>
<td>49.75±3.10</td>
<td>50.25±4.99</td>
<td>52.00±5.89</td>
</tr>
<tr>
<td>LT</td>
<td>5</td>
<td>40.00±6.68</td>
<td>42.50±10.38</td>
<td>42.75±9.54</td>
<td>46.00±8.76</td>
</tr>
<tr>
<td>LDH (U/L)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NT</td>
<td>5</td>
<td>672.25±90.60</td>
<td>829.25±74.20</td>
<td>1010.75±143.87</td>
<td>1240.00±116.62</td>
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<tr>
<td>LT</td>
<td>5</td>
<td>640.75±52.81</td>
<td>699.00±82.00</td>
<td>705.75±51.33</td>
<td>984.00±68.72</td>
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</tbody>
</table>

Compared with baseline, *P<0.05, †P<0.01; compared with the NT group, ∆P<0.05, ∆P<0.01.

Table 2. Changes of hepatic ATP enzyme activity in the LT and NT groups (mean ±SD)

<table>
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<tr>
<th>Parameters</th>
<th>n</th>
<th>ROSC 24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺-K⁺-ATP enzyme (U)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NT</td>
<td>5</td>
<td>3.274±0.710</td>
</tr>
<tr>
<td>LT</td>
<td>5</td>
<td>8.614±3.317</td>
</tr>
<tr>
<td>Ca²⁺-ATP enzyme (U)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NT</td>
<td>5</td>
<td>2.746±0.778</td>
</tr>
<tr>
<td>LT</td>
<td>5</td>
<td>10.918±2.122</td>
</tr>
</tbody>
</table>

Compared with baseline, *P<0.05, †P<0.01.

the NT group which was significant at 2 and 4 hours after ROSC (P<0.01). Though the level of AST after ROSC was increased more significantly in the LT group than the baseline value, it was significantly lower than that in the NT group at 4 hours after ROSC (P<0.05). And so was LDH. However, the level of ALT was not changed much (Table 1).

**Hepatic ATP enzyme activity**

The hepatic ATP enzyme activity in the LT group was increased more significantly than that in the NT group (P<0.05) (Table 2).

**Hepatic histology**

Under a light microscope, hepatic cells in the NT group showed marked edema, pyknosis and necrosis. Furthermore, we noted narrowing hepatic sinusoid, extensive infiltration and adherence of inflammatory cells in the hepatic lobule. In contrast, the hepatic cells were largely normal, and there were fewer inflammatory cells in the LT group (Figure 2). Under a transmission electron microscope (TEM), the NT group showed indistinct mitochondria, with increased electron density. However, normal mitochondria morphology was shown in the LT group (Figure 3).

Figure 2. Hepatic pathology of the LT and NT groups after CPR. NT: A, B; LT: C, D. A: Pyknosis and necrosis of hepatic cells; B: Narrowing hepatic sinusoid, extensive infiltration and adherence of inflammatory cells in the hepatic lobule; C: Hepatic cells were tight; D: Hepatic cells were tight.

Figure 3. Hepatic ultra microstructure of the LT and NT groups after CPR. NT: A, B; LT: C, D. A: A necrotic hepatic cell; B: The morphous of mitochondria in hepatic cell was irregular, and electron density increased; C: A normal hepatic cell; D: The morphous of mitochondria was regular, with mitochondrial cristae.
DISCUSSION

Recently, therapeutic hypothermia has been more generally used in patients surviving CPR, and cold saline infusion (CSI) is thought to be the most effective method to induce hypothermia.\[7\] Although there have been many successful studies on cerebrum protection by hypothermia after CPR, there are few reports on whether hypothermia protects the liver. Therefore, we induced the hypothermic state after ROSC by intravenous infusion of 4 ºC NS, and compared with a control group given NS at a 'normal' temperature in an attempt to determine its effect on the liver.

Our results demonstrated that continuous infusion of 4 ºC NS after ROSC lowered the temperature by 1.5 ºC in a short period of time. This is similar to Johanna Nordmark's study, in which the core temperature was lowered by 1.6 ºC.\[6\] In the present study, mean arterial pressure, cerebral perfusion pressure and systemic vascular resistance did not exhibit any changes after ROSC, maintaining the stable hemodynamic status, and avoiding aggravation of cardiac dysfunction after ROSC. The reason may be related to decreased metabolic rate due to hypothermia, which causes a parallel decrease in heart rate.\[9\] The oxygen supply in the LT group decreased, while the oxygen consumption decreased to a greater extent, thus the oxygen uptake in the LT group was still lower than that in the NT group. It was reasonable to reduce oxygen metabolism under low blood pressure conditions and low cardiac output state after ROSC so as to satisfy the oxygen supply during organ perfusion and avoid post-cardiac arrest syndrome, thereby improving organ function. The serum levels of AST and LDH in the NT group increased post-ROSC, which is due to the ischemic, swell, and necrosis of hepatic cells after CPR. In the LT group, however, the levels of AST and LDH were significantly lower, because oxygen consumption of hepatic cells decreased during the hypothermia state. Thus it could enable hepatic cells maintain its normal oxygen and energy metabolism,\[10\] decrease the damage from oxygen radicals, and decrease the level of microcirculation dysfunction or epithelial damage.\[11,12\] We found that hepatic ATP enzyme activity in the LT group increased more significantly than that in the NT group. We thought that hepatic cells may have enough energy reserve during the hypothermia state, thus inhibiting the production of oxygen radicals, lowering their activity, and preserving the activity of metabolic enzyme.\[13\]

To further examine the effects of hypothermia on the liver, we studied the histology after ROSC. Compared to the NT group, the hepatic cells in the LT group were broader normal, and there were fewer inflammatory cells. Under an electron microscope, mitochondrial morphosis of hepatic cells was observed in the LT group with intact capsules, visible ridge structures and integrated basement membranes. The amount and ridge structure integrity of mitochondria are necessary conditions for the normal function of mitochondria, which produce ATP. Morphological analysis of the liver showed that the structure of mitochondria in the LT group was healthier than that in the NT group. Swelling, necrosis and inflammatory infiltration of cells were all decreased in the LT group compared with the NT group.\[14,15\] These findings suggested that hypothermia induced by 4 ºC NS was protective to the liver after ROSC.

There are some limitations in this study. Because of the small sample, there are a few potential controls missing from the experimental design, which need for further investigation.

In conclusion, using a CA-CPR model in swine and infusing 4 ºC NS continuously after ROSC could quickly lower the core body temperature by 1.5 ºC, maintain a stable hemodynamic state, and balance oxygen metabolism. On the basis of stable vital signs, the normal function of mitochondria in hepatic cells was ensured, adequate energy supply to the liver was guaranteed, and acute hepatic injury or acute hepatic failure was avoided.

Funding: None.

Ethical approval: The present study was approved by Animal Care and Use Committee of Beijing Chaoyang Hospital, Capital Medical University, Beijing, China.

Conflicts of interest: The authors have no competing interests relevant to the present study.

Contributors: Han Y designed the research, analyzed the data, and wrote the paper. All authors read and approved the final version.

REFERENCES


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