Original Article

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

Yi Han¹, Zhen-ju Song¹, Chao-yang Tong¹, Chun-sheng Li²

¹Emergency Department, Shanghai Zhongshan Hospital, Fudan University, Shanghai, China

²Emergency Department, Beijing Chaoyang Hospital, Capital Medical University, Beijing, China

Corresponding Author: Chao-yang Tong: tong.chaoyang@zs-hospital.sh.cn

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 $\text{Na}^+\text{-K}^+\text{-ATPase}$ and $\text{Ca}^{2+}\text{-ATPase}$ 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 ($\text{Na}^+\text{-K}^+\text{-ATP}$ enzyme: 8.64 ± 3.32 U vs. 3.28 ± 0.71 U; $\text{Ca}^{2+}\text{-ATP}$ 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

World J Emerg Med 2013;4(4):298–303

DOI: 10.5847/wjem.j.issn.1920-8642.2013.04.011

INTRODUCTION

Cardiac arrest (CA) is often followed by serious hepatic dysfunction and multiple organs dysfunction syndrome (MODS), which cause a low survival rate.^[1] 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.^[2] However, there is few studies on whether therapeutic hypothermia after CPR can protect the liver.^[3,4] 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 P_{CO_2} ($EtPCO_2$) 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,^[5] manual CPR was carried out at a frequency of 100 compressions/min with mechanical ventilation at FiO_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 biphasic 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.^[5] 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.^[6] 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_2), oxygen consumption (VO_2), oxygen extraction ratio (ERO_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 $Na^+-K^+-ATPase$ and $Ca^{2+}-ATPase$ 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. $Na^+-K^+-ATPase$ and $Ca^{2+}-ATPase$ 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 ultrastructure 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 $\pm$ 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 DO_2 decreased after ROSC in both groups, while 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

Figure 1. Changes of temperature, CO, CPP, SVR and DO_2 , VO_2 , ERO_2 at baseline and each time point after ROSC in the LT and NT groups. Temperature in the LT group began to decrease at 10 minutes after ROSC, and was significantly lower than that in the NT group from 30 minutes to 4 hours after ROSC; CO in the LT group was significantly decreased from 30 minutes to 1 hour after ROSC, while there were no differences in the NT group; SVR in the LT and NT groups was not obviously changed before and after ROSC. DO_2 in the LT group was decreased compared to the NT group, while VO_2 in the LT group was more descent, ERO_2 in the LT group increased more significantly than that in the NT group after ROSC, which was comparable to the result 2 hours after ROSC.

Table 1. Changes of AST, ALT and LDH in the LT and NT groups after CPR (mean±SD)

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

Compared with baseline, * $P<0.05$, [#] $P<0.01$; compared with the NT group, ^Δ $P<0.05$, ^Δ $P<0.01$.

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

Parameters	n	ROSC 24 h
Na ⁺ -K ⁺ -ATP enzyme (U)		
NT	5	3.274±0.710
LT	5	8.614±3.317*
Ca ²⁺ -ATP enzyme (U)		
NT	5	2.746±0.778
LT	5	10.918±2.122 [#]

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 crista.

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 °C 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 °C NS after ROSC lowered the temperature by 1.5 °C in a short period of time. This is similar to Johanna Nordmark's study, in which the core temperature was lowered by 1.6 °C.^[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 broadly 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 °C 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 °C NS continuously after ROSC could quickly lower the core body temperature by 1.5 °C, 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

- 1 Geocadin RG, Ritzl EK. Seizures and status epilepticus in post cardiac arrest syndrome: therapeutic opportunities to improve outcome or basis to withhold life sustaining therapies? *Resuscitation* 2012; 83: 791–792.
- 2 Zeiner A, Holzer M, Sterz F, Behringer W, Schörkhuber W, Müllner M, et al. Mild resuscitative hypothermia to improve neurological outcome after cardiac arrest. A clinical feasibility trial. *Hypothermia After Cardiac Arrest (HACA) Study Group. Stroke* 2000; 31: 86–94.
- 3 Choi S, Noh J, Hirose R, Ferrell L, Bedolli M, Roberts JP, et al. Mild hypothermia provides significant protection against

- ischemia/reperfusion injury in livers of obese and lean rats. *Ann Surg* 2005; 241: 470–476.
- 4 Kulstad EB, Courtney MD, Waller D. Induction of therapeutic hypothermia via the esophagus: a proof of concept study. *World J Emerg Med* 2012; 3: 118–122.
- 5 Stadlbauer KH, Rheinberger K, Wenzel V, Raedler C, Krismer AC, Strohmenger HU, et al. The effects of nifedipine on ventricular fibrillation mean frequency in a porcine model of prolonged cardiopulmonary resuscitation. *Anesth Analg* 2003; 97: 226–230.
- 6 Nordmark J, Rubertsson S. Induction of mild hypothermia with infusion of cold (4 degrees C) fluid during ongoing experimental CPR. *Resuscitation* 2005; 66: 357–365.
- 7 Kulstad EB, Courtney DM, Waller D. Induction of therapeutic hypothermia via the esophagus: a proof of concept study. *World J Emerg Med* 2012; 3: 118–122.
- 8 Post H, Schmitto JD, Steendijk P, Christoph J, Holland R, Wachter R, et al. Cardiac function during mild hypothermia in pigs: increased inotropy at the expense of diastolic dysfunction. *Acta Physiol (Oxf)* 2010; 199: 43–52.
- 9 Vijlbrief DC, Benders MJ, Kemperman H, van Bel F, de Vries WB. Cardiac biomarkers as indicators of hemodynamic adaptation during postasphyxial hypothermia treatment. *Neonatology* 2012; 102: 243–248.
- 10 Gundersen Y, Vaagenes P, Pharo A, Valø ET, Opstad PK. Moderate hypothermia blunts the inflammatory response and reduces organ injury after acute haemorrhage. *Acta Anaesthesiol Scand* 2001; 45: 994–1001.
- 11 Westermann S, Vollmar B, Thorlacius H, Menger MD. Surface cooling inhibits tumor necrosis factor-alpha-induced microvascular perfusion failure, leukocyte adhesion, and apoptosis in the striated muscle. *Surgery* 1999; 126: 881–889.
- 12 Zhang C, Gao GR, Jiang HY, Lv CG, Zhang BL, Xie MS, et al. Effects of environmental hypothermia on hemodynamics and oxygen dynamics in a conscious swine model of hemorrhagic shock. *World J Emerg Med* 2012; 3: 128–134.
- 13 Hildebrand F, Giannoudis PV, van Griensven M, Chawda M, Pape HC. Pathophysiologic changes and effects of hypothermia on outcome in elective surgery and trauma patients. *Am J Surg* 2004; 187: 363–371.
- 14 Peliowski-Davidovich A; Canadian Paediatric Society, Fetus and Newborn Committee. Hypothermia for newborns with hypoxic ischemic encephalopathy. *Paediatr Child Health* 2012; 17: 41–46.
- 15 Lauriat S, Linas SL. The role of neutrophils in acute renal failure. *Semin Nephrol* 1998; 18: 498–504.

Received May 12, 2013

Accepted after revision October 20, 2013