Short-term, Mild Hypothermia Can Increase the Beneficial Effect of Permissive Hypotension on Uncontrolled Hemorrhagic Shock in Rats

Tao Li, Ph.D.,* Xiulai Lin, Ph.D.,* Yu Zhu, M.S.,† Lijie Li, M.S.,† Liangming Liu, M.D., Ph.D.‡

ABSTRACT

Background: Our previous and other studies have shown that hypotensive or hypothermic resuscitation have beneficial effects on uncontrolled hemorrhagic shock. Whether hypothermia can increase the beneficial effect of hypotensive resuscitation on hemorrhagic shock is not known.

Methods: Two-hundred and twenty Sprague-Dawley rats were used to make uncontrolled hemorrhagic shock. Before bleeding was controlled, rats received normotensive or hypotensive resuscitation (target mean arterial pressure at 80 or 50 mmHg) in combination with normal (37°C) or mild hypothermia (34°C) (phase II). After bleeding was controlled, rats received whole blood and lactated Ringer's solution resuscitation for 2 h (phase III). The animal survival, blood loss, fluid requirement, cardiac output, and coagulation functions, as well as vital organ function, mitochondrial function, and energy metabolism of liver, kidney and intestines, were noted.

Results: Short-term, mild hypothermia before bleeding was controlled increased the beneficial effect of hypotensive resuscitation. Hypothermia further decreased blood loss, oxygen consumption, and functional damage to the liver, kidney, and intestines during hypotensive resuscitation, protected mitochondrial function and energy metabolism (activity of Na^+-K^+-ATPase), and further improved survival time and survival rate (hypothermic/hypotensive combined group: survival rate, 9/10; survival time, 616 min; normothermic/normotensive group: 1/10, 256 min; hypothermic/normotensive group: 4/10, 293 min). Hypothermia slightly inhibited coagulation function.

Conclusion: Mild hypothermia before bleeding is controlled can increase the beneficial effect of hypotensive resuscitation on uncontrolled hemorrhagic shock. The mechanism underlying the benefits of short-term hypothermia may be related to the decrease in oxygen consumption and metabolism, and protection of mitochondrial and organ functions.

What We Already Know about This Topic

- Permissive hypotensive resuscitation is advocated for traumatic, hemorrhagic shock

What This Article Tells Us That Is New

- The combination of permissive hypotension and mild hypothermia in anesthetized rats increased their survival time and survival rate compared with normotensive and normothermic rats, all exposed to similar states of hemorrhagic shock

IT is estimated that 15–20% of traumatic deaths are potentially preventable, and that 66–80% of these deaths are from hemorrhagic shock.[1,2] Effective fluid resuscitation is the key step in follow-up therapy for hemorrhagic shock.[3–5]

Based on optimal fluid resuscitation and transfusion to patients with hemorrhagic shock and life-threatening injuries, the concept of damage-control resuscitation has been developed.[6–8] The main principles are permissive hypotensive resuscitation, rapid definitive/surgical control of bleeding, prevention/treatment of hypothermia, acidosis, and hypocalcemia. The goal of damage-control resuscitation is to minimize iatrogenic resuscitation injury, prevent worsening of the presenting traumatic shock, and to obtain definitive hemostasis. Research has shown that permissive hypotensive resuscitation results in a good resuscitative effect for uncontrolled hemorrhagic shock.[9] Permissive hypotensive resuscitation is aimed to reduce blood loss, prevent the translocation of a forming thrombus, and rebleeding.[10] Our previous

* Associate Professor, † Research Assistant, ‡ Professor, State Key Laboratory of Trauma, Burns and Combined Injury, Second Department of Research Institute of Surgery, Daping Hospital, Third Military Medical University, Chongqing, China.

Received from the State Key Laboratory of Trauma, Burns and Combined Injury, Second Department of Research Institute of Surgery, Daping Hospital, Third Military Medical University, Chongqing, China. Submitted for publication May 28, 2011. Accepted for publication January 31, 2012. Supported by grant no. 06Z030 from the Scientific Research Foundation of Medical Science and Public Health of the People's Liberation Army, Beijing, China; grant no. 30625037 from the National Natural Science Foundation of China for Distinguished Young Scholars, Beijing, China; grant no. CSTC2011jjjq0019 from the Natural Science Foundation of Chongqing for Distinguished Young Scholars, Chongqing, China; and grant no. 30830053 from the Key Project, Beijing, China.

Address correspondence to Dr. Liu: Second Department of Research Institute of Surgery, Daping Hospital, Third Military Medical University, Daping, Chongqing 400042, People's Republic of China. liangmingliu@yahoo.com. Anesthesiology 2012; 116:1288–98

study[11] showed that 50–60 mmHg of mean arterial pressure (MAP) is an ideal resuscitative pressure during uncontrolled hemorrhage.

Damage-control resuscitation advocates prevention of a decrease in body temperature during resuscitation after shock,[12] but reports have shown that short-term and mild-to-moderate controlled hypothermic resuscitation is beneficial in the treatment of uncontrolled hemorrhagic shock.[13–16] Our previous study also demonstrated that short-term application of mild or moderate hypothermia to uncontrolled hemorrhagic shock improved the final resuscitative effect, whereas too low and long-term hypothermia did not improve the resuscitative effect. No study has investigated whether short-term and mild hypothermia results in beneficial effects of hypotensive resuscitation or elucidated the underlying mechanism. Based on other and our previous studies, we hypothesized that short-term and mild hypothermia during hypotensive resuscitation improves survival compared with hypotensive resuscitation, and protects organ function *via* decreasing oxygen consumption and tissue metabolism.

To test this hypothesis, the effects of permissive hypotension (50 mmHg) in combination with mild hypothermia on uncontrolled hemorrhagic shock (as well as the effects on hemodynamics, coagulation parameters, vital organ and mitochondrial function, and energy metabolism) were observed.

Materials and Methods

Ethical Approval of the Study Protocol

The study protocol was approved by the Research Council and Animal Care and Use Committee of the Research Institute of Surgery, Daping Hospital, Third Military Medical University (Chongqing, China). None of the authors are members of this committee.

Animal Management

Two-hundred and twenty Sprague-Dawley rats (220–260 g) were used in the present study. They were fasted for 12 h, but allowed water *ad libitum* before experiments. The number of animals used had to be sufficient so that we could discover if short-term and mild hypothermia during hypotensive resuscitation improved survival compared with hypotensive resuscitation. The sample number ($n = 8$ or 10) in each group was calculated by power analyses based on our previous study and results from pilot studies (α at 0.05, power at 80%, two-tailed).

On the day of the experiment, rats were anesthetized with sodium pentobarbital (30 mg/kg). This agent was then added until the rats had no response to a needle stimulus. Rats were spontaneously breathing without mechanical ventilation. The right femoral arteries and veins were catheterized with a polyethylene catheter (OD, 0.965 mm; ID, 0.58 mm) for monitoring the MAP and bleeding, respectively. Rats underwent left ventricular catheterization with an appropriately sized catheter *via* the right carotid artery. To monitor hemodynamics and prevent clot formation, rats were given 500 U/kg of heparin and all catheters were filled with physiologic saline (0.9%) containing 30 U/ml of heparin. An uncontrolled hemorrhagic shock model was induced by transection of the splenic parenchyma and one of the branches of the splenic artery, as previously described by our research team.[11] Briefly, after the completion of catheterization, the spleen was exposed after laparotomy and a cross-transection was made in the splenic parenchyma between the two major branches of the splenic artery. One of the major branches of the splenic artery was also transected. Blood was allowed to flow into the abdominal cavity. When the MAP decreased to 40 mmHg, uncontrolled hemorrhagic shock was established for subsequent experiments.

Experimental Protocol

Experiments were defined as four phases. Phase I was the uncontrolled hemorrhagic period (model stage) in which blood was freely hemorrhaged to the abdomen. This phase was achieved when the MAP decreased to 40 mmHg, and this period was maintained for approximately 20–30 min. Phase II was the hypotensive and hypothermic resuscitation period in which rats were maintained at different target MAPs (including 50 mmHg and 80 mmHg) in combination with mild hypothermia (34°C) or normal temperature (37°C) with infusion of 130 kD of hydroxyethyl starch and lactated Ringer's solution at a ratio of 1:2. Phase III was the definitive treatment period; after bleeding was completely controlled *via* ligation of the spleen artery, rats received whole blood and lactated Ringer's solution (1:2) to maintain the MAP at 90–100 mmHg for 2 h. Phase IV was the observation period, in which the follow-through effects of hypotensive and hypothermic resuscitation were observed for 2 h. In phase II, rats in the mild hypothermia (34°C) resuscitation group underwent external application of ice on the head and abdomen to keep the rectal temperature at 34°C. (fig. 1).

Hemodynamics, Blood Gases, and Animal Survival

Fifty Sprague-Dawley rats were randomly divided into five groups of 10 as follows: sham-operated; 50 mmHg at 34°C; 50 mmHg at 37°C; 80 mmHg at 34°C; and 80 mmHg at 37°C. Animal management and resuscitation regimens were described in the section of experimental phases. The animal number in each group was based on our previous work and power analyses. MAP, hemodynamic parameters (including left intraventricular systolic pressure, maximal change rate of left intraventricular pressure [$\pm dp/dt_{max}$]), and blood gases (including pH and partial pressure of oxygen and carbon dioxide in arterial blood [Pa_{O_2}, P_{CO_2}]) were determined at baseline, at the end of phase I (model phase), phase II (hypotensive and hypothermic resuscitation), phase III (definitive treatment stage), and 2 h during phase IV (observation

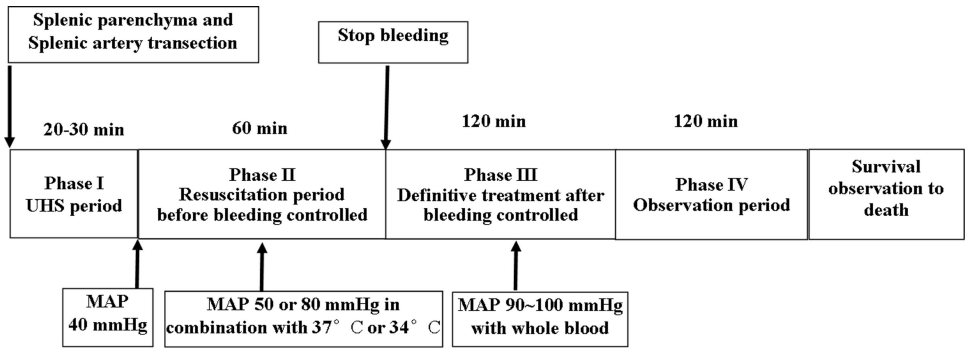

Fig. 1. Timeline of the experimental phases. MAP = mean arterial blood pressure; UHS = uncontrolled hemorrhagic shock.

period) with a polygraph physiologic recorder (SP844, Power Laboratory; AD Instruments, Castle Hill, NSW, Australia) and a blood gas analyzer (Phox plus L; Nova Biomedical, Waltham, MA). All hemodynamic parameters were obtained by Power Laboratory software, Chart V5. Blood loss, the amount of fluid infusion, and animal survival were recorded. The amount of blood loss was measured at the end of phase II using the method of cotton weighing.

Cardiac Output and Oxygen Consumption (VO_2)

In this experiment, another 50 Sprague-Dawley rats underwent catheterization of the left ventricle and right external jugular vein for measurement of cardiac output. The other procedures (including grouping, production of hemorrhage shock, and fluid infusion) were the same as described in experiment series 1. Each fluid tested at every target MAP involved experiments on 10 rats. Two hours after fluid infusion during phase III, cardiac output was measured using a Cardiomax-III machine (Columbus Instruments, Columbus, OH). Blood was sampled from the femoral artery and vein to analyze arterial and venous blood gases, respectively, using a blood gas analyzer. The values of VO_2 in tissues were calculated using the following formulas:

$$VO_2 = CI \times 13.4 \times \text{hemoglobin} \times (Sa_{O_2} - Sv_{O_2}),$$

with VO_2 = oxygen consumption, CI = cardiac index, Sa_{O_2} = oxygen saturation of the artery, and Sv_{O_2} = oxygen saturation of the vein.

Vital Organ and Mitochondrial Function and Energy Metabolism

Eighty Sprague-Dawley rats were randomly divided into five groups (n = 16/group; each time-point, 8 rats) as follows: sham-operated; 50 mmHg + 34°C; 50 mmHg + 37°C; 80 mmHg + 34°C; and 80 mmHg + 37°C. Blood was sampled to measure the function of the liver and kidneys, and the level of D-lactic acid in the plasma at the end of phase II and III. Thereafter, rats were killed to remove the liver, kidneys, and small intestine for the measurement of mitochondrial function and energy metabolism. The variables of liver function (total bilirubin), kidney function (blood urea nitrogen), and serum creatinine), as well as the variables of hepatic cell damage (alanine aminotransferase and aspartate aminotransferase), were measured using a biochemical analyzer (DX800; Biochemical Analyzer, Beckman, Fullerton, CA). The intestinal barrier function was reflected by plasma D-lactic acid, which was determined by an enzymatic technique.[17]

Mitochondrial function was measured by a mitochondrial function analyzer (MT 200; Strathkelvin, Lanarkshire, Scotland). Mitochondrial function reflected the respiration control rate of mitochondria, which is the rate of consumed oxygen of mitochondria with and without adenosine diphosphate. The respiration control rate is a relevant index to reflect the construction integrity and oxidative phosphorylation function of mitochondria. Energy metabolism was reflected by Na^+-K^+-ATPase, which was determined using an enzyme-linked immunosorbent assay.

Measurement of Mitochondrial Function in the Liver and Kidney

Samples of liver or kidney tissue (5 g) were put into 20 ml of ice-cold isolation buffer (sucrose 0.25 M, Na_2EDTA 0.1 mM, Tris 0.01 M, pH 7.6). They were cut into small pieces, and washed three times to remove blood. Tissue with isolation buffer was homogenized and then centrifuged at 1600 × *g* for 12 min at 4°C. The supernatant was further centrifuged twice at 25,000 × *g* for 15 min at 4°C. The pellet was collected and resuspended in 1 ml isolation buffer. The concentration of mitochondrial protein was measured by the Lowry method; 1.4 ml of measurement buffer (Tris 0.2 M, pH 7.6, KCl 15 mM, KH_2PO_4 15 mM, Na_2EDTA 1 mM, $MgCl_2$ 5 mM, sucrose 0.25 M) warmed to 30°C was added into the reaction chamber and equilibrated for 2 min. Then 0.2 ml of 3 mg/ml of a mitochondrial mixture was put into the reaction chamber and equilibrated for 20 s. Ten microliters of 0.5 M sodium malate ($C_4H_4Na_2O_5 \cdot H_2O$) and sodium glutamate ($C_5H_8NNaO_4$) and 5 μl of adenosine diphosphate (400 nM) were added in succession. The rate of oxygen consumption was determined using the mitochondrial function analyzer.

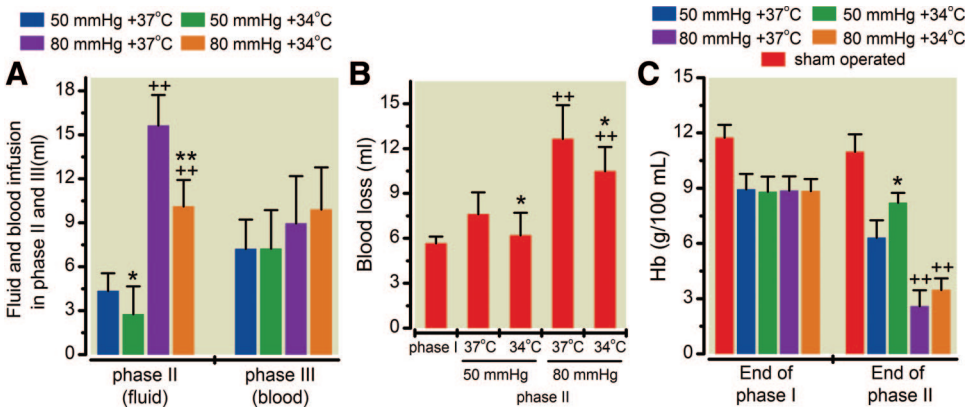

Fig. 2. Fluid requirement, blood loss, and hemoglobin. Data represent the mean ± SD of 10 observations. (*A*) Fluid requirement, (*B*) blood loss, and (*C*) hemoglobin are shown. *$P < 0.05$, **$P < 0.01$, comparison of different temperatures at same pressure; +$P < 0.05$, ++$P < 0.01$ comparison of different pressure at same temperature. Hb = Hemoglobin.

Measurement of the Activity of Na^+-K^+-ATPase in the Liver, Kidneys, and Intestine

Five grams of tissue from the liver, kidneys, or intestine were homogenized and centrifuged at 1,000 × *g* for 30 min. The supernatant was collected and the protein concentration measured by the Lowry method. Ten microliters of diluted testing sample was added into each well of a 96-well plate and incubated at 37°C for 30 min. Fifty microliters of horseradish peroxidase-conjugated reagent was then added to each well and mixed, and incubated for another 30 min at 37°C. Chromogen solution was then added to each well, and incubated for 15 min at 37°C. The optical density was measured at 450 nm after adding stop buffer with a Multiscan Spectrum 1500 machine (Thermo Electron Corporation, Waltham, MA). The activity of Na^+-K^+-ATPase in each sample (μmol/mg) was calculated by the linear regression equation of the standard curve based on the optical density value of the sample.

Coagulation Parameters

Forty Sprague-Dawley rats were randomly divided into four groups (n = 10/group): 50 mmHg + 34°C; 50 mmHg + 37°C; 80 mmHg + 34°C; and 80 mmHg + 37°C group. To avoid the interference of heparin on coagulation function, heparin was not used in this experiment. Blood was sampled to measure coagulation parameters such as the thrombin time (TT), prothrombin time (PT), international normalized ratio of prothrombin time (PT-INR), activated partial prothrombin time (APTT), fibrinogen, platelet count, and aggregation rate at baseline, at the end of phase I, II, III, and 2 h of phase IV.

Statistical Analyses

Data (hemodynamic parameters, blood gas parameters, coagulation parameters, fluid and blood requirements, blood loss, hemoglobin, cardiac output, oxygen consumption [VO_2], mitochondrial respiratory control rate, organ function parameters and Na^+-K^+-ATPase) are presented as the mean ± SD of n observations. Fluid and blood requirements were analyzed by one-way ANOVA, followed by the *post hoc* Tukey test (SPSS 15.0; SPSS, Inc., Chicago, IL). Hemodynamic parameters, blood gas parameters, coagulation parameters, blood loss, hemoglobin, cardiac output, VO_2, mitochondrial respiratory control rate, organ function parameters, and Na^+-K^+-ATPase were analyzed by two-way ANOVA, followed by the repeated *post hoc* Tukey test. Animal survival time and survival rate were analyzed by median and interquartile range, Kaplan–Meier survival analyses, and the log-rank test. $P < 0.05$ was considered significant (two-tailed).

Results

Fluid Requirement, Blood Loss, and Hemoglobin Concentration

Fluid Requirement. The mean fluid requirement (hydroxyethyl starch + lactated Ringer's, ratio of 1:2) in the hypotensive resuscitation group was less than in normotensive resuscitation group at normothermia (4.3 ml *vs.* 15.6 ml) during phase II ($P < 0.01$). Application of hypothermia in phase II further reduced the average fluid requirement of the hypotensive resuscitation group (reduced to 2.7 ml from 4.3 ml; fig. 2A). Blood requirements in the two hypotensive resuscitation groups during phase III was less than in the two normotensive resuscitation groups, but there were no differences among all four groups ($P > 0.05$) (fig. 2A).

Blood Loss. The mean blood loss of uncontrolled hemorrhagic shock for each rat in phase I was 5.66 ± 0.44 ml (mean ± SD). Permissive hypotensive resuscitation (50 mmHg) during phase II slightly increased blood loss; blood loss was increased by only 0.53 ml and 1.94 ml in the 50 mmHg target MAP group at 34°C or at 37°C, respectively. Normotensive resuscitation (80 mmHg) increased blood loss as compared with hypotensive resuscitation ($P < 0.01$); blood loss was increased by 4.82 ml and 6.98 ml in the 34°C or 37°C group in normotensive resuscitation, respectively (fig. 2B).

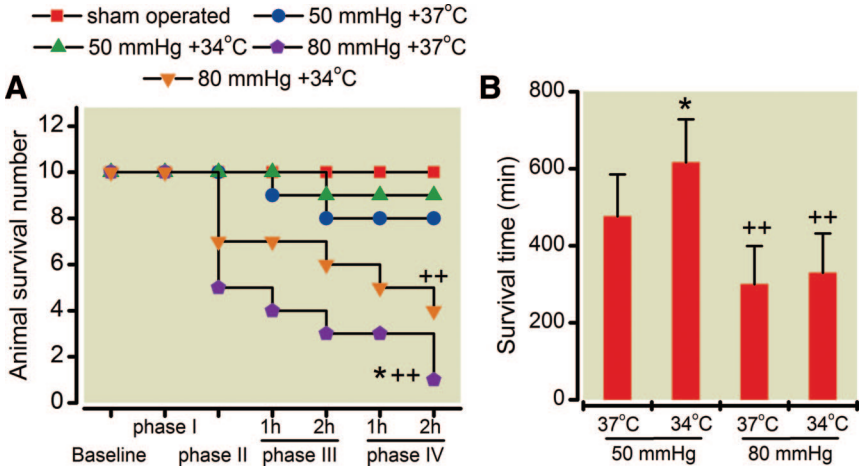

Fig. 3. Survival rate (*A*) and survival time (*B*), represented by the median and Kaplan–Meier survival line and analyzed by the interquartile range and Kaplan–Meier survival analyses, respectively (n = 10/group). *$P < 0.05$, comparison of different temperatures at the same pressure; ++$P < 0.01$, comparison of different pressures at the same temperature.

Hemoglobin Concentration. As compared with the sham-operated group, the mean hemoglobin concentration was decreased at the end of phase I (from approximately 11.7 g/100 ml to approximately 8.8 g/100 ml). Hypotensive resuscitation only slightly decreased the concentration of hemoglobin, whereas normotensive resuscitation significantly decreased the concentration of hemoglobin during phase II (from approximately 8.8 g/100 ml to 2.5–3.4 g/100 ml) ($P < 0.01$). In the same resuscitation pressure groups, the concentration of hemoglobin in the hypothermia group was higher than in the normothermia group (fig. 2C).

Animal Survival

As compared with the normotensive/normothermia resuscitation group, short-term and mild hypothermic/hypotensive resuscitation increased the survival time and survival rate of Sprague-Dawley rats. The number of rats surviving was 9/10, 8/10, 4/10, and 1/10 in the 50 mmHg at 34°C or 37°C and 80 mmHg at 34°C or 37°C groups at the 2 h time-point of phase IV, respectively. Two hypotensive resuscitation groups were significantly superior to the two normotensive resuscitation groups ($P < 0.05$ or 0.01). Mild hypothermia in combination with hypotensive resuscitation had the best survival time and survival rate (fig. 3).

Hemodynamics, Cardiac Output, and VO_2

Hemodynamic Parameters. MAP, left intraventricular systolic pressure, and $\pm dp/dt_{max}$ in all groups at baseline and at the end of phase I were not different. During phase II, because of the difference in target pressure, these hemodynamic parameters in normotensive resuscitation groups were higher than in hypotensive resuscitation groups. However, during phases III and IV, these hemodynamic parameters in normotensive groups were lower than in the hypotensive resuscitation groups. Among all groups, hypotensive resuscitation in combination with mild hypothermia (50 mmHg at 34°C group) had the best hemodynamic parameters during phases III and IV (table 1).

Blood Gases. There were no differences in blood pH, Pa_{O_2}, and Pa_{CO_2} at baseline and at the end of phase I among all groups. At the end of phase II, the blood pH value in all groups decreased slightly as compared with baseline and at the end of phase I. During phase III and phase IV, the pH value in hypotensive resuscitation groups (irrespective of 34°C or 37°C) was restored to a normal level, which was higher than the two normotensive resuscitation groups ($P < 0.05$). Pa_{O_2} values in the two hypotensive resuscitation groups were higher than the two normotensive groups at all time-points (phases II, III, and IV) ($P < 0.05$ or 0.01). Pa_{CO_2} values in the hypotensive resuscitation groups were lower than in the normotensive groups at the end of phase II ($P < 0.05$). In the same resuscitation pressure groups, Pa_{O_2} in the mild hypothermia group was higher than in the normothermia group ($P < 0.05$) (table 2).

Cardiac Output. In phase II, cardiac output in normotensive resuscitation groups (at 34°C and 37°C) was slightly higher than in the hypotensive resuscitation groups (at 34°C and 37°C) in the same target pressure group. That is, hypothermia resuscitation (34°C) had no impact on cardiac output. During phase III, cardiac output among all groups was not obviously different ($P > 0.05$) (fig. 4A).

VO_2. In the normotensive resuscitation groups, VO_2 was higher than in the hypotensive resuscitation groups at the end of phase II ($P < 0.01$). Hypothermia decreased VO_2 (fig. 4B). There were no significant differences among all groups for VO_2 at the end of phase III ($P > 0.05$). (fig. 4B).

Vital Organ and Mitochondrial Function

Intestinal Barrier and Liver and Kidney Function. Hypotensive resuscitation in combination with mild hypothermia could better protect vital organ function (including intestinal barrier function). The plasma level of D-lactic acid, the con-

Table 1. The Changes in Hemodynamic Parameters

Group	Baseline	End of Phase I	End of Phase II	End of Phase III	End of Phase IV
MAP (mmHg)					
50 mmHg-37°C	115.3 ± 15.5	40.3 ± 2.5*	49.5 ± 3.3	93.4 ± 10.4	84.3 ± 15.3
50 mmHg-34°C	114.8 ± 13.6	40.8 ± 3.6*	50.8 ± 2.1	87.8 ± 13.3	90.4 ± 10.7
80 mmHg-37°C	113.5 ± 12.3	39.1 ± 2.3*	79.3 ± 1.6**	90.7 ± 7.3	74.0 ± 12.8#
80 mmHg-34°C	109.5 ± 11.9	39.5 ± 3.9*	80.37 ± 1.8**	88.9 ± 7.3	76.0 ± 10.9*
LVSP (mmHg)					
50 mmHg-37°C	140.3 ± 14.1	76.3 ± 11.1*	86.5 ± 13.5	111.4 ± 14.9	115.5 ± 18.8
50 mmHg-34°C	155.0 ± 13.9	75.0 ± 13.9*	87.4 ± 15.8	147.1 ± 19.1‖	149.9 ± 14.0‖
80 mmHg-37°C	144.2 ± 12.1	74.2 ± 12.1*	109.4 ± 21.9**†	111.9 ± 13.4	93.1 ± 10.5**
80 mmHg-34°C	141.2 ± 15.3	77.2 ± 9.3*	104.2 ± 11.2**‡	116.9 ± 11.6**	108.1 ± 20.3#
$+dp/dt_{max}$ (mmHg/s)					
50 mmHg-37°C	4,429 ± 614.0	1,429 ± 414*	1926.7 ± 525†	3,439 ± 857	3,542 ± 629
50 mmHg-34°C	4,615 ± 1,302	1,615 ± 302*	1,818 ± 708	4,369 ± 1,111§	5,849 ± 1,143§
80 mmHg-37°C	4,229 ± 1,302	1,422 ± 334*	3,052 ± 1,090#‡	3,188 ± 1,064	2,987 ± 478#
80 mmHg-34°C	4,524 ± 1,003	1,524 ± 403*	2,836 ± 991#‡	3,238 ± 936#	3,289 ± 997**
$-dp/dt_{max}$ (mmHg/s)					
50 mmHg-37°C	−4,096 ± 850	−1,296 ± 350*	−1,404.4 ± 557	−3,221 ± 616	−3,038 ± 421
50 mmHg-34°C	−4,113 ± 1,192	−1,113 ± 292*	−1,674 ± 736	−3,939 ± 1,060	−4,234 ± 904§
80 mmHg-37°C	−4,158 ± 1,108	−1,158 ± 308*	−1,477.0 ± 650	−2,313 ± 468#	−2,155 ± 348#
80 mmHg-34°C	−4,058 ± 1,209	−1,258 ± 209*	−1,587.0 ± 650	−2,654 ± 768#	−3,055 ± 898#

Data represent the mean ± SD of 10 observations (n = 10/group).

* $P < 0.01$ as compared with baseline; † $P < 0.05$, ‡ $P < 0.01$ as compared with phase II (hypothermia-hypotensive resuscitation period); § $P < 0.05$, ‖ $P < 0.01$ comparison of different temperatures at the same pressure; # $P < 0.05$, ** $P < 0.01$, comparison of different pressures at the same temperature.

$\pm dp/dt_{max}$ = the maximal increase and decrease rate of left intraventricular pressure; LVSP = left intraventricular systolic pressure; MAP = mean arterial blood pressure.

centration of total bilirubin, blood urea nitrogen, serum creatinine, alanine aminotransferase, and aspartate aminotransferase were significantly lower than in the normotensive/normothermia, normotensive/hypothermia, and hypotensive/normothermia groups, and almost close to the normal level (sham-operated group; figs. 5A–F). In the same target MAP group, the variables of the intestinal barrier, liver, and kidney function in the mild hypothermia group were superior to that in the normothermia group, whereas in the same temperature group, the hypotensive group was superior to the normotensive group.

Mitochondrial Function of the Liver, Kidney, and Intestinal Mucosa. Hypotensive resuscitation in combination with mild hypothermic resuscitation could better pro-

Table 2. The Changes in Arterial pH, Pa_{O_2} (mmHg), and Pa_{CO_2} (mmHg)

Group	Baseline	End of Phase I	End of Phase II	End of Phase III	End of Phase IV
pH					
50 mmHg-37°C	7.39 ± 0.04	7.37 ± 0.06	7.27 ± 0.07†	7.31 ± 0.06	7.34 ± 0.05
50 mmHg-34°C	7.35 ± 0.05	7.41 ± 0.07	7.22 ± 0.06†	7.35 ± 0.06	7.35 ± 0.04
80 mmHg-37°C	7.39 ± 0.02	7.35 ± 0.08	7.15 ± 0.08‡	7.18 ± 0.05#	7.23 ± 0.09#
80 mmHg-34°C	7.36 ± 0.03	7.36 ± 0.08	7.13 ± 0.09‡	7.15 ± 0.06#	7.22 ± 0.03#
Pa_{O_2} (mmHg)					
50 mmHg-37°C	96.73 ± 14.2	123.2 ± 10.9*	101.8 ± 12.5†	99.8 ± 8.8	97.1 ± 10.5
50 mmHg-34°C	97.06 ± 10.3	125.7 ± 9.8*	105.7 ± 8.6†	103.0 ± 11.1	108.2 ± 9.7§
80 mmHg-37°C	97.5 ± 14.6	124.3 ± 9.1*	94.3 ± 7.9#†	76.1 ± 7.9**	75.4 ± 12.1**
80 mmHg-34°C	96.3 ± 7.9	120.5 ± 9.9*	95.7 ± 7.8#†	80.2 ± 12.1**	80.3 ± 8.5**
P_{ACO_2} (mmHg)					
50 mmHg-37°C	45.11 ± 4.36	38.52 ± 2.82*	31.21 ± 3.01†	30.45 ± 3.43	33.38 ± 4.85
50 mmHg-34°C	41.21 ± 6.25	41.86 ± 2.98	32.17 ± 2.52†	32.64 ± 3.61	35.66 ± 2.84
80 mmHg-37°C	42.53 ± 4.21	46.32 ± 2.87	30.31 ± 3.59†	35.87 ± 2.72#	36.27 ± 3.27
80 mmHg-34°C	45.23 ± 7.61	42.65 ± 3.01	33.18 ± 3.65†	37.91 ± 3.68#	38.95 ± 4.62

Data represent the mean ± SD of 10 observations (n = 10/group).

* $P < 0.01$ as compared with baseline; † $P < 0.05$, ‡ $P < 0.01$ as compared with phase II (hypothermia-hypotensive resuscitation period); § $P < 0.05$, comparison of different temperatures at the same pressure; # $P < 0.05$, ** $P < 0.01$, comparison of different pressures at the same temperature.

P_{ACO_2} = arterial pressure of carbon dioxide; Pa_{O_2} = arterial pressure of oxygen.

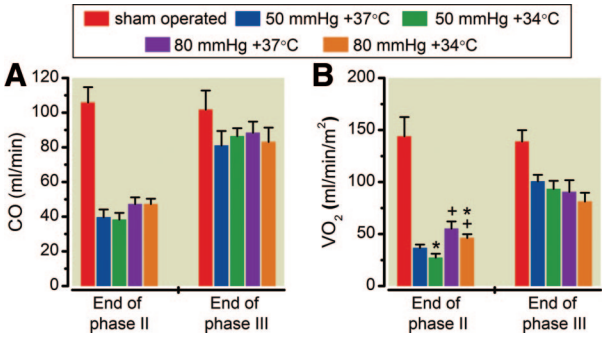

Fig. 4. Cardiac output (*A*) and VO_2 (*B*). Data represent the mean ± SD of 10 observations (n = 10 group). *$P < 0.05$, comparison of different temperatures at the same pressure; +$P < 0.05$, comparison of different pressures at the same temperature. CO = cardiac output; VO_2 = oxygen consumption.

tect the mitochondrial function of the liver, kidneys, and intestinal mucosa. The respiratory control rates of mitochondria from these organs were close to the normal level (sham-operated group). Two hypotensive resuscitation groups (50 mmHg at 34°C and 37°C) were superior to the two normotensive groups (80 mmHg at 34°C and 37°C) ($P < 0.05$ or 0.01). In the same target pressure resuscitation groups, the mild hypothermia group (34°C) was superior to the normothermia group (37°C; figs. 6A–C) ($P < 0.05$).

Activity of Na^+-K^+ ATPase in Liver, Kidneys, and Intestinal Mucosa

The activity of Na^+-K^+ ATPase in the liver, kidneys, and intestinal mucosa was increased after hemorrhagic shock as compared with the control group (sham-operated). Hypotension in combination with mild hypothermic resuscitation reduced the activity of Na^+-K^+ ATPase in the liver, kidneys, and intestinal mucosa. In the same target MAP group, the activity of Na^+-K^+ ATPase in the hypothermia resuscitation group was lower than in the normothermia group (figs. 7A–C) ($P < 0.05$ or 0.01).

Coagulation Parameters

At the end of phase I, TT, PT, and PT-INR did not change obviously as compared with baseline. Fibrinogen decreased slightly ($P > 0.05$). APTT was prolonged, platelet count was decreased, and platelet aggregation increased ($P < 0.05$). During phase II, TT, PT, PT-INR, and APTT were prolonged or increased as compared with the end of phase I ($P < 0.05$ or 0.01); fibrinogen, platelet count, and aggregation were decreased ($P < 0.05$ or 0.01). Among these parameters, PT, PT-INR, and APTT in the hypothermia group (34°C group) were slightly higher than in the normothermia group (37°C group) at the same pressure level ($P < 0.05$), and platelet aggregation in the hypothermia group was lower

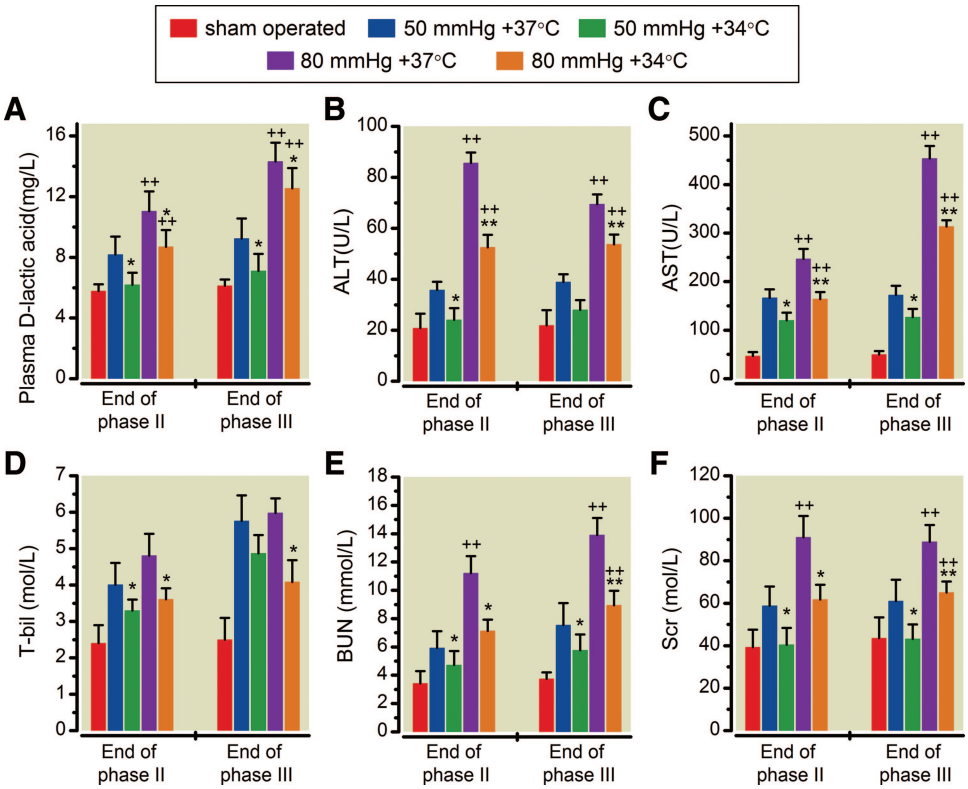

Fig. 5. Intestinal barrier, liver, and kidney function. Data represent the mean ± SD of eight observations (n = 8/group). (*A*) Plasma D-lactic acid, (*B*) alanine aminotransferase, (*C*) aspartate aminotransferase, (*D*) total bilirubin, (*E*) blood urea nitrogen, and (*F*) serum creatinine are shown. *$P < 0.05$, **$P < 0.01$, comparison of different temperatures at the same pressure; ++$P <$ 0.01 comparison of different pressures at the same temperature. ALT = alanine aminotransferase; AST = aspartate aminotransferase; BUN = blood urea nitrogen; T-bil = total bilirubin; Scr = serum creatinine.

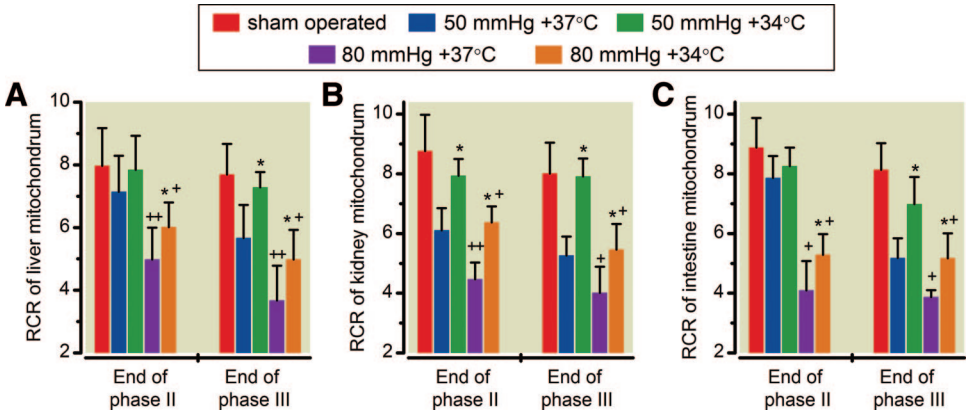

Fig. 6. Mitochondrial function of liver, kidneys, and intestine. Data represent the mean ± SD of eight observations (n = 8/group). (*A*) Liver, (*B*) kidneys, and (*C*) intestine are shown. *$P < 0.05$, comparison of different temperatures at the same pressure; +$P < 0.05$, ++$P < 0.01$ comparison of different pressures at the same temperature. RCR = respiratory control rate.

than in normothermia group. During phases III and IV, some parameters appeared to be slightly increased and some parameters appeared to be slightly decreased, but there were no significant differences in most parameters between hypothermia and normothermia groups (table 3) ($P > 0.05$).

Discussion

Traumatic hemorrhagic shock occurs often in civilian and military settings. Traumatic hemorrhagic shock accounts for approximately 50% of deaths of battle personnel. Fluid resuscitation is the common and very important treatment for many types of circulatory shock (particularly for traumatic hemorrhagic shock).[18] A great deal of research has shown that permissive hypotensive resuscitation and short-term hypothermic resuscitation results in a good resuscitative effect for uncontrolled hemorrhagic shock before bleeding is controlled. Long-term and deep hypothermia do not achieve a good effect.[19] Whether short-term and mild hypothermia can produce a beneficial effect of hypotensive resuscitation before bleeding is controlled (and how it is enforced) are not understood.

The present study showed that permissive hypotensive resuscitation in combination with mild hypothermia had a good effect on uncontrolled hemorrhagic shock; short-term and mild hypothermia can potentiate this beneficial effect of hypotensive resuscitation. Permissive hypotensive resuscitation in combination with mild hypothermia can decrease the blood loss and functional damage of vital organs, decrease tissue oxygen consumption and metabolism, protect mitochondrial function, stabilize hemodynamic parameters, and maintain the acid–base balance of shocked animals ($P <$ 0.05 or 0.01). The beneficial effect of permissive hypotension is mainly related to preventing blood loss and hemodilution. The beneficial effect of hypothermia is mainly related to decreasing metabolism and protecting organs.

The literature shows that subhypothermia has been successfully applied to cranial trauma patients.[20] However, application of hypothermia in trauma patients or hemorrhagic shock victims is controversial.[21] Some authors suggest that trauma patients are already hypothermic, and further application of hypothermia can interfere with cardiac function, the activity of many enzymes, and with coagulation.[21–23]

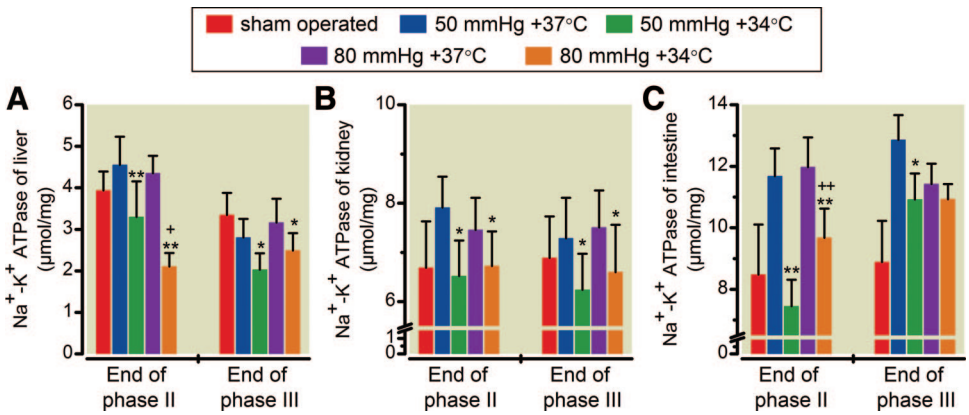

Fig. 7. The activity of Na^+-K^+-ATPase of liver, kidneys, and intestine. Data represent the mean ± SD of eight observations (n = 8/group). (*A*) Liver, (*B*) kidneys, and (*C*) intestine are shown. *$P < 0.05$, **$P < 0.01$, comparison of different temperatures at the same pressure; +$P < 0.05$, ++$P < 0.01$ comparison of different pressures at the same temperature.

Table 3. The Changes of Coagulation Parameters

—	Baseline	End of Phase I	End of Phase II	End of Phase III	2 h of Phase IV
TT					
50 mmHg-37°C	33.66 ± 0.73	36.88 ± 1.41*	48.68 ± 1.85‡	58.59 ± 1.05‡	54.85 ± 2.08‡
50 mmHg-34°C			52.36 ± 2.08‡§	63.25 ± 2.99‡§	56.36 ± 2.96‡
80 mmHg-37°C			49.83 ± 3.29‡	60.23 ± 3.89‡	56.76 ± 3.83‡
80 mmHg-34°C			54.85 ± 2.86‡	68.96 ± 4.56‡§	57.48 ± 2.84‡
PT(s)					
50 mmHg-37°C	8.82 ± 0.91	8.95 ± 0.98	10.67 ± 1.38†	12.36 ± 2.31‡	13.25 ± 2.01‡
50 mmHg-34°C			14.86 ± 2.11†§	15.84 ± 2.84‡	15.58 ± 3.64‡
80 mmHg-37°C			11.28 ± 1.46†	13.25 ± 3.61†	13.01 ± 3.25
80 mmHg-34°C			15.88 ± 3.25‡§	16.25 ± 2.88‡	16.38 ± 3.26‡
PT-INR					
50 mmHg-37°C	0.72 ± 0.21	0.76 ± 0.32	0.83 ± 0.22	1.08 ± 0.35†	1.18 ± 0.11†
50 mmHg-34°C			0.98 ± 0.19†	1.18 ± 0.18†	1.38 ± 0.25†
80 mmHg-37°C			0.89 ± 0.12	0.98 ± 0.13†	1.12 ± 0.2†
80 mmHg-34°C			1.08 ± 0.18†§	1.16 ± 0.12†	1.25 ± 0.35†
FIB (g/L)					
50 mmHg-37°C	2.51 ± 0.35	2.29 ± 0.23	1.15 ± 0.12‡	1.35 ± 0.09‡	1.38 ± 0.05‡
50 mmHg-34°C			0.93 ± 0.11‡	1.34 ± 0.12‡	1.36 ± 0.13‡
80 mmHg-37°C			1.05 ± 0.12‡	1.42 ± 0.13‡	1.38 ± 0.12‡
80 mmHg-34°C			0.90 ± 0.09‡	1.40 ± 0.09‡	1.35 ± 0.14‡
APTT(s)					
50 mmHg-37°C	18.77 ± 1.28	26.54 ± 2.46*	32.35 ± 3.52‡	45.88 ± 3.38‡	56.64 ± 2.84‡
50 mmHg-34°C			46.54 ± 3.65‡‖	54.52 ± 3.56‡§	60.38 ± 2.85‡
80 mmHg-37°C			44.52 ± 5.45‡	53.83 ± 2.83‡	58.86 ± 3.65‡
80 mmHg-34°C			52.83 ± 6.27‡§	61.85 ± 5.39‡§	62.38 ± 4.89‡
Platelet count (1,061;10^9/L)					
50 mmHg-37°C	887 ± 88	631 ± 75*	477 ± 36‡	522 ± 83†	530 ± 64†
50 mmHg-34°C			504 ± 38†	538 ± 57†	558 ± 68†
80 mmHg-37°C			326 ± 28‡#	498 ± 56†	499 ± 54†
80 mmHg-34°C			398 ± 39‡#	512 ± 45†	523 ± 59†
Platelet aggregation (%)					
50 mmHg-37°C	24.22 ± 3.51	31.53 ± 3.41*	9.33 ± 1.32‡	15.62 ± 2.84‡	13.75 ± 4.62‡
50 mmHg-34°C			6.82 ± 1.23‡§	13.23 ± 2.86‡	12.84 ± 1.21‡
80 mmHg-37°C			9.53 ± 2.44‡	15.85 ± 3.23‡	14.93 ± 2.22‡
80 mmHg-34°C			6.65 ± 1.34‡§	12.44 ± 0.94‡	13.14 ± 3.15‡

Data represent the mean ± SD of 10 observations (n = 10/group).

* $P < 0.01$ as compared with baseline; † $P < 0.05$, ‡ $P < 0.01$ as compared with phase II (hypothermia-hypotensive resuscitation period); § $P < 0.05$, ‖ $P < 0.01$ as compared with northermia group at the same pressure group; # $P < 0.05$ as compared with hypotensive group at the same temperature group.

APTT = activated partial prothrombin time; FIB = fibrinogen; PT = prothrombin time; PT-INR = international normalized ratio of prothrombin time; TT = thrombin time.

However, other authors suggest that short-term and mild hypothermia would not significantly interfere with cardiovascular function and the activity of so many enzymes. In contrast, it may protect organ function by decreasing the metabolic rate.[24–26] The present study demonstrated that short-term application of mild hypothermia limited to before bleeding has stopped can decrease the energy metabolism, decrease the activity of Na^+-K^+-ATPase, decrease oxygen consumption, and protect mitochondrial and vital-organ function. However, it affected coagulation function only slightly. The present study further confirmed the efficiency of short-term and mild hypothermia in the early treatment of traumatic hemorrhagic shock. It also showed that hypothermia can increase the beneficial effect of hypotensive resuscitation on uncontrolled hemorrhagic shock.

There are many approaches to decreasing the body temperature of traumatic victims (*e.g.*, external application of ice or alcohol, use of a hypothermic bed, or intravenous infusion of cold fluid). For example, Takasu and Wu used alcohol and ice to implement hypothermic treatment on uncontrolled hemorrhagic shock in rats.[27,28] Wladis *et al.*[29] used a hypothermia bed to observe the effect of hypothermia on acute metabolism and endocrine responses after hemorrhagic shock. Gotberg *et al.* utilized intravenous infusions of cold saline to induce hypothermia to observe the beneficial effect on cardiogenic shock in pigs.[30] Other studies also demonstrated that endovascular cooling achieved a significant reduction in core body temperature.[31] External application of ice was used in the present study to induce hypothermia because this method is easy to implement. Even if the trauma team is outside the hospital, as long as there is an ice bag, the

temperature-decreasing process can be done. If inside a hospital, intravenous infusion of cold saline can be selected to better control the temperature-decreasing process.

Hemorrhagic coagulopathy is a significant complication after traumatic injury, which has an important impact on the development and therapy of traumatic-hemorrhagic shock.[3,32–33] Studies have shown that hypothermia has a very important effect on coagulation if the core temperature is less than 34°C,[34,35] whereas hypothermia more than 34°C has no or little effect on coagulation. Hence, 34°C of hypothermia was selected in the present study. Resnick[36] reported that hypothermia more than 34°C did not affect the coagulation in hemorrhagic-shock pigs; all coagulation parameters, including TT, APTT, platelet count, activated clotting time, and thromboelastogram did not differ between that at 34°C and at 37°C. To observe the influence of the resuscitation regimen in the present study on coagulation function in hemorrhagic shock, we observed changes of coagulation parameters such as TT, PT, PT-INR, APTT, fibrinogen, platelet count, and platelet aggregation. The results showed that after hemorrhagic shock, coagulation functions had different levels of impairment: it appeared that APTT was significantly prolonged. During hypothermia and hypotensive resuscitation, TT, PT, PT-INR and APTT were further prolonged or increased as compared with the end of phase I. However, among these parameters, PT, PT-INR, and APTT in the hypothermia (34°C) group were slightly higher than in the normothermia (37°C) group, and platelet aggregation in the hypothermia group was slightly lower than in the normothermia group at the same pressure level. These results showed that severe hemorrhage impairs the coagulation function, and that mild hypothermia applied for a limited period before bleeding only slightly inhibited coagulation function.

The present study showed that permissive hypotensive resuscitation in combination with hypothermia had a beneficial effect upon uncontrolled hemorrhagic shock, and that short-term and mild hypothermia could increase the beneficial effect of hypotensive resuscitation. Nevertheless, the present study had limitations. First, only small animals were used; whether this effect can be extrapolated to larger animals and humans needs further investigation. Second, the duration of hypotensive and hypothermic resuscitation during uncontrolled hemorrhagic shock was only 1 h; discovering the optimal duration needs further study.

Conclusion

Permissive hypotensive resuscitation in combination with mild hypothermia had a synergistic effect upon uncontrolled hemorrhagic shock in rats. Short-term and mild hypothermia could increase the beneficial effect of hypotensive resuscitation. The latter could reduce blood loss and stabilize the concentration of hemoglobin. Mild hypothermia could protect mitochondrial function and decrease oxygen consumption and energy metabolism, but did not cause severe damage to coagulation function. This combination had a synergistic effect, including protection of organ function, hemodynamic stabilization, and animal survival.

The authors thank Tang Shihai, M.D., Associate Professor in Statistics in the editorial office of the Journal of Trauma Surgery, Chongqing, China, for assistance in the statistical analysis in the preparation of this manuscript.

References

1. Esposito TJ, Sanddal TL, Reynolds SA, Sanddal ND: Effect of a voluntary trauma system on preventable death and inappropriate care in a rural state. J Trauma 2003; 54:663–9
2. Hess JR, Thomas MJ: Blood use in war and disaster: Lessons from the past century. Transfusion 2003; 43:1622–33
3. Hoyt DB, Dutton RP, Hauser CJ, Hess JR, Holcomb JB, Kluger Y, Mackway-Jones K, Rizoli SB, Yukioka T, Bouillon B: Management of coagulopathy in the patients with multiple injuries: Results from an international survey of clinical practice. J Trauma 2008; 65:755–64
4. Repine TB, Perkins JG, Kauvar DS, Blackborne L: The use of fresh whole blood in massive transfusion. J Trauma 2006; 60:S59–69
5. Hess JR, Holcomb JB, Hoyt DB: Damage control resuscitation: The need for specific blood products to treat the coagulopathy of trauma. Transfusion 2006; 46:685–6
6. Holcomb JB, Jenkins D, Rhee P, Johannigman J, Mahoney P, Mehta S, Cox ED, Gehrke MJ, Beilman GJ, Schreiber M, Flaherty SF, Grathwohl KW, Spinella PC, Perkins JG, Beekley AC, McMullin NR, Park MS, Gonzalez EA, Wade CE, Dubick MA, Schwab CW, Moore FA, Champion HR, Hoyt DB, Hess JR: Damage control resuscitation: Directly addressing the early coagulopathy of trauma. J Trauma 2007; 62:307–10
7. Beekley AC: Damage control resuscitation: A sensible approach to the exsanguinating surgical patient. Crit Care Med 2008; 36:S267–74
8. Holcomb JB: Fluid resuscitation in modern combat casualty care: Lessons learned from Somalia. J Trauma 2003; 54: S46–51
9. Cannon WB, Fraser J, Cowell E: The preventive treatment of wound shock. JAMA 1918; 70:618–21
10. Sondeen JL, Coppes VG, Holcomb JB: Blood pressure at which rebleeding occurs after resuscitation in swine with aortic injury. J Trauma 2003; 54:S110–7
11. Lin XL, Li T, Yang GM, Liu LM: Comparison of effects of 3 kinds of hydroxyethyl- starch with different molecular weight in resuscitation of uncontrolled hemorrhagic shock in rats. J Traumatic Surg 2008; 10:203–6
12. Duchesne JC, Hunt JP, Wahl G, Marr AB, Wang YZ, Weintraub SE, Wright MJ, McSwain NE Jr: Review of current blood transfusions strategies in a mature level I trauma center: Were we wrong for the last 60 years? J Trauma 2008; 65: 272–6
13. Yang X, Hachimi-Idrissi S, Nguyen DN, Zizi M, Huyghens L: Effect of resuscitative mild hypothermia and oxygen concentration on the survival time during lethal uncontrolled haemorrhagic shock in mechanically ventilated rats. Eur J Emerg Med 2004; 11:210–6
14. Prueckner S, Safar P, Kentner R, Stezoski J, Tisherman SA: Mild hypothermia increases survival from severe pressure-controlled hemorrhagic shock in rats. J Trauma 2001; 50: 253–62
15. Takasu A, Stezoski SW, Stezoski J, Safar P, Tisherman SA: Mild or moderate hypothermia, but not increased oxygen breathing, increases long-term survival after uncontrolled hemorrhagic shock in rats. Crit Care Med 2000; 28:2465–74
16. Kim SH, Stezoski SW, Safar P, Capone A, Tisherman S: Hypothermia and minimal fluid resuscitation increase survival

after uncontrolled hemorrhagic shock in rats. J Trauma 1997; 42:213–22

17. Brandt RB, Siegel SA, Waters MG, Bloch MH: Spectrophotometric assay for D-(−)-lactate in plasma. Anal Biochem 1980; 102:39–46
18. Bellamy RF: The causes of death in conventional land warfare: Implications for combat casualty care research. Mil Med 1984; 149:55–62
19. Handrigan MT, Bentley TB, Oliver JD, Tabaku LS, Burge JR, Atkins JL: Choice of fluid influences outcome in prolonged hypotensive resuscitation after hemorrhage in awake rats. Shock 2005; 23:337–43
20. Steinemann S, Shackford SR, Davis JW: Implications of admission hypothermia in trauma patients. J Trauma 1990; 30:200–2
21. Kheirbek T, Kochanek AR, Alam HB: Hypothermia in bleeding trauma: A friend or a foe? Scand J Trauma Resusc Emerg Med 2009; 17:65
22. Haman F: Shivering in the cold: From mechanisms of fuel selection to survival. J Appl Physiol 2006; 100:1702–8
23. Mizushuma Y, Wang P, Cioffi WG, Bland KI, Chaudry IH: Should normothermia be restored and maintained during resuscitation after trauma and hemorrhage? J Trauma 2000; 48:58–65
24. George ME, Mulier KE, Beilman GJ: Hypothermia is associated with improved outcomes in a porcine model of hemorrhagic shock. J Trauma 2010; 68:662–8
25. Deniz T, Agalar C, Ozdogan M, Edremitlioglu M, Eryilmaz M, Devay SD, Deveci O, Agalar F: Mild hypothermia improves survival during hemorrhagic shock without affecting bacterial translocation. J Invest Surg 2009; 22:22–8
26. Shuja F, Tabbara M, Li Y, Liu B, Butt MU, Velmahos GC, DeMoya M, Alam HB: Profound hypothermia decreases cardiac apoptosis through Akt survival pathway. J Am Coll Surg 2009; 209:89–99
27. Takasu A, Sakamoto T, Okada Y: Effect of induction rate for mild hypothermia on survival time during uncontrolled hemorrhagic shock in rats. J Trauma 2006; 61:1330–5
28. Wu X, Stezoski J, Safar P, Nozari A, Tisherman SA: After spontaneous hypothermia during hemorrhagic shock, continuing mild hypothermia (34°C) improves early but not late survival in rats. J Trauma 2003; 55:308–16
29. Wladis A, Hjelmqvist H, Brismar B, Kjellström BT: Acute metabolic and endocrine effects of induced hypothermia in hemorrhagic shock: An experimental study in the pig. J Trauma 1998; 45:527–33
30. Götberg M, van der Pals J, Olivecrona GK, Götberg M, Koul S, Erlinge D: Mild hypothermia reduces acute mortality and improves hemodynamic outcome in a cardiogenic shock pig model. Resuscitation 2010; 81:1190–6
31. Dixon SR, Whitbourn RJ, Dae MW, Grube E, Sherman W, Schaer GL, Jenkins JS, Baim DS, Gibbons RJ, Kuntz RE, Popma JJ, Nguyen TT, O'Neill WW: Induction of mild systemic hypothermia with endovascular cooling during primary percutaneous coronary intervention for acute myocardial infarction. J Am Coll Cardiol 2002; 40:1928–34
32. Ho AM, Karmakar MK, Dion PW: Are we giving enough coagulation factors during major trauma resuscitation? Am J Surg 2005; 190:479–84
33. Brohi K, Cohen MJ, Ganter MT, Schultz MJ, Levi M, Mackersie RC, Pittet JF: Acute coagulopathy of trauma: Hypoperfusion induces systemic anticoagulation and hyperfibrinolysis. J Trauma 2008; 64:1211–7
34. Reed RL 2nd, Johnson TD, Hudson JD, Judy D, Fischer RP: The disparity between hypothermic coagulopathy and clotting studies. J Trauma 1992; 33:465–70
35. Patt A, McCroskey BL, Moore EE: Hypothermia-induced coagulopaties in trauma. Surg Clin North Am 1988; 68:775–85
36. Resnick DK, Marion DW, Darby JM: The effect of hypothermia on the incidence of delayed traumatic intracerebral hemorrhage. Neurosurgery 1994; 34:252–5