Original Article

Effect of Induced and Spontaneous Hypothermia on Survival Time of Uncontrolled Hemorrhagic Shock Rat Model

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We examined the hypothesis that mild hypothermia (rectal temperature 34℃) results in the same survival time, whether induced spontaneously or intentionally, during untreated, lethal, uncontrolled hemorrhagic shock in rats. Sixty-four Sprague-Dawley male rats were randomly assigned to normothermia (Nth) (n=19), spontaneous mild hypothermia (Sp.Hth) (n=25) or controlled mild hypothermia (Con.Hth) (n=20) groups. After blood withdrawal of 3 mL/100 g over 15 minutes, followed by 75% tail amputation under spontaneous breathing and light anesthesia by i.p. injection of pentobarbital sodium, rats were observed without fluid resuscitation or hemostasis for 180 minutes or until death. The initial temperature of the Nth group was artificially maintained throughout the experiment. For the mild hypothermia groups, the Sp.Hth group was exposed to ambient temperature while the Con. Hth group was actively cooled to a target rectal temperature of 34℃. In the Con.Hth group, all rats except one died before 180 minutes. All rats in the Nth group died within 38 minutes, and within 67 minutes in the Sp.Hth group. The average survival time was shortest in the Nth group at 20.3 ± 5.3 minutes, followed by the Sp.Hth group at 30.1 ± 13.5 minutes, and the Con.Hth group at 81.9 ± 39.8 minutes ($p <$ 0.01). Tail bleed out volume was 0.51 ± 0.19, 0.26 ± 0.15 and 0.19 ± 0.12 mL/100 g in the Nth, Sp.Hth and Con.Hth groups, respectively ($p < 0.05$). In conclusion, spontaneous mild hypothermia did not prolong the survival time as much as controlled mild hypothermia in the rat model for untreated, lethal, uncontrolled hemorrhagic shock.

Key Words: Uncontrolled hemorrhagic shock, mild hypothermia

INTRODUCTION

In trauma patients, uncontrolled hemorrhage is a major cause of early death.[1] The purpose of prehospital and emergency department (ED) care is to maintain the pulse and prevent cardiac arrest until more advanced medical care, i.e. resuscitative operative hemostasis, is available. Although the integrated trauma care system, trauma center designation, and advanced trauma life support (ATLS) training with the "golden-hour" concept have improved the outcome of severely injured patients, there is still an inevitable time lag between the insult and the definitive treatment which may lead to exsanguination death.

There is a general concern about spontaneous, uncontrolled hypothermia in trauma patients. Patients suffering from hypothermia and hemorrhagic shock (HS) do not respond normally to blood and fluid resuscitation and often develop coagulopathy. Trauma patients are predisposed to hypothermia because of the necessity for removal of clothing, opening of body cavities and administration of cold intravenous fluids and blood products. In addition they have a decreased ability to maintain body temperature because of shock, anesthetic agents, or alcohol or drug intoxication. Consequently, they are often already cool in the ED and the more severely injured patients

are at the greatest risk for developing hypothermia.[2] Accordingly, in the hospital ED, body temperature is an important vital sign to monitor during the initial assessment.[3]

Recently, there has been renewed interest in therapeutic hypothermia for resuscitation (i.e. treatment after the insult), starting with cerebral resuscitation from cardiac arrest (i.e. temporary complete global brain ischemia) to HS (i.e. systemic hypoperfusion).[4] Safar's group reported that mild (33 - 36℃) as well as moderate (28 - 32℃) hypothermia prolonged the survival time in otherwise untreated rats after lethal, uncontrolled HS (UHS).[5,6] In contrast, clinical data suggest that uncontrolled(i.e. spontaneous or accidental) hypothermia is deleterious to the outcome of trauma patients.[7,8]

The objective of the present study was to test whether spontaneous mild hypothermia can produce the same effect in prolonging the survival time as controlled mild hypothermia during untreated, lethal UHS in rats.

MATERIALS AND METHODS

We designed the study with 75 Sprague-Dawley male rats, each weighing 300 - 400 g, after the approval by our Institutional Animal Care Committee.

Preparation

The rats were housed in a controlled environment with free access to food and water. At the same time each day, general anesthesia was induced with an intraperitoneal injection of pentobarbital sodium (50 mg/kg), and the animals were placed supine and allowed to breathe spontaneously in room air throughout the experiment. PE-50 catheters were inserted into the right femoral artery for continuous monitoring of blood pressure and into the right carotid artery for blood withdrawal. Electrocardiogram, and blood pressure were monitored with Polygraph (Harvard Apparatus, Holliston, MA, USA). A rectal temperature probe (YSI 400 series, Harvard Apparatus, MA, USA) was inserted to monitor the core temperature. The ambient room temperature was maintained at 25 ± 1.0℃.

Induction of UHS

After preparation and baseline measurement, HS was induced by blood withdrawal of 3 mL/ 100 g at a constant rate over 15 minutes from the right carotid artery. The starting time of bleeding was designated as time 0. At 5 minutes, a 75% tail cut was performed to mimic uncontrolled hemorrhage. Without any fluid infusion, all animals were observed until death (no pulse, apnea) or until 180 minutes (Fig. 1).

Induction of hypothermia

Animals were randomly assigned to the normothermia group (Nth), spontaneous mild hypothermia group (Sp.Hth) and controlled mild
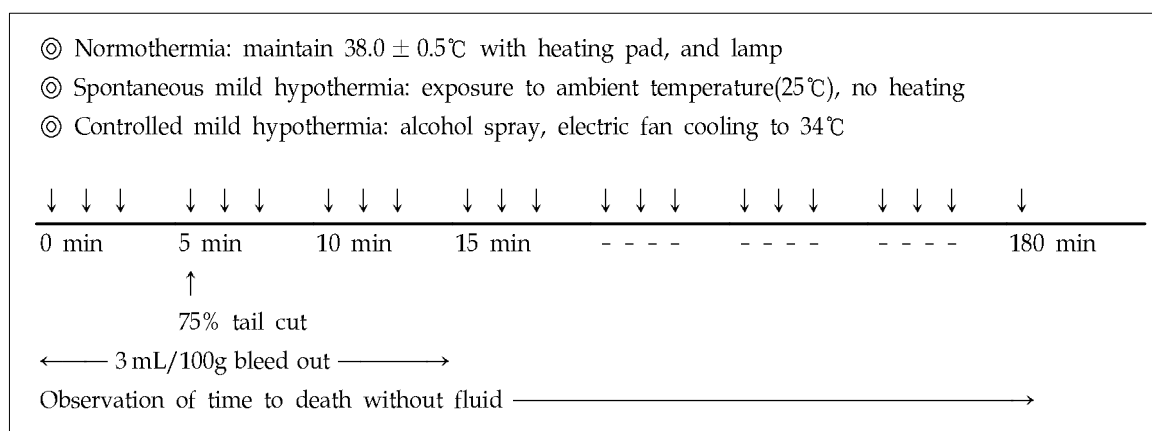
◎ Normothermia: maintain 38.0 ± 0.5℃ with heating pad, and lamp
◎ Spontaneous mild hypothermia: exposure to ambient temperature(25℃), no heating
◎ Controlled mild hypothermia: alcohol spray, electric fan cooling to 34℃

↓ ↓ ↓   ↓ ↓ ↓   ↓ ↓ ↓   ↓ ↓ ↓   ↓ ↓ ↓   ↓ ↓ ↓   ↓ ↓ ↓   ↓

0 min   5 min   10 min   15 min   - - - -   - - - -   - - - -   180 min

              ↑

            75% tail cut

←——— 3 mL/100g bleed out ———→

Observation of time to death without fluid ————————————————→

Fig. 1. Experimental scheme.

hypothermia group (Con.Hth). In the Nth group, rectal temperature was maintained at 38.0 ± 0. 5℃ with an intermittent heating pad and heat lamp application throughout the experiment. In both mild Hth groups, the heating pad was turned off and the rectal temperature was targeted to 34.0 ±0.5℃, by alcohol spray and electric fan in the Con.Hth group, and by exposure to ambient temperature in the Sp.Hth group.

Measurements

The mean arterial pressure (MAP), and electrocardiogram were continuously monitored throughout the experiment. Rectal temperature, and bleed out volume were recorded at regular intervals of 5 minutes.

Statistical analysis

Data was expressed as the mean ± SD. The primary end point was survival time, which was compared with the log-rank test. MAP, rectal temperature, tail bleed out volume, and time to reach 34.0℃ were all compared with ANOVA. Levels of $p < 0.05$ were accepted as statistically significant.

RESULTS

Among the 75 rats, we lost 11 due to exsanguination during catheterization. Consequently, 64 rats were randomly assigned into three groups, the Nth (n=19), Sp.Hth (n=25) and Con.Hth (n=20) groups. The body weight, ambient temperature, baseline MAP and rectal temperature showed no differences among the three groups.

MAP

After increasing during the first 5 minutes in all animals due to nociceptive stimuli after tail amputation, MAP declined continuously until death. In the Con.Hth group, compared to the Sp.Hth group, MAP maintained a significantly higher level throughout the experiment.

Rectal temperature

At 20 minutes, all Con.Hth rats but no Sp.Hth rats had reached below 34℃. Throughout the experiment, only 3 Sp.Hth rats reached below 34. 0℃ before death. The Con.Hth rats reached 34℃ in 12.1 ± 4.5 minutes (range: 6-18 min), in contrast to 30.7 ± 9.0 minutes (range : 22-40 min) for the 3 rats in the Sp.Hth group.

Tail bleed out volume

The Nth group showed significantly more bleed out volume than both mild hypothermia groups ($p < 0.05$). The Con.Hth group bleed less than the Sp.Hth group but without statistical difference (0.19 ± 0.12 vs. 0.26 ± 0.15 ml/100g, respectively).

Survival time

Eighteen of the 19 rats in the Nth group died within 25 minutes. In the Sp.Hth group, all 25 rats died within 67 minutes but only half of them survived over 25 minutes. The Con.Hth group rats showed the longest survival time and one rat survived to 180 minutes. The mean survival times were 20.3 ± 5.3, 30.1 ± 13.5 and 81.9 ± 39.8 minutes in the Nth, Sp.Hth and Con.Hth groups respectively ($p < 0.05$) (Table 1, Fig. 2).

DISCUSSION

In spite of many years of shock research, nothing much seems to have been added, at least in terms of the initial management of the trauma victim to the time-honored principles of controlling hemorrhage and replacing lost blood in the clinical management.[1]

Recently, there has been renewed interest in therapeutic hypothermia.[4]

Mechanism and outcome-oriented studies in animal models have documented benefits from controlled mild-to-moderate hypothermia during HS and resuscitation.[5,6,9-12] However, the widespread use of therapeutic moderate hypothermia during and after states of hypoperfusion has not been embraced by the medical community. The

Table 1. Demography and Results

	Nth (n=19)	Sp.Hth (n=25)	Con.Hth (n=20)
Body weight(g)	339 ± 70.6	364.0 ± 93.8	344.3 ± 39.8
MAP(mmHg)			
baseline	100.5 ± 21.7 (n=19)	112.7 ± 22.2 (n=25)	109.3 ± 19.4 (n=20)
15 minutes	17.7 ± 9.3 (n=19)	23.1 ± 11.0 (n=25)	33.3 ± 9.7*,† (n=20)
20 minutes	12.3 ± 7.1 (n=9)	20.8 ± 8.5* (n=18)	26.8 ± 7.7* (n=20)
25 minutes	29.0 (n=1)	20.6 ± 5.1 (n=13)	27.6 ± 7.4† (n=20)
30 minutes	23.0 (n=1)	19.4 ± 7.2 (n=11)	28.0 ± 9.6† (n=20)
Rectal temp.(℃)			
baseline	37.8 ± 0.3 (n=19)	37.8 ± 0.3 (n=25)	37.8 ± 0.3 (n=20)
15 minutes		36.4 ± 0.5 (n=25)	33.8 ± 0.3† (n=20)
20 minutes		35.9 ± 0.8 (n=17)	33.6 ± 0.3† (n=20)
25 minutes		35.5 ± 0.9 (n=13)	33.8 ± 0.3 (n=20)
30 minutes		35.2 ± 1.0 (n=11)	33.9 ± 0.2 (n=20)
Time to 34.0℃ (min)	-	30.7 ± 9.0 (n=3) (range: 22 - 40)	12.1 ± 4.5† (n=20) (range: 6 - 18)
Tail bleed out (ml/100g)	0.51 ± 0.19	0.26 ± 0.15*	0.19 ± 0.12*
Survival time (min)	20.3 ± 5.3	30.1 ± 13.5‡	81.9 ± 39.8‡,§

*$p<0.05$ Nth vs. Sp.Hth, Con.Hth.
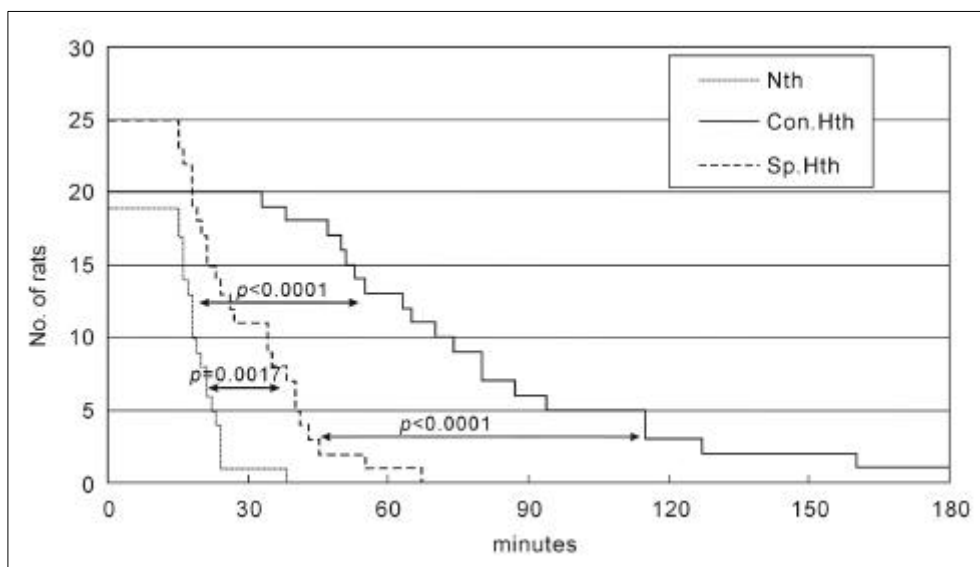†$p<0.05$ Sp.Hth vs. Con.Hth.
‡$p<0.01$ Nth vs. Sp.Hth, Con.Hth.
§$p<0.01$ Sp.Hth vs. Con.Hth.
Nth, normothermia group; Sp.Hth, spontaneous mild hypothermia group; Con.Hth, controlled mild hypothermia group; MAP, mean arterial pressure.

Fig. 2. Survival time.

potential negative effects of hypothermia, particularly with respect to coagulopathy, cannot be ignored. Core temperature below 30℃, even without shivering, can cause this complication and lead to life-threatening dysrhythmias, including ventricular fibrillation. However, clinical data suggest that significant changes in clotting do not occur unless body temperature is less than 34℃. Another concern has been infection but this would seem to be a minor issue if survival is improved with therapeutic hypothermia.[8,13]

So far, there has been a significant disparity between the results of laboratory and clinical studies with regard to the effects of hypothermia on HS outcome. In clinical studies, it was suggested that uncontrolled (i.e. spontaneous or accidental) hypothermia is deleterious to trauma patients.[7,8] Steinmann et al.[14] reported that patients with a core temperature less than 35℃ had a higher injury severity score (ISS), lower trauma score and systolic pressure and greater fluid requirement and base deficit. But when the patients were stratified according to their probability of survival, there was no significant difference in survival and length of stay in the intensive care unit (ICU) between the hypothermic and normothermic groups. This result suggests that rather than hypothermia being a primary factor in mortality, it is the underlying shock process that decreased survival and led to hypothermia. Although they concluded that hypothermia is associated with increased mortality in trauma patients, Jurkovich et al.[15] questioned, "whether it is the hypothermia per se or the severity of the injury producing the hypothermia that is responsible for the subsequent mortality".

It is important to distinguish between a situation in which hypothermia has occurred only during hemorrhage and one in which hypothermia persists after resuscitation. As reported in trauma articles, trauma patients have a certain degree of hypothermia when they arrive at ED and hypothermia is aggravated if any countermeasures are not initiated. Maybe this is the cause of the discrepancy between the results of clinical and laboratory animal studies. In laboratory animal studies, the control or normothermia group maintains normal temperature throughout the experiment, but in reality it counteracts the

normal defense mechanisms against the bleeding; vasoconstriction, increased heart rate, decreased urine output etc. To maintain normothermia, investigators used a heating pad, and lamp, causing vasodilation which led to a further decrease of perfusion pressure due to increased bleeding from the damaged vessel. The hypothermic reaction is a normal defense mechanism as revealed in Bergstein et al's study,[16] and one of us has observed increased temperature without heating during fluid resuscitation study in hypothermic animals.

When considering therapeutic hypothermia, one must differentiate between uncontrolled (i.e. spontaneous, accidental) hypothermia - which can be deleterious because of initial shivering, sympathetic discharge, increased oxygen demand, and vasoconstriction - and controlled (i.e. therapeutic) hypothermia with poikilothermia induced by insult or drugs, which can be beneficial.[17] The level of hypothermia can be divided as mild (33-36℃), moderate (28-32℃), deep (10-20℃), profound (5-10℃), and ultraprofound hypothermia (0-5℃).[4]

Our model is originated from a three-phase model developed by Capone et al. of UHS administered by tail cut in rats.[18] The pressure- and volume-controlled HS models do not adequately duplicate the physiologic changes that occur in the trauma patient with ongoing bleeding. One of us has examined the effects of increased oxygen breathing and moderate hypothermia (30℃) in a model of lethal UHS in rats, using only phase 1 of the three-phase model.[12] The study simulated clinical conditions with the victims temporarily experiencing uncontrolled bleeding while waiting for definitive treatment. The moderate hypothermia prolonged the survival time, but 100% oxygen breathing did not. Previous studies have shown that hyperoxia can increase blood pressure during mild to moderate HS.[19,20] Interestingly, a study by Brod et al.[21] showed that hyperoxia could be detrimental if hemorrhage were uncontrolled.

Although we did not attempt to explore the specific mechanisms of hypothermia, it may offer another way to overcome a shock-induced metabolic imbalance of oxygen supply and demand. One mechanism for such protection is the reduc-

tion of the metabolic rate by 6% for each 1℃ depression of body temperature. Wladis et al.[22] found that with 30℃ core temperature in the volume-controlled HS porcine model, hypothermia slowed the heart rate and induced a further reduction of cardiac output while the stroke volume did not change. Their conclusion was that the hypothermia aggravated the hypokinetic situation resulting from HS, but without increasing the mortality rate. But tissue oxygen demand at 34℃ (mild hypothermia) is expected to be only slightly lower than that at normothermia, and therefore there must be other mechanisms to explain the benefit. In a pressure-controlled HS rat model, Prueckner et al.[23] claimed that it is the hypothermia, not a blood pressure effect, that increases the survival time and rate. Nevertheless hypothermia is thought to be associated with both a decrease in oxygen delivery and a proportional decrease in oxygen consumption. Hypothermia is also known to be a powerful short-term stimulator of the sympathetic nervous system.[24] However, we did not observe the increments of pulse rate in hypothermic rats and it was rather the normothermic rats which showed marked tachycardia until impending death.

In conjunction with serial UHS studies of the Safar group,[5,6,11,12] we investigated whether the survival prolonging effect of mild hypothermia could be applied to the mild spontaneous hypothermia which occurs in trauma patients exposed to ambient temperature in the ED and prehospital phase. In this experiment, although only 3 out of 25 rats reached 34℃ before death, we found that spontaneous mild hypothermia is detrimental to survival unlike controlled mild hypothermia. So, the difference in survival time between the Sp.Hth and Con.Hth groups may be interpreted as resulting from the difference in the degree of hypothermia; only 3 in Sp.Hth rats, versus all 20 Con.Hth rats, reached below 34℃ before death. Nevertheless, we cannot conclude that survival time is entirely related to bleed out volume. Even with higher blood pressure, Con.Hth rats showed less bleed out than Sp.Hth rats. The mechanism behind this disparity could be vasoconstriction, cellular/tissue protective effect of hypothermia, maintenance of viable tissue perfusion pressure in Con.Hth, or combina-

tion of these effects. The determination of the true nature of this mechanism represents a further research agenda.

The twin limitations of our study were firstly, the use of pentobarbital sodium as an anesthetic agent which may have affected the physiologic response to hemorrhage, and secondly, the achievement of spontaneous mild hypothermia to 34℃ in only 3 rats with the majority of rats retaining a rectal temperature of over 34℃ until death. To minimize the effect of body temperature difference, further research under ambient temperature below 25℃ is needed.

The following three questions are suitable for future research. First, the question of the ideal duration of mild therapeutic hypothermia during HS and resuscitation remains unanswered. Although controlled hypothermia prolonged survival, the issue of how to combine the spontaneous hypothermia inevitably occurring in the prehospital phase with the controlled hypothermia in the ED and operating room remains to be settled. Second, almost all laboratory studies used the rat model which can be easily driven into hypothermia, whereas large animal studies such as pig have demonstrated that surface cooling is an ineffective cooling method. Two related issues requiring resolution concern - the appropriate method of cooling that is feasible in large animal models for which the results could be applied to clinical study, and the optimal method to provide rapid, simple and safe cooling. Third, the determination of the optimal timing and level of hypothermia is necessary to confirm whether more prolonged hypothermia during resuscitation will improve the outcome.

The clinical implications of uncontrolled versus controlled hypothermia in trauma patients are still unclear. A prospective trial of therapeutic, controlled hypothermia during traumatic HS and resuscitation is needed. Starting in the prehospital phase, mild hypothermia will not only decrease the immediate mortality rate, but will also possibly protect cells and reduce the likelihood of secondary inflammatory response syndrome, multiple organ failure, and late deaths.

In conclusion, we found that mild controlled hypothermia prolonged the survival time more than mild spontaneous hypothermia in lethal,

untreated, UHS in rats. A further finding was that spontaneous hypothermia is detrimental to survival, although it did result in better survival than normothermia which significantly hastened death.

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