

# Ideal Permissive Hypotension to Resuscitate Uncontrolled Hemorrhagic Shock and the Tolerance Time in Rats

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## ABSTRACT

**Background:** Studies have shown that permissive hypotension for uncontrolled hemorrhagic shock can result in good resuscitation outcome. The ideal target mean arterial pressure (MAP) and the tolerance time for permissive hypotension have not been determined.

**Methods:** To elucidate the ideal target MAP and tolerance time for permissive hypotension with uncontrolled hemorrhagic shock rats, the effects of different target MAPs (40, 50, 60, 70, 80, and 100 mmHg) and 60-, 90-, and 120-min permissive hypotension (50 mmHg) on uncontrolled hemorrhagic shock were observed.

**Results:** Rats in normotensive groups (80 and 100 mmHg) had increased blood loss (101%, 126% of total blood volume), decreased hematocrit, decreased vital organ (liver and kidney) and mitochondrial function, and decreased animal survival rate (1 of 10). Rats in the 50- and 60-mmHg target MAP groups had decreased blood loss (52% and 69%, respectively), good hematocrit and vital organ and mitochondrial function, stable hemodynamics, and increased animal survival (8 of 10 and 6 of 10, respectively). Rats in the 40-mmHg target MAP group, although having decreased blood loss (39%), appeared to have very inferior organ function and animal survival (2 of 10). Animal survival (1 of 10) and vital

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## What We Already Know about This Topic

- Control of blood pressure during resuscitation for hemorrhagic shock affects outcome and permissive hypotension may be beneficial.

## What this Article Tells Us that is New

- In rats, survival and organ function after uncontrolled hemorrhagic shock were best when a resuscitation target pressure of 50–60 mmHg was applied during a maximum period of 90 min.

organ function in the 120-min permissive hypotension group were significantly inferior to the 60- and 90-min groups. The 60- and 90-min groups had similar animal survival (8 of 10 and 6 of 10) and vital organ function.

**Conclusion:** A target resuscitation pressure of 50–60 mmHg is the ideal blood pressure for uncontrolled hemorrhagic shock. Ninety minutes of permissive hypotension is the tolerance limit; 120 min of hypotensive resuscitation can cause severe organ damage and should be avoided.

**T**RAUMATIC hemorrhagic shock is often seen in civilian and military situations. It is the major cause of early death in injured soldiers, accounting for approximately 50% of deaths of battle personnel.<sup>1</sup> Besides dressing, immobilization, and hemostasis for early emergency treatment, fluid resuscitation is the common and very important treatment for many types of circulatory shock, particularly for traumatic hemorrhagic shock. Effective fluid resuscitation of shock is the key to success for follow-up therapy.

Many studies, including animal studies and clinical trials, suggest that over- and underresuscitation can increase mortality.<sup>2–4</sup> Early aggressive fluid resuscitation can cause severe hemodilution, clot dislocation, and a decrease in platelet and coagulant factors, resulting in an increase in blood loss.<sup>5</sup> Permissive hypotension has been advocated as a better means to carry out field resuscitation of penetrating trauma<sup>4</sup> and has been shown to decrease mortality in models of uncontrolled

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hemorrhagic shock in anesthetized pigs and rats compared with resuscitation designed to normalize blood pressure (normotensive resuscitation).<sup>6,7</sup>

Ascertaining the ideal target blood pressure and how long the body can tolerate permissive hypotension has not been determined. Based on the previous reports and our pilot study that too-low target mean arterial pressure (MAP) (less than 40 mmHg) can cause tissue hypoperfusion for uncontrolled hemorrhagic shock, whereas too high or normal MAP (more than 80 mmHg) may greatly increase the blood loss,<sup>6–8</sup> we speculated that the ideal target MAP to resuscitate uncontrolled hemorrhagic shock may be 50–70 mmHg, and only for an appropriate shorter time at this target MAP, can a good resuscitation effect be obtained. To confirm this hypothesis, the effects of different target blood pressures (40, 50, 60, 70, 80, and 100 mmHg) and different durations (60, 90, and 120 min) of permissive hypotension on uncontrolled hemorrhagic shock were observed in the current study.

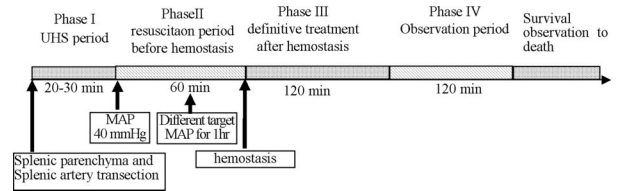
## Materials and Methods

### Ethical Approval of the Study Protocol

The current study 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).

### Animal Management

Sprague-Dawley (SD) rats (200–250 g) were fasted for 12 h but allowed water *ad libitum* before the experiment. Rats were first anesthetized with sodium pentobarbital (30 mg/kg). This was added until the rats had no response to a needle stimulus. Rats were not given respiration control, and none of them developed apnea. The right femoral artery and left and right femoral vein were catheterized with a polyethylene catheter (outer diameter, 0.965 mm; inner diameter, 0.58 mm) for monitoring the mean MAP, bleeding, and infusion. The normal MAP of rats is approximately 100–120 mmHg. Left ventricular catheterization *via* the right carotid artery was done for observation of hemodynamics. To prevent clot formation, the artery tubing was filled with normal (0.9%) saline containing 30  $\mu$ /ml heparin. To maintain the body temperature at 37°C, rats were placed on a warming plate. An uncontrolled hemorrhagic shock model was induced by transection of splenic parenchyma and one of the branches of the splenic artery as described before in our research team.<sup>9</sup> 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. In addition, one of the major branches of the splenic artery was 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.



**Fig. 1.** The timeline of the experiment phases. MAP = mean arterial blood pressure; UHS = uncontrolled hemorrhagic shock.

### Experimental Protocol

**Experimental Phases.** Experiments were classified into four phases. Phase I was the uncontrolled hemorrhagic shock (model stage). This phase was achieved when the MAP decreased to 40 mmHg. Blood was allowed to hemorrhage freely into the abdominal cavity. This phase was usually maintained for 20–30 min. Phase II was the resuscitation period before hemostasis, in which rats were resuscitated at different target MAPs (40, 50, 60, 70, 80, and 100 mmHg) for 1 h with infusion of hydroxyethyl starch and lactated Ringer's solution at a ratio of 1:2. Phase III was the definitive resuscitation period. After complete hemostasis was achieved by full ligation of the splenic artery, rats received whole blood and lactated Ringer's solution at a ratio of 1:2 to maintain the MAP at 90–100 mmHg for 2 h. Whole blood and lactated Ringer's solution were simultaneously infused *via* left and right femoral venous catheters at 1:2 infusion rate. Whole blood was acquired from donor normal rats. Phase IV was the observation period, during which the effects of the experiments detailed above were observed (fig. 1). Experiments were carried out in three parts as follows.

**Fluid Requirements, Blood Loss, and Animal Survival and the Changes in the Hematocrit, Hemodynamics, and Blood Gases after Different Target MAP Resuscitation.** Eighty SD rats were randomly divided into eight groups of 10 before complete hemostasis: sham-operated group; no treatment group; and 40-, 50-, 60-, 70-, 80-, and 100-mmHg target MAP groups at phase II. The MAP and left intraventricular systolic pressure and blood gases (including blood pH and PaO<sub>2</sub>) were determined at baseline and at the end of phase I (model phase), phase II (resuscitation before hemostasis), phase III (definitive resuscitation), and phase IV (observation period) using a polygraph physiologic recorder (SP844, Power Laboratory; AD Instruments, Castle Hill, NSW, Australia) and blood gas analyzer (Phox plus L; Nova Biomedical, Waltham, MA). Blood loss, hematocrit, the amount of fluid infusion, and animal survival were recorded. The amount of blood loss and hematocrit were measured at the end of phase II using the method of cotton weighing and centrifuge, respectively.

**Changes in the Function of the Liver, Kidney, and Mitochondria after Different Target MAP Resuscitation.** SD rats (120) were randomly divided into six groups (n = 10 for each group at each time point) before complete hemostasis: sham-operated group and 40-, 50-, 60-, 70-, and 80-mmHg

target MAP groups. At the end of phase II and phase III, after blood was sampled to measure liver and kidney function, the rats were killed to obtain liver and kidney tissue for measurement of mitochondrial function by a mitochondrial function analyzer (MT 200; Strathkelvin, Lanarkshire, Scotland). Mitochondrial function was reflected with the respiration control rate of mitochondria, which is the consumed oxygen rate of mitochondria with and without adenosine diphosphate. It is a sensible index to reflect the construction integrity and oxidative phosphorylation function of mitochondria. Variables of liver function (total bilirubin), kidney function (blood urea nitrogen and serum creatinine), and hepatic cell damage (alanine aminotransferase and aspartate aminotransferase) were measured by a biochemical analyzer (DX800 Biochemical Analyzer; Beckman, Fullerton, CA). Most rats in the no-treatment control group and 100-mmHg group died before the definitive resuscitation phase in the first experiment, so these two groups were not designed in this part of the experiment.

**Measurement of Mitochondrial Function in the Liver and Kidney.** Samples of liver or kidney tissue (5 g) were put into 20 ml ice-cold isolation buffer (0.25 M sucrose, 0.1 mM Na<sub>2</sub>EDTA, 0.01 M Tris, 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  $1,600 \times g$  for 12 min at 4°C. The supernatant was further centrifuged twice at  $25,000 \times g$  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 measurement buffer (0.2 M Tris, pH 7.6, 15 mM KCl, 15 mM KH<sub>2</sub>PO<sub>4</sub>, 1 mM Na<sub>2</sub>EDTA, 5 mM MgCl<sub>2</sub>, 0.25 M sucrose) warmed to 30°C was added into the reaction chamber and equilibrated for 2 min. Then 0.2 ml of 3 mg/ml mitochondrial mixture was put into the reaction chamber and equilibrated for 20 s. Ten microliters of 0.5 M sodium malate (C<sub>4</sub>H<sub>4</sub>Na<sub>2</sub>O<sub>5</sub>·H<sub>2</sub>O) and sodium glutamate (C<sub>5</sub>H<sub>8</sub>NNaO<sub>4</sub>) and 5 μl adenosine diphosphate (400 nM) were added in succession. The rate of oxygen consumption was determined by a mitochondrial function analyzer (MT 200; Strathkelvin, Lanarkshire, Scotland). Mitochondrial function was reflected by the respiration control rate (consumed oxygen rate with and without adenosine diphosphate).

**Effects of Different Durations of Permissive Hypotension on Uncontrolled Hemorrhagic Shock.** Based on the results of the first experiment in the current study, 50-mmHg permissive hypotension had the best resuscitative effect on uncontrolled hemorrhagic shock and was therefore used to investigate the tolerance limit of the body to permissive hypotension. Two parts of the experiments were conducted in this section. In part I, 30 SD rats were randomly divided into three groups of 10: 60-, 90-, and 120-min hypotensive (50 mmHg) duration. Blood loss, survival time, and survival were recorded during the period of permissive hypotensive

resuscitation and after the experiment. In part II, 60 SD rats were randomly divided into three groups: 60-, 90-, and 120-min duration groups. At the end of hypotensive (phase II) and definitive resuscitation (phase III), rats (n = 10 in each group at each time point) were killed. Samples of blood and liver and kidney tissue were taken to measure the function of the liver, the kidney, and mitochondria.

### Statistical Analyses

Data including MAP, left ventricular systolic pressure, PO<sub>2</sub>, blood pH value, fluid requirement, blood loss, hematocrit, mitochondrial control rate, and organ function variables were presented as mean ± SD of n observations. Statistical differences were analyzed by a repeated measures one-way or two-way ANOVA followed by the *post hoc* Tukey test (SPSS 15.0; SPSS Incorporated, Chicago, IL). Survival time and survival rate were analyzed by median and interquartile range and Kaplan–Meier survival analysis and log-rank test. A *P* value less than 0.05 was considered significant (two-tailed).

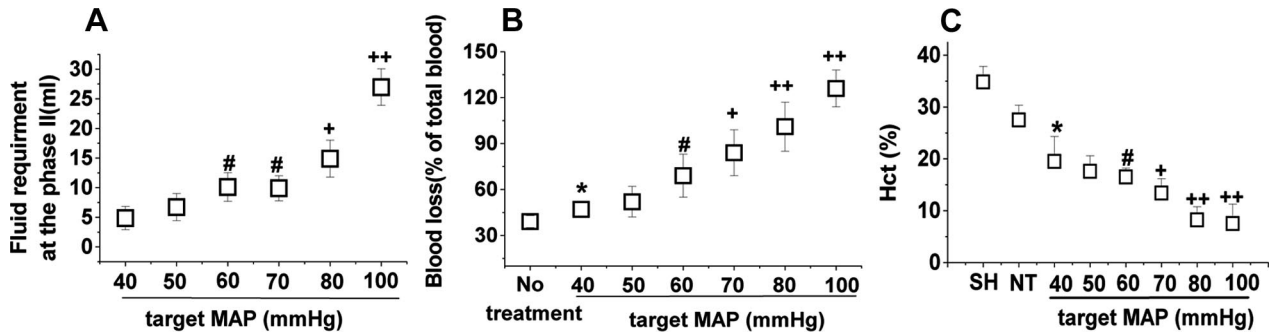
## Results

### Fluid Requirements, Blood Loss, Animal Survival, and Changes in the Hematocrit, Hemodynamics, and Blood Gases

**Fluid Requirement.** There were significant differences in fluid requirements in the 40-, 50-, 60-, 70-, 80-, and 100-mmHg target MAP groups during phase II (before hemostasis). With the increase of target MAP, the fluid requirement was increased. The average amount of fluid infusion in the 100-mmHg resuscitation group was  $26.9 \pm 3.1$  ml, approximately six times more than in the 40-mmHg group,  $4.8 \pm 1.7$  ml. There was no significant difference in the fluid requirement in the 50-, 60-, and 70-mmHg target MAP groups (fig. 2A). In addition, there were no significant differences in the fluid requirements in all groups during phase III (data not shown).

**Blood Loss.** The total blood loss in the no-treatment control group was  $39.4 \pm 4.3\%$  of the total estimated blood volume (70 ml/kg). The blood loss was significantly increased with the increase in target blood pressure. The blood loss in the 50- and 60-mmHg target MAP resuscitation groups was  $52.3 \pm 10.1\%$  and  $69.0 \pm 14.2\%$  of the estimated total blood volume, respectively, whereas in the 80- and 100-mmHg target MAP groups, the blood losses reached  $101.3 \pm 16.2\%$  and  $126 \pm 14.8\%$ , respectively, which was significantly higher than those in the 50- and 60-mmHg resuscitation groups. The average blood loss of rats at the end of phase I was approximately 38.3% (fig. 2B).

**Hematocrit.** At the end of phase II, the increase in target blood pressure was accompanied with a gradual decrease in the hematocrit. The hematocrit in the 50- and 60-mmHg target MAP groups was  $17.6 \pm 3.0\%$  and  $16.5 \pm 1.7\%$ , respectively, whereas the hematocrit in the 80- and 100-mmHg target MAP groups was significantly reduced to  $8.2 \pm 2.5\%$  and  $7.5 \pm 3.8\%$ , respectively (fig. 2C).



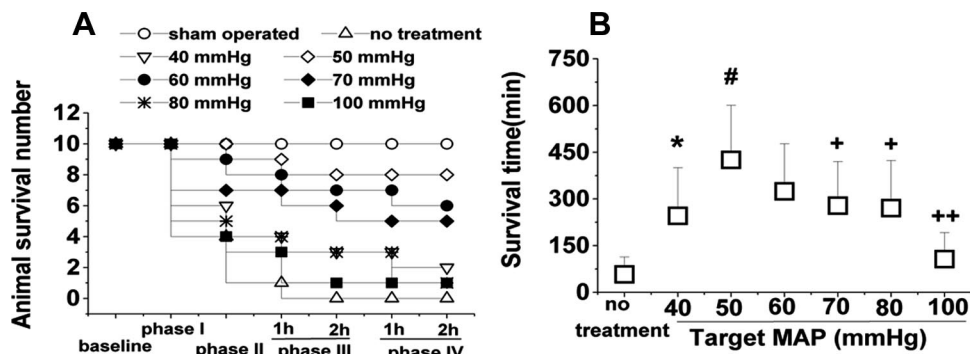
**Fig. 2.** Effect of different target MAPs on fluid requirements, blood loss, and hematocrit after uncontrolled hemorrhagic shock in rats. (A) Fluid requirement. (B) Blood loss (percentage of estimated total blood volume). (C) Hematocrit. Data are presented as mean  $\pm$  SD ( $n = 10$ ). Hct = hematocrit; MAP = mean arterial blood pressure; NT = no treatment group; SH = sham operated group. \*  $P < 0.05$ , versus no-treatment group; #  $P < 0.05$ , versus 40-mmHg group; +  $P < 0.05$ , ++  $P < 0.01$ , versus 50-mmHg group.

**Survival.** Six of 10 rats in the no-treatment control group died at the end of phase II; the average survival time was 60 min, and only one rat survived for 1 h of phase III. Five, 6, and eight rats of 10 survived in phase IV in the 70-, 60-, and 50-mmHg target MAP groups, respectively. Only one or two rats in the 40-, 80-, and 100-mmHg target MAP groups survived in phase IV. Animal survival and survival time in the 60- and 50-mmHg target MAP groups were significantly higher than in the 40-, 80-, and 100-mmHg target MAP groups and no-treatment group ( $P < 0.05$ ) (figs. 3A and B).

**MAP and Left Intraventricular Systolic Pressure.** During phase II (uncontrolled hemorrhagic shock), the MAP in the 40-, 50-, 60-, 70-, and 80-mmHg groups could be maintained at a set concentration. The MAP in the 100-mmHg group could be maintained at 100 mmHg at the early stage of this phase. However, at the late stage of this phase, the MAP could not be maintained at 100 mmHg; it fell to only 53 mmHg at the end of this phase. During phase III (definitive resuscitation phase), the MAP in all groups could be maintained at 90–100 mmHg. At the end of fluid infusion (phase IV), the MAP in the 40-, 70-, 80-, and 100-mmHg group could not be stabilized; it gradually decreased in the 50- and 60-mmHg groups, and the MAP maintained a stable and higher concentration. The trend in change in the left intraventricular systolic pressure was similar to the trend in change in the MAP (table 1).

**Blood pH and PaO<sub>2</sub>.** Blood pH in the 40-mmHg resuscitation group was significantly lower than that in the control group during the entire experiment. Although pH in other groups decreased after uncontrolled hemorrhagic shock and fluid infusion, it was significantly higher than that in the no-treatment and 40-mmHg resuscitation groups. PaO<sub>2</sub> in each group was decreased compared with that in the control group at the end of phase II, but the difference was not significant. After definitive fluid resuscitation (phase III), PaO<sub>2</sub> in all groups increased. In phase IV, PaO<sub>2</sub> in the 40-, 70-, 80-, and 100-mmHg resuscitation groups was significantly decreased; only in the 50- and 60-mmHg resuscitation groups was PaO<sub>2</sub> maintained at a higher concentration (table 2).

**Changes in the Function of the Liver, Kidney, and Mitochondria after Different Target MAP Resuscitation Mitochondrial Function in the Liver and Kidney.** The mitochondrial respiratory control rate in the liver and kidney in the 40-, 70-, and 80-mmHg groups was significantly reduced compared with the sham-operated group. Of these groups, the respiratory control rate of the liver ( $3.9 \pm 0.9\%$  and  $2.1 \pm 0.5\%$ ) and kidney ( $3.1 \pm 0.9\%$  and  $2.4 \pm 0.7\%$ ) in the 40-mmHg group was lowest at the end of phase II and phase III. There was a good mitochondrial respiratory control rate in the liver ( $8.8 \pm 1.1\%$  and  $6.3 \pm 1.2\%$ ) and kidney ( $6.9 \pm 1.5\%$



**Fig. 3.** Effect of different target MAPs during uncontrolled hemorrhagic shock on survival. (A) Survival number. (B) Survival time. Data are presented as median and interquartile range (IQR) ( $n = 10$ ). MAP = mean arterial blood pressure. \*  $P < 0.05$ , versus no-treatment group; #  $P < 0.05$ , versus 40-mmHg group; +  $P < 0.05$ , ++  $P < 0.01$ , versus 50-mmHg group.

**Table 1.** Changes in MAP and LVSP after Fluid Resuscitation at Different Target MAPs in Uncontrolled Hemorrhagic Shock Rats

Group	MAP (mmHg)			LVSP (mmHg)		
	End of Phase II	End of Phase III	End of Phase IV	End of Phase II	End of Phase III	End of Phase IV
Sham-operated	111.3 ± 18.2	109.6 ± 18.7	109.8 ± 17.0	147.8 ± 21.4	147.1 ± 15.5	132.1 ± 18.7
No treatment	30.2 ± 12.3	None survived	None survived	60.6 ± 14.5	None survived	None survived
40 mmHg	40.0 ± 2.4	100.7 ± 5.2	80.7 ± 9.5	118.1 ± 37.6	132.8 ± 9.8	128.7 ± 11.1
50 mmHg	49.3 ± 4.1	96.2 ± 9.6	101.5 ± 5.5	100.4 ± 17.9	132.2 ± 10.8	122.7 ± 5.7
60 mmHg	58.1 ± 5.3*	98.2 ± 10.2	102.2 ± 5.7	125.4 ± 23.7*	142.5 ± 18.0	117.9 ± 15.2
70 mmHg	70.7 ± 1.9*‡§	96.0 ± 8.6	83.3 ± 12.7§	124.1 ± 39.8*	124.6 ± 15.5	116.2 ± 13.9
80 mmHg	81.5 ± 11.4†‡§	97.7 ± 6.2	80.5 ± 11.4	122.0 ± 31.2	125.0 ± 17.1	103.0 ± 14.4*§
100 mmHg	53.0 ± 10.1	98.0 (n = 1)	87.0 (n = 1)	86.1 ± 13.1	100.5 (n = 1)	127.1 (n = 1)

Phase II = the resuscitation period before hemostasis; phase III = the definitive resuscitation period; phase IV = the observation period. Data are presented as mean ± SD, n = 10 in each group.

\*  $P < 0.05$ ; †  $P < 0.01$ , vs. no treatment group; ‡  $P < 0.01$ , vs. 40-mmHg group; §  $P < 0.05$ ; ||  $P < 0.01$ , vs. 50-mmHg group.

LVSP = left intraventricular systolic pressure; MAP = mean arterial pressure.

and  $5.9 \pm 1.6\%$ ) in the 50-mmHg resuscitation groups, and they were significantly superior to the values in the 40- and 80-mmHg resuscitation groups (figs. 4A and B).

**Function of the Liver and Kidney and the Hepatic Cell Damage Index.** The function of the liver and kidney in all resuscitation groups appeared to be reduced after uncontrolled hemorrhagic shock. A reduction in the function of the liver and kidney was most severe in the 40-mmHg group; concentrations of alanine aminotransferase, aspartate aminotransferase, total bilirubin, blood urea nitrogen, and serum creatinine were significantly higher than in the sham-operated group. Concentrations of alanine aminotransferase, aspartate aminotransferase, total bilirubin, blood urea nitrogen, and serum creatinine in other groups also appeared to increase, but they were significantly lower than the concentrations in the 40-mmHg group (fig. 5).

#### Effects of Different Durations of Permissive Hypotension on Uncontrolled Hemorrhagic Shock

**Survival.** Eight of 10 and 6 of 10 rats in the 60- and 90-min permissive hypotension resuscitation groups survived phase

IV, and the mean survival time was  $440 \pm 98$  min and  $340 \pm 89$  min, respectively. These survival times were significantly superior to those in the 120-min persistence time group (1 of 10 rat survived phase IV; the survival time was only  $260 \pm 86$  min [figs. 6A and B]).

**Blood Loss.** Average blood loss in the 60-min hypotensive resuscitation group during uncontrolled hemorrhagic shock was  $40.9 \pm 5.1\%$ . With prolongation of hypotensive resuscitation time, blood loss gradually increased. The mean blood loss in the 90- and 120-min resuscitation group was  $51.4 \pm 12.3\%$  and  $56.3 \pm 9.2\%$ , respectively; these values were significantly more than the mean blood loss in the 60-min hypotensive resuscitation group (fig. 6C).

**Function of the Liver, Kidney, and Mitochondria.** Mitochondrial function in the liver and kidney in the 120-min resuscitation group was significantly lower than that in the 60- and 90-min resuscitation groups. There was no significant difference in mitochondrial function between the 60- and 90-min resuscitation groups. Concentrations of alanine

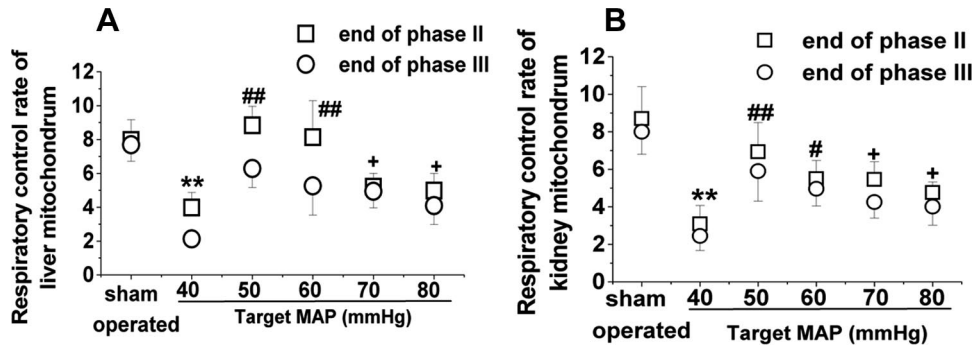
**Table 2.** Changes in Blood pH and Pao<sub>2</sub> after Fluid Resuscitation at Different Target MAPs in Uncontrolled Hemorrhagic Shock Rats

Group	pH			Pao <sub>2</sub> (mmHg)		
	End of Phase II	End of Phase III	End of Phase IV	End of Phase II	End of Phase III	End of Phase IV
Sham-operated	7.39 ± 0.11	7.38 ± 0.07	7.34 ± 0.07	112.1 ± 16.1	112.3 ± 16.1	119.8 ± 16.5
No treatment	7.12 ± 0.02*	None survived	None survived	87.8 ± 15.3*	None survived	None survived
40 mmHg	7.13 ± 0.04*	7.28 ± 0.03*	7.13 ± 0.04*	90.1 ± 15.1*	92.3 ± 12.4*	79.8 ± 13.1*
50 mmHg	7.25 ± 0.05†	7.33 ± 0.05†	7.34 ± 0.08†	96.6 ± 17.1	105.7 ± 18.7	96.4 ± 11.6
60 mmHg	7.27 ± 0.07†	7.33 ± 0.05†	7.33 ± 0.03†	96.9 ± 15.3	104.3 ± 18.2	97.2 ± 13.9
70 mmHg	7.28 ± 0.04	7.32 ± 0.02†	7.33 ± 0.07†	94.4 ± 14.5	98.6 ± 17.7	87.1 ± 12.5*
80 mmHg	7.28 ± 0.12	7.32 ± 0.11†	7.12 ± 0.03‡	88.5 ± 14.1	94.8 ± 15.4*	76.6 ± 13.3*
100 mmHg	7.23 ± 0.04	7.27 (n = 1)	7.12 (n = 1)	87.1 ± 13.9	87.3 (n = 1)	83.4 (n = 1)

Phase II = the resuscitation period before hemostasis; phase III = the definitive resuscitation period; phase IV = the observation period. Data are presented as mean ± SD, n = 10 in each group.

\*  $P < 0.05$  vs. sham-operated group; †  $P < 0.05$ , vs. 40-mmHg group; ‡  $P < 0.05$ , vs. 50-mmHg or 60-mmHg group.

MAP = mean arterial pressure.



**Fig. 4.** Effect of different target MAPs on mitochondrial function in the liver and kidney after uncontrolled hemorrhagic shock. (A) Respiration control rate of liver mitochondria. (B) Respiration control rate of kidney mitochondria. Data are presented as mean  $\pm$  SD ( $n = 10$ ). MAP = mean arterial blood pressure. \*\*  $P < 0.01$ , versus sham-operated group; #  $P < 0.05$ , versus 40-mmHg group; ##  $P < 0.01$ , versus 40-mmHg group; +  $P < 0.05$ , versus 50-mmHg group.

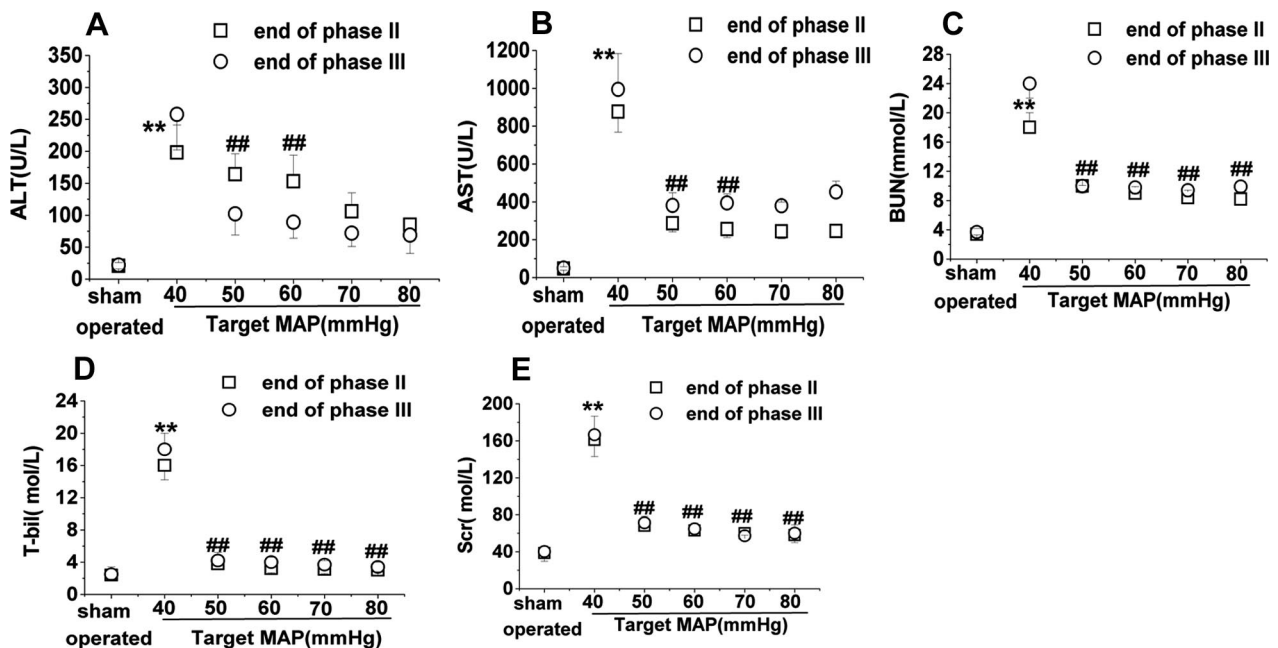
aminotransferase, aspartate aminotransferase, total bilirubin, blood urea nitrogen, and serum creatinine in the 120-min resuscitation group were significantly higher than those in the 60- and 90-min resuscitation group. In addition, there was no significant difference in liver function and kidney function between the 60- and 90-min resuscitation group (figs. 6D–J).

## Discussion

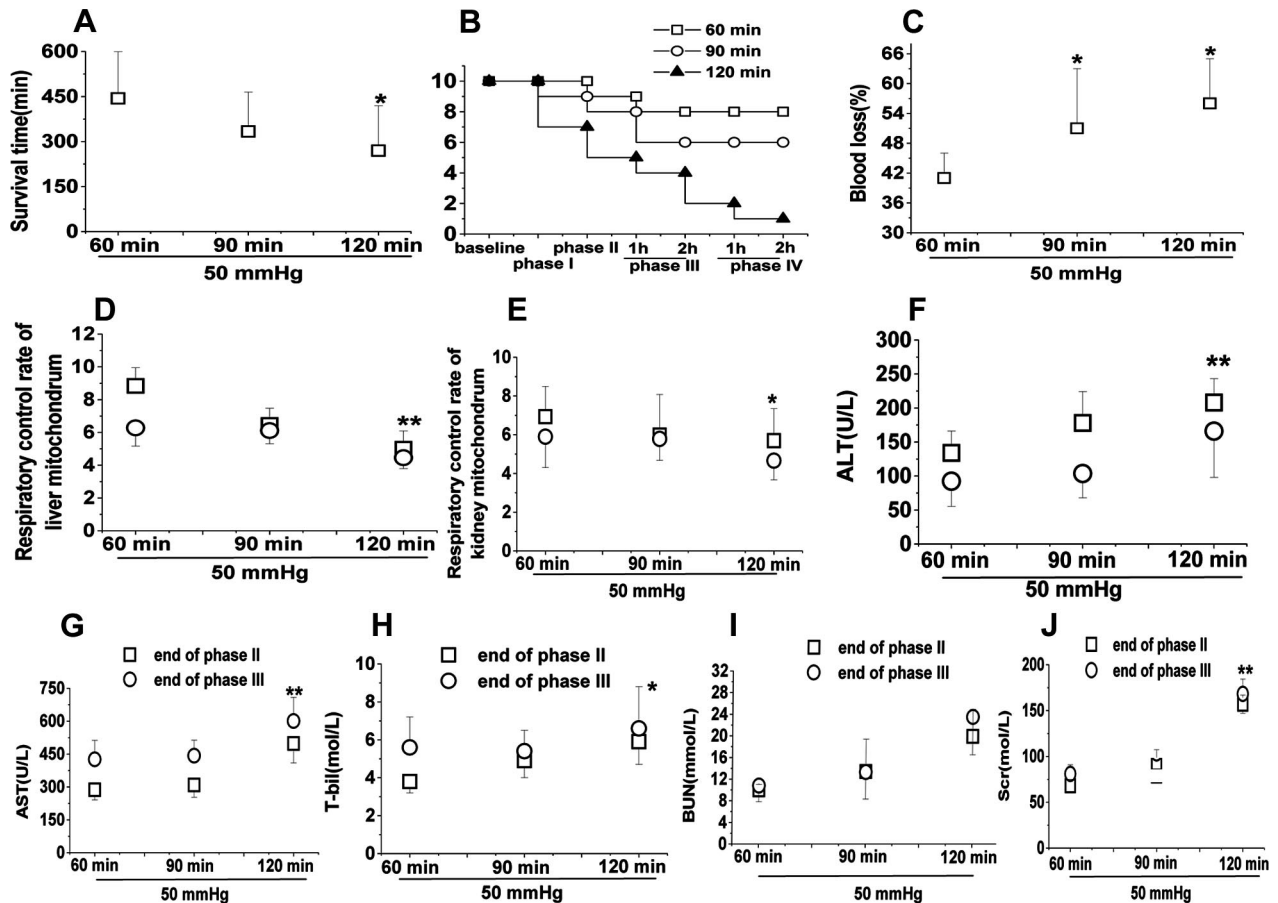
Aggressive fluid resuscitation for uncontrolled hemorrhagic shock before hemostasis could cause severe hemodilution and clot dislocation and increase the loss of platelet and coagulant factors, thereby increasing the risk of death. Limited or hypotensive resuscitation has been advocated as a better

resuscitation approach for penetrating trauma. The ideal target blood pressure and duration for hypotensive resuscitation are not known.

We compared the effects of a series of target MAP (40, 50, 60, 70, 80, and 100 mmHg) and different durations (60, 90, and 120 min) of permissive hypotension on the resuscitation outcome of uncontrolled hemorrhagic shock. We found that normotensive resuscitation (80 and 100 mmHg) significantly decreased survival and survival time, increased blood loss and hemodilution, and caused severe damage to vital organ functions (including mitochondrial function). In contrast, permissive hypotension (50- and 60-mmHg MAP) resulted in longer survival time and higher survival in uncontrolled hemorrhagic shock rats. The hematocrit, hemodynamic



**Fig. 5.** Effect of different target MAPs on liver function and kidney function after uncontrolled hemorrhagic shock. (A) ALT. (B) AST. (C) BUN. (D) T-bil. (E) Scr. Data are presented as mean  $\pm$  SD ( $n = 10$ ). ALT = alanine aminotransferase; AST = aspartate aminotransferase; BUN = blood urea nitrogen; MAP = mean arterial blood pressure; T-bil = total bilirubin; Scr = serum creatinine. \*\*  $P < 0.01$ , versus sham-operated group; #  $P < 0.05$ , versus 40 mmHg group; ##  $P < 0.01$ , versus 40 mmHg group.



**Fig. 6.** Effect of different durations of permissive hypotension on resuscitation outcome after uncontrolled hemorrhagic shock. Animal survival time was presented as median and interquartile range (IQR); other variables are presented as mean  $\pm$  SD ( $n = 10$ ). \*  $P < 0.05$ , versus 60 min for 50 mmHg group. (A) Survival time. (B) Survival number. (C) Bleeding rate. (D) Respiratory control rate of liver mitochondria. (E) Respiratory control rate of kidney mitochondria. (F) ALT. (G) AST. (H) BUN. (I) T-bil. (J) Scr. ALT = alanine aminotransferase; AST = aspartate aminotransferase; BUN = blood urea nitrogen; T-bil = total bilirubin; Scr = serum creatinine.

parameters, and organ function of rats given appropriate hypotensive resuscitation were significantly superior to normotensive resuscitation groups. Having too-low target MAP (40 mmHg) during uncontrolled hemorrhagic shock was not good; survival and survival time were significantly lower than those in the 50-, 60-, and 70-mmHg target MAP groups. Among different duration of permissive hypotensive resuscitation, rats tolerated 60- and 90-min hypotensive resuscitation better than 120 min; their survival, survival time, and organ function were almost identical in the 60- and 90-min hypotensive resuscitation groups. Hypotensive resuscitation lasting more than 90 min (e.g., 120-min hypotensive resuscitation) caused severe damage to vital organ functions; survival and survival time were significantly inferior to the values in the 60- and 90-min hypotensive resuscitation groups. These results suggested that 50–60 mmHg target MAP was the ideal resuscitation pressure for uncontrolled hemorrhagic shock in rats. A too-low (40 mmHg) or too-high (more than 80 mmHg) target MAP for uncontrolled hemorrhagic shock was not good. Ninety minutes was the maximal tolerance limit of hypotensive resuscitation; 120 min of hypotensive

resuscitation could cause severe organ damage during uncontrolled hemorrhagic shock.

Capone *et al.* compared the effect of 40- and 80-mmHg pressure resuscitation on uncontrolled hemorrhagic shock in rats.<sup>6</sup> They found that the 40-mmHg group had a significantly higher survival time than that of the 80-mmHg group. Wang *et al.* found that bleeding rate and mortality were higher in rabbits undergoing 90-mmHg resuscitation than rabbits undergoing 50- and 70-mmHg resuscitation before hemostasis, although suffering uncontrolled hemorrhagic shock.<sup>10</sup> Stern found that early aggressive resuscitation (80 mmHg) resulted in an increase in bleeding rate and mortality and did not improve hemodynamic parameters in the brain arteries of pigs after aortic artery injury in combination with brain injury.<sup>11</sup> These studies compared the effects of hypotensive and normotensive or hypertensive resuscitation on uncontrolled hemorrhagic shock, but they did not observe the effect of a series of resuscitation pressures. These studies, therefore, did not find the ideal resuscitation pressure for uncontrolled hemorrhagic shock. By comparison of the effects of a series of target resuscitation pressures (40, 50, 60,

70, 80, and 100 mmHg MAP) on the resuscitation outcome of uncontrolled hemorrhagic shock, we found that a too-low (40 mmHg) or too-high (more than 80 mmHg) resuscitation pressure during uncontrolled hemorrhagic shock was not good. The ideal target MAP for uncontrolled hemorrhagic shock in rats was 50–60 mmHg.

Normotensive resuscitation significantly decreased the animal survival. It may be closely related to the large amount of blood loss, resulting in a severe hemodilution and severe ischemia and hypoxia in tissues. A too-low target blood pressure of fluid resuscitation can cause severe tissue ischemia and hypoxia because it cannot meet the demand of tissue perfusion, so animal survival was low. Permissive hypotensive resuscitation resulted in a good resuscitation effect (including good animal survival), good organ function and acid-base balance, and stable hemodynamics. It may not only be related to reducing blood loss and keeping a stable hematocrit and  $\text{PaO}_2$ , but also to maintaining the basic perfusion of vital organs and alleviating tissue ischemia and hypoxia, thereby preventing damage to vital organs and tissue metabolism.

Uncontrolled hemorrhagic shock is usually established by injury to the parenchymal tissue of organs (including impairment of the liver or spleen) or by vascular injury.<sup>12–15</sup> Organ damage in combination with vascular injury can imitate uncontrolled hemorrhagic shock, but this model is not easy to reproduce because of severe impairment of tissue.<sup>16</sup> Single vascular damage-induced uncontrolled hemorrhagic shock resulted in the same hemodynamic changes, coagulation disorders, and inflammation reaction as a model using organ damage and vascular injury.<sup>17–19</sup> However, this model cannot completely mimic the clinical scenario because the tissue damage in this model is not severe. The model of uncontrolled hemorrhagic shock adopted in the current study was induced by transection of splenic parenchyma and the splenic artery. The mean concentration of bleeding for each rat in this model was approximately 29.5 ml/kg, which accounted for approximately 38.3% of the estimated total blood volume of each rat. The current study showed that this model was stable and repeatable. We previously used this model to investigate the effect of initial fluid resuscitation on uncontrolled hemorrhagic shock and achieved satisfactory data.<sup>8</sup>

Traumatic coagulopathy is a hypocoagulable state that often occurs in the most severely injured. There are multiple factors that may contribute to coagulopathy after severe trauma or shock. These include the increase of anticoagulation factors, such as hyperfibrinolysis and tissue plasminogen activator, and the decrease of the concentration of plasminogen activator and thrombin activatable fibrinolysis inhibitor.<sup>20,21</sup> In addition, hemodilution induced by excessive infusion of crystalloids and banked erythrocytes would worsen shock-induced hypocoagulation.<sup>22–25</sup> The current study showed that higher target resuscitation pressure (more than 80 mmHg of the MAP) could greatly increase blood loss, resulting in severe hemodilution, but whether it was related

to coagulopathy and the precise mechanism needs further investigation.

Research has shown that different anesthetics have different effects on the cardiovascular system, especially on vascular tone, because of different physiologic and pathophysiological states and different vascular beds. Most general anesthetics decreased the vascular resistance in peripheral circulations.<sup>26</sup> Some research showed that thiopental, propofol, and ketamine can induce local vasodilator effects by inhibition of L-type voltage-gated  $\text{Ca}^{2+}$  channels.<sup>27</sup> So, in anesthetized animals, at the beginning of bleeding, the blood vessel is in a vasodilated state, which is different from human traumatic shock patients, whose bleeding is in a state of maximal vasoconstriction. The anesthetic that we used in the current study was sodium pentobarbital. One study showed that sodium pentobarbital could cause compensatory vasoconstriction because of myocardial depression and systemic hypotension.<sup>28</sup> It was therefore assumed that the vasodilatation in the current model may not be obvious, and with continued hemorrhage, the blood vessel will constrict. Nevertheless, the extent of the effects of anesthetics on vascular tone after hemorrhagic shock (and whether preanesthesia can increase the tolerance of rats to hemorrhage) needs experimental confirmation.

Although we found that the target MAP of 50–60 mmHg is the ideal pressure for uncontrolled hemorrhagic shock in rats and that 90 min is the maximal tolerance limit of the body to permissive hypotension, there were several limitations in the current study. First, this study was mainly limited to small and anesthetized animals (rats); whether this model can completely reflect uncontrolled hemorrhagic shock in humans, and whether the ideal target MAP and duration in animals are completely suitable to humans, need further confirmation. Second, the rats we used in the current study were healthy and young; whether these parameters can be appropriate to elderly patients and patients with cardiovascular comorbidity also need further investigation. Third, we only observed the tolerance time of the body to permissive hypotension; we did not observe the effects of different persistence time at different target MAPs on uncontrolled hemorrhagic shock. Fourth, severe injury to tissue, shock, and coagulation have obvious interactions; it may be an important area for future research. We did not observe their interaction and precise mechanism in the current study.

## Conclusion

The current study suggested that normotensive resuscitation for uncontrolled hemorrhagic shock before hemostasis would increase blood loss and result in suboptimal resuscitation outcome. Appropriate hypotensive resuscitation is beneficial in uncontrolled hemorrhagic shock, and 50–60 mmHg may be the ideal hypotensive resuscitation target MAP. A too-low resuscitation pressure for uncontrolled hemorrhagic shock was not good. Ninety minutes of hypotensive resuscitation may be the maximal tolerance limit of the body; more than 120 min of hypotensive resuscitation can cause severe organ damage and



should be avoided. Whether these parameters can be completely suitable to human patients, especially for elderly patients and patients with other comorbidities, needs further confirmation and investigation.

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