Effect of Mild Hypothermia on Coagulatory Function and Survival in Sprague-Dawley Rats Exposed to Uncontrolled Hemorrhagic Shock

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Effect of Mild Hypothermia on Coagulatory Function and Survival in Sprague-Dawley Rats Exposed to Uncontrolled Hemorrhagic Shock

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This certifies that the dissertation of

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감사의 글

이 논문이 나오기까지 바쁘신 와중에도 아낌없는 지도를 해 주신 이강현 교수님께 가슴 속 깊이 감사드립니다. 또한 귀중한 시간을 내어 지도해 주신 김현 교수님, 박종택 교수님, 어영 교수님, 이종인 교수님께 감사드립니다.

동물 실험을 하는 동안 근무에 대해 많은 배려를 해 주신 제주대학교병원 응급의학과 김우정 교수님, 강영준 교수님, 박주옥 교수님께도 감사드립니다. 바쁜 근무 중에도 실험을 도와준 이성근 전공의, 무엇보다 실험을 시작할 때부터 열심히 보조해준 현혜진 연구원에게 감사의 마음을 전합니다.

박사 논문을 완성시킨 기쁨을 누리게 해 주신 위의 모든 분들께 감사드립니다.

실험을 시작했을 때, 잘 진행되지 않아 마음 고생이 심할 때 위로해주신 부모님, 동생 경화, 경석에게도 고마움을 전합니다.

마지막으로 본 논문은 2012 년 INJURY 에 출판될 예정이며 (DOI information: 10.1016/j.injury.2011.11.016), 본 학위 논문에는 더 추가된 내용이 있습니다.

2011 년 12 월 저자 씀

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ABSTRACT

Effect of Mild Hypothermia on Coagulatory Function and Survival in Sprague-Dawley Rats Exposed to Uncontrolled Hemorrhagic Shock

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Background: Acute coagulopathy, hypothermia, and acidosis are the lethal triad of conditions manifested by major trauma patients. Recent animal studies have reported that hypothermia improves survival in animals subjected to controlled hemorrhagic shock.

Purpose: The objective of this study was to investigate the effect of hypothermia on coagulation in rats subjected to uncontrolled hemorrhagic shock.

Subjects and Methods: Thirty-two male Sprague–Dawley rats were randomly divided into four groups: normothermia (control, group N), hypothermia (group H), hypothermic hemorrhagic shock (group HS), and normothermic hemorrhagic shock (group NS). Hemorrhagic shock was induced by splenic laceration. Capacity for coagulation was

measured by rotation thromboelastometry (ROTEM®), and was measured at baseline as well as the end of the shock and resuscitation periods. Survival was observed for 48 hours post-trauma.

Results: Baseline parameters were not different among the groups. Rats exposed to hypothermia alone did not differ in coagulation capacity compared to the control group. Clot formation time (CFT) and maximal clot firmness (MCF) in group HS decreased as the experiment progressed. Maximal clot firmness time (MCFt) in groups H and HS was significantly prolonged during shock and resuscitation compared with that in group NS. In group NS, MCF did not change significantly, but MCFt was reduced compared with baseline. Group HS had poor survival when compared with normovolemic groups.

Conclusion: Blood clotted less firmly in traumatic hemorrhagic shock, and hypothermia prolonged clotting. However, clot firmness maximized rapidly under normothermic hemorrhagic shock. Hemorrhage would continue for a longer time in hypothermic hemorrhagic shock. Survival of hypothermic shock was not significantly different compared to that of normothermic hemorrhagic shock.

Key Words: Hemorrhagic shock, Traumatic shock, Thromboelastography, Hypothermia, Coagulopathy

I. Introduction

Trauma is the third greatest cause of mortality in Korea following neoplasm and cardiovascular disease, and the most common cause in individuals under 40 years of age (1). Despite advances in trauma care, the leading cause of trauma death is exsanguination in up to 30% of cases (2). In particular, coagulopathy, hypothermia, and acidosis are well known as the lethal triad contributing to trauma mortality (3).

Traumatic hemorrhagic shock increases the rate of coagulation, which leads to the use of fibrinogen and reduced clot strength (4). Intravascular consumption of clotting components during continuous bleeding as well as blood dilution during massive transfusion and fluid resuscitation leads to post-traumatic coagulopathy (5). These hemostatic functional changes continue, causing alterations in platelet function and, finally, serious coagulopathy such as disseminated intravascular coagulation (4).

Hypothermia is another important factor determining the prognosis of trauma patients. Trauma patients lose heat by evaporation through wet clothing in the field or by radiation and convection following exposure at the trauma scene. Body temperature is lowered by massive fluid resuscitation, transfusion of cold blood products, and irrigation of open wounds. Hypothermia is a huge contributing factor to mortality in trauma patients, regardless of injury severity and age (6). Patients who undergo rapid rewarming require less resuscitation fluid and have significantly less early mortality compared to those experiencing slow rewarming (7). For that reason, the Advanced Trauma Life Support Guidelines of the American College of Surgeons recommend that hypothermia should be avoided in trauma patients. Furthermore, warming of fluids to 39°C before infusion and the use of a blood warmer are strongly recommended (8).

Hypothermia itself is not only a strong determinant of poor prognosis in trauma patients but also has direct effects on clotting. Hypothermia attenuates the initiation phase of thrombin generation and fibrinogen availability (9). Severely injured patients with hypothermia and acidosis develop clinically significant bleeding, even though blood products such as red blood cells, plasma, and platelets are replaced (10).

Recent experimental studies have reported that hypothermia improves survival in hemorrhagic shock (11, 12). However, in this work controlled hemorrhagic shock was induced by blood withdrawal, which is different to the clinical reality of uncontrolled bleeding, and hypothermia was induced only by external cooling (11, 12). In addition, the mechanisms eliciting these results were unclear. Artificial hypothermia therapy is effective for cardiac-arrest victims who remain comatose after restoration of spontaneous circulation (13). As previously stated, hypothermia clinically aggravates traumatic hemorrhagic shock in patients.

The objective of this study was to investigate the effects of mild hypothermia on coagulation capacity using rotational thromboelastometry and survival in a rat model of hemorrhagic shock with spleen injury.

II. Subjects and Methods

1. Subjects

This study was approved by the Animal Care and Use Committee in Jeju National University (No. 2010-0045).

Thirty-two male Sprague–Dawley rats (weight, 350 ± 50 g; age, 9–10 weeks old; Charles-River, Montreal, Quebec, Canada) were used. The rats had unlimited access to food and water before the experiment.

2. Methods

2.1. Animal preparation

Animals were anesthetized with an intramuscular injection of 15 mg/kg tiletamine/zolazepam (Zoletil®, Virbac, France). A temperature probe (MLT 1403, AD Instruments, Bella Vista, New South Wales, Australia) was placed rectally for continuous measurement of central body temperature. Rectal temperature was maintained at 37.0–38.0°C with a heating pad and heating lamp. Warm saline infusion or intraperitoneal irrigation with warm saline was used for normothermia groups. Hypothermia was targeted at 33.0–34.0°C using wet alcohol gauze and an electric fan, or a cold saline infusion and intraperitoneal irrigation.

After a sterile right-groin dissection, a 24-gauge catheter was inserted into the femoral artery for blood pressure monitoring and blood sampling. A polyethylene catheter (PE 50)

was inserted into the femoral vein to withdraw blood and for fluid infusion and transfusion. Heparin (80 IU/kg) was administered to prevent clotting in the catheters. The femoral artery catheter was connected to a blood pressure transducer (MLT 0380/D, AD Instruments), and systolic and diastolic blood pressure, mean arterial pressure (MAP), and heart rate were continuously recorded using PowerLab® (AD Instruments).

2.2. Blood sampling and measurements

Animals were randomly assigned to four groups of eight rats each: 1) normothermia (37.0–38.0°C), with only catheterization and blood sampling (group N); 2) hypothermia (33.0–34.0°C), with only catheterization and blood sampling (group H); 3) hypothermia with hemorrhagic shock (group HS); and 4) normothermia with hemorrhagic shock (group NS). The animals were randomized using a free online calculator offering simple random allocation into equal-sized groups.

Hemoglobin, HCO_3, lactic acid, and excess base were measured at baseline, after administration of shock, and post-resuscitation. Laboratory testing was conducted using a point-of-care laboratory instrument (i-STAT®, Abbott, Abbott Park, IL, USA) and test cartridges (CG4+, EG7+; Abbott).

2.3. Assessment of coagulation

To analyze coagulatory function using the hep-TEM protocol as a guide (Pentapharm GmbH, Munich, Germany), rotational thromboelastometry (ROTEM®, Pentapharm) tests were performed at baseline, after administration of shock, and after resuscitation in each rat. Blood samples (300 μL) were temporarily preserved in a citrate tube and added to hep-TEM reagent (20 μL) and in-TEM reagent (20 μL), then immediately returned to the

ROTEM® instrument, which incubated samples at the holding temperature adjusted for each group: 37.0°C for normothermia, and 33.0°C for hypothermia, groups. Although the default temperature of ROTEM® is 37.0°C, temperature is adjustable. Coagulation capacity altered with temperature (9). Temperature-adjusted measurements were performed in a previous study and indicated that blood sampled at 32°C was applied at 32°C-adjusted thrombelastogram (TEG®, Hemoscope Corp, Skokie, IL, USA) (14).

ROTEM® has eight assay modes, in which hep-TEM is indicated for specific detection of heparin; the modified in-TEM test adds heparinaze for heparin inactivation (15). Hep-TEM evaluates coagulatory function without potential effects from the presence of heparin in the blood.

ROTEM® measures the dynamics of clot (fibrin) formation by measuring the restriction in movement of a rotating rod placed in a cup of clotting blood. A beam of light, focused on the reflective surface of the rod, moves as the reflective surface rotates through 4°45' over 10 seconds. As a clot forms, torque on the rod increases, restricting the rod's movement and limiting light displacement. From these changes in light displacement the following measurements can be obtained: clotting time (CT), clot formation time (CFT), α-angle, maximal clot firmness (MCF), and maximal clot firmness time (MCFt). CT is the time from the start of measurement to initial fibrin formation, and CFT is the time necessary to reach 20 mm of clot strength. The MCF is a measure of maximal clot strength, which is dependent on platelet function and, to a lesser extent, on fibrinogen level. MCFt is the duration to reach MCF. The α-angle is the slope of the tangent at a 2-mm amplitude and measures the speed of fibrin buildup and cross-linking, which resembles the speed of clot strengthening. CT is mainly dependent on coagulation factor activity. The CFT and α-angle depend on plasma coagulation and the interaction of

platelets with fibrin, which increases clot stability. Tests were halted after MCF was calculated, and the raw data collected for analysis.

2.4. Surgical procedures

A sterile midline laparotomy was performed via a midline incision, and the spleen gently exposed. Grade III spleen injury was performed with a 5-mm deep incision along the length of the spleen's dorsal capsule, not for complete resection of the spleen, but to create uncontrolled bleeding. The wound was temporarily closed with sutures to maintain body temperature and avoid blood spillage. Unless MAP decreased to 40 mmHg within 10 minutes after spleen injury, blood was withdrawn through the femoral vein. Normal saline was administered when MAP was <30 mmHg. A splenectomy was performed during the early resuscitation period, and the abdominal wound was closed. The volume of blood lost from the spleen was measured. Rats were infused with normal saline for fluid resuscitation, and the blood removed from the femoral vein was additionally reinfused to restore MAP to >90 mmHg.

The animals were moved to a cage after completion of resuscitation and splenectomy. Rats in the hypothermia groups were rewarmed to 37°C. Gentamicin sulfate (Daesung Microbiological Labs, Seoul, South Korea) was administered to rats that had undergone laparotomy. Rats were allowed free access to food and water after experimental completion. Acetaminophen (Children's Tylenol suspension®, Janssen, Seoul, Republic of Korea) was mixed with water to control pain in all rats. Observations were performed every 8 hours for 48 hours to assess survival. A gross necropsy was performed in rats that died before 48 hours. Survivors to 48 hours were reanesthetized and killed by cervical dislocation. All experimental processes are summarized in Fig. 1.

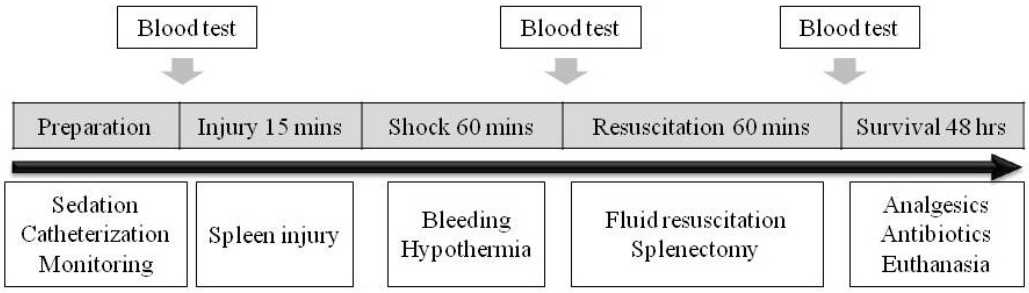


Fig. 1. Experimental protocol.

3. Statistical analysis

Data are presented as medians and ranges, unless otherwise stated. The Friedman test was used to analyze the repeated-measures values within the same group. Comparisons between groups were performed using the Kruskal–Wallis test with a Bonferroni correction for multiple comparisons; a probability value <0.0083 was considered significant. Survival analysis was determined using the Kaplan–Meier procedure. SPSS 15.0 (SPSS, Inc., Chicago, IL, USA) was used to analyze data. Differences were considered statistically significant at $p < 0.05$.

III. Results

1. Laboratory assessment

The mean arterial blood pressure and mean body temperatures of each group during experiment are shown in Fig. 2, 3. No significant differences in laboratory values were found at baseline among the four groups (Table 1). Hemorrhagic shock reduced bicarbonate, base excess, lactate, and hemoglobin, all of which differed significantly from values in non-shock groups at the end of the treatment period (Table 1). Lactate recovered by the end of resuscitation in groups HS and NS, and no difference was observed among the groups, as expected (Table 1). Base excess and hemoglobin remained lower than those of the shock groups (Table 1). When group H was compared with group N, no significant differences were found in bicarbonate, base excess, or lactate (Table 1).

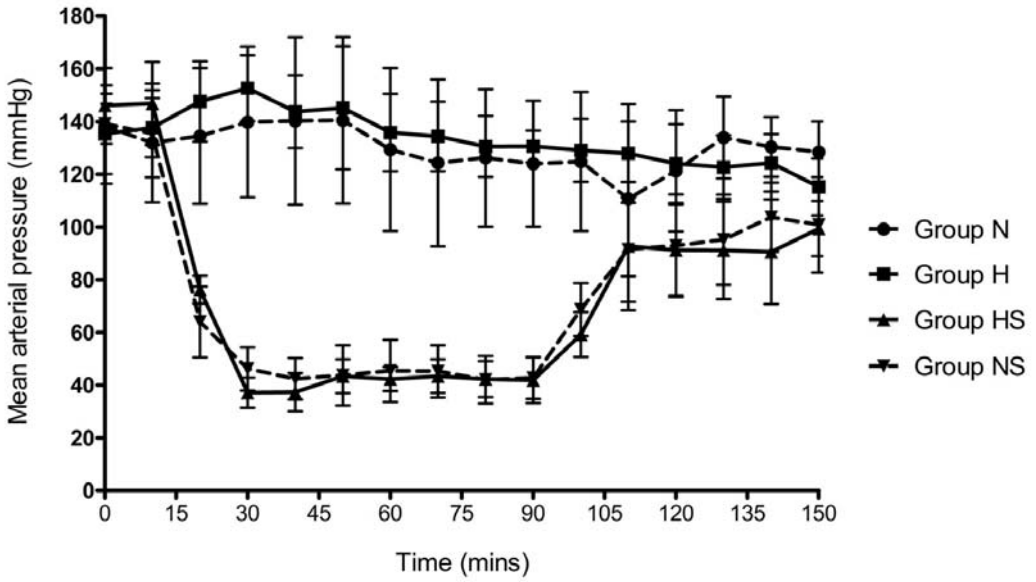


Fig.2. Mean arterial pressure in the four groups during shock and resuscitation.

Values are mean ± SE.

Group N, normothermia without shock; group H, hypothermia without shock; group HS, hypothermia with shock; group NS, normothermia with shock.

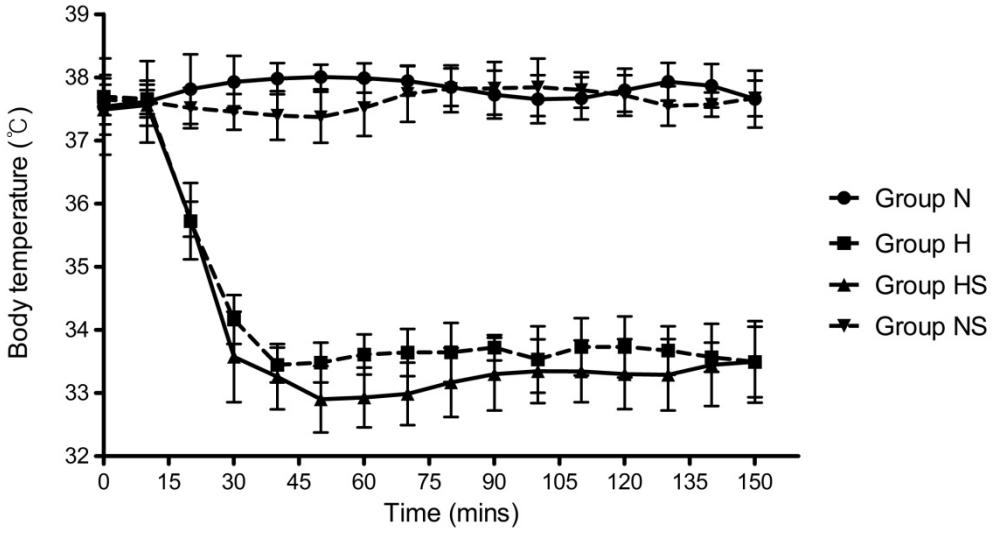


Fig. 3. Core body temperature in the four groups during shock and resuscitation.

Values are mean ± SE.

Group N, normothermia without shock; group H, hypothermia without shock; group HS, hypothermia with shock; group NS, normothermia with shock.

Table 1. Laboratory variables at baseline, at the end of shock period, and at the end of the resuscitation period

	Group N	Group H	Group HS	Group NS	P
Baseline					
HCO_3^- (mmol/L)	24.7 (21.6, 27.5)	25.0 (22.8, 29.4)	26.1 (21.6, 27.6)	25.1 (23.2, 28.4)	0.63
Base excess (mmol/L)	0 (-3, 3)	0 (-2, 5)	1.5 (-3, 3)	0.5 (-1, 4)	0.79
Lactate (mmol/L)	2.2 (1.8, 3.2)	2.2 (1.1, 3.9)	2.9 (1.1, 3.2)	2.7 (2.9, 3.2)	0.83
Hemoglobin (g/dL)	13.9 (11.2, 14.6)	13.6 (12.9, 15.3)	13.8 (9.5, 15.0)	12.6 (10.9, 13.6)	0.11
Shock period					
HCO_3^- (mmol/L)	23.2*† (19.3, 26.7)	24.8*† (21.2, 27.1)	15.4 (7.7, 19.1)	11.5 (6.6, 18.8)	<0.001
Base excess (mmol/L)	-2*† (-6, 2)	-0.5*† (-5, 2)	-11.5 (-20, -1)	-15.5 (-12, -6)	<0.001
Lactate (mmol/L)	2.1*† (1.4, 3.0)	1.5*† (0.9, 2.0)	6.0 (3.1, 11.3)	8.9 (4.0, 13.8)	<0.001
Hemoglobin (g/dL)	12.9*† (11.6, 14.3)	13.8*† (12.2, 15.3)	7.8 (5.4, 9.2)	7.1 (5.8, 10.5)	<0.001
Resuscitation period					
HCO_3^- (mmol/L)	24.1* (22.5, 26.6)	24.7*† (22.7, 25.6)	14.9 (12.7, 19.7)	14.5 (11.8, 24.9)	<0.001

Base excess (mmol/L)	-0.5*† (-2, 2)	-1*† (-3, 0)	-12 (-18, 7)	-11 (-15, 0)	<0.001
Lactate (mmol/L)	1.6 (1.05, 2.06)	1.4 (1, 2.95)	2.4 (1.04, 7.28)	3.8 (0.91, 5.49)	0.12
Hemoglobin (g/dL)	13.1*† (10.5, 14.3)	13.6*† (12.6, 16)	5.6 (3.3, 11.2)	5.8 (4.8, 9.2)	<0.001

Data are presented as median and range.

Group N; normothermia without shock, Group H; hypothermia without shock, Group HS; hypothermia with shock, Group NS; normothermia with shock

* $p<0.0083$ when compared with Group NS, †$p<0.0083$ when compared with Group HS

2. Thrombelastometry

The baseline CT, CFT, α-angle, MCF, and MCFt results did not differ among groups. CT was no different within the duration of the experiment among the four groups. CFT in group NS was significantly shortened at the end of the shock period relative to that in group H. After resuscitation, CFT in group HS diminished more than that in group H. CFT decreased gradually during shock and resuscitation in group HS. α-angles at the end of the shock period in group H, and after resuscitation in group N, were reduced compared with those of group NS (Table 2).

At the end of resuscitation, MCF in group H had increased with respect to that in groups HS and NS. MCF in group HS decreased significantly as the experiment progressed. MCFt in groups H and HS was significantly prolonged compared with that in group NS during shock and resuscitation. Hypothermia delayed the time to maximum clot firmness during shock and resuscitation. MCF did not change significantly in group NS, but MCFt was shortened during shock and resuscitation compared with that at baseline. Rats subjected to hypothermia alone did not have different coagulatory function compared with that in the control group (Table 2). Fig. 4. shows the representative ROTEM® of the rat in the hypothermic hemorrhagic shock goup.

Table 2. Coagulation parameters measured with the rotational thrombelastometry at baseline, end of shock period, and end of resuscitation period.

	Group N	Group H	Group HS	Group NS	P
Clotting time (sec)					
Baseline	202.0 (165, 253)	201.0 (146, 429)	179.5 (151, 231)	197.5 (151, 242)	0.86
Shock period	201.5 (88, 242)	188.5 (170, 215)	191.0 (163, 206)	149.5 (125, 202)	0.06
Resuscitation period	195.0 (161, 241)	195.0 (157, 226)	203.5 (173, 385)	179.0 (139, 327)	0.37
Clot formation time (sec)					
Baseline	53.0 (45, 109)	61.5 (37, 182)	53.5[a] (38, 110)	54.5 (31, 108)	0.87
Shock period	61.0 (21, 97)	60.5* (47, 78)	48.0[a] (40, 73)	36.0 (29, 50)	0.02
Resuscitation period	62.5* (47, 107)	50.5 (40, 64)	47.0[a] (34, 126)	41.5 (32, 70)	0.03
α-angle (°)					
Baseline	79 (70, 81)	77 (59, 83)	79 (69, 82)	78.5 (68, 84)	0.81
Shock period	77.5 (70, 86)	77.5* (74, 81)	80 (75, 82)	82.5 (80, 84)	0.01
Resuscitation period	77* (69, 80)	80 (77, 82)	80.5 (65, 83)	81.5 (76, 83)	0.02

Maximal clot firmness (mm)					
Baseline	71.0 (67, 76)	72.0 (67, 76)	72.5[b] (68, 75)	72.0 (66,76)	0.87
Shock period	70.5 (67, 75)	71.5 (65, 73)	68.5[b] (63, 72)	69.0 (66, 72)	0.16
Resuscitation period	70.5 (68, 75)	72.5†* (68, 74)	67.0[b] (65, 71)	67.0 (61, 72)	0.002
Maximal clot firmness time (sec)					
Baseline	1913.5 (1462, 3066)	1966 (1418, 2260)	1861 (1618, 2196)	1853[c] (1432, 2411)	0.88
Shock period	1971.5 (1440, 2349)	2159* (1750, 2574)	2120.5* (1872, 2635)	1486[c] (1339, 1888)	0.004
Resuscitation period	1995.5* (1676, 2241)	2117* (1659, 2620)	1936* (1669, 2243)	1525[c] (1116, 2020)	0.004

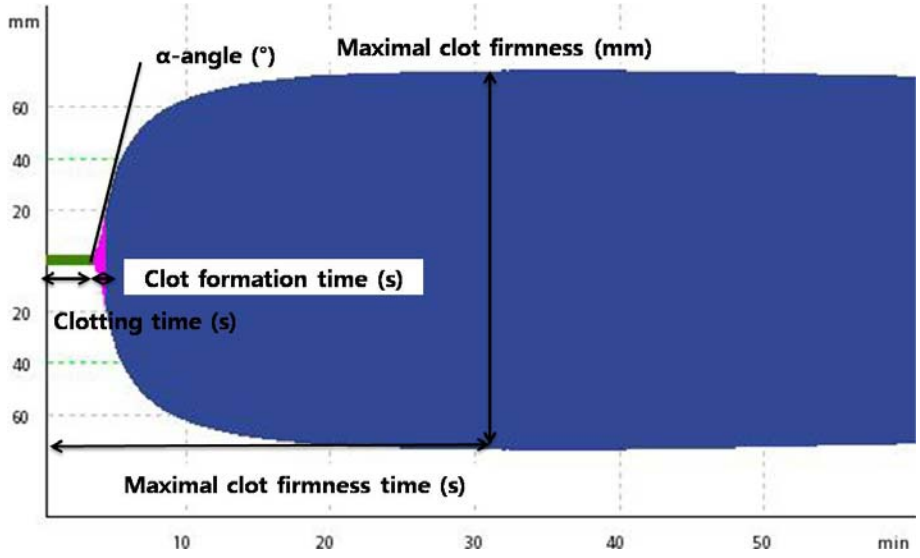
Data are presented as median and range.

Group N; normothermia without shock, Group H; hypothermia without shock, Group HS; hypothermia with shock, Group NS; normothermia with shock.

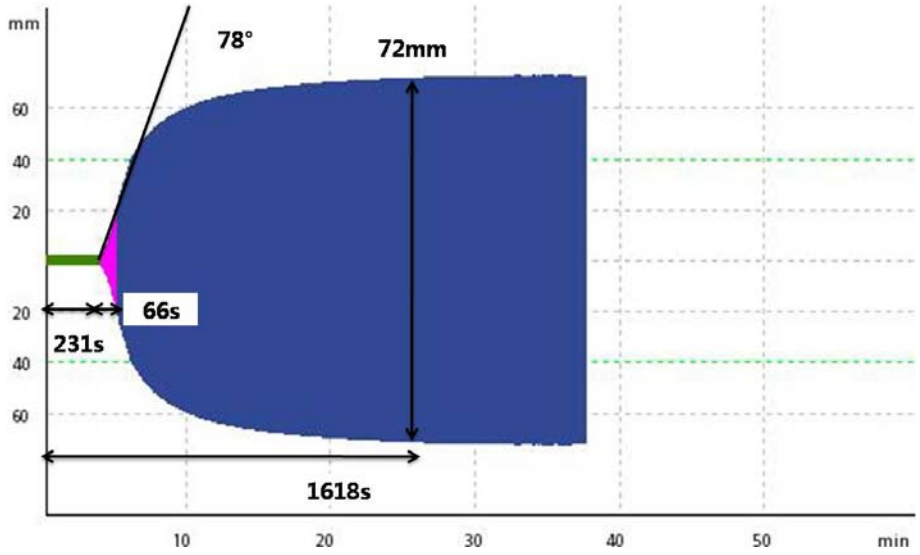
* $p<0.0083$ when compared with Group NS, †$p<0.0083$ when compared with Group HS

[a, b, c] $p<0.05$, Friedman test

A


mm
α-angle (°)
Maximal clot firmness (mm)
60
40
20
Clot formation time (s)
20
Clotting time (s)
40
60
Maximal clot firmness time (s)
10
20
30
40
50
min

B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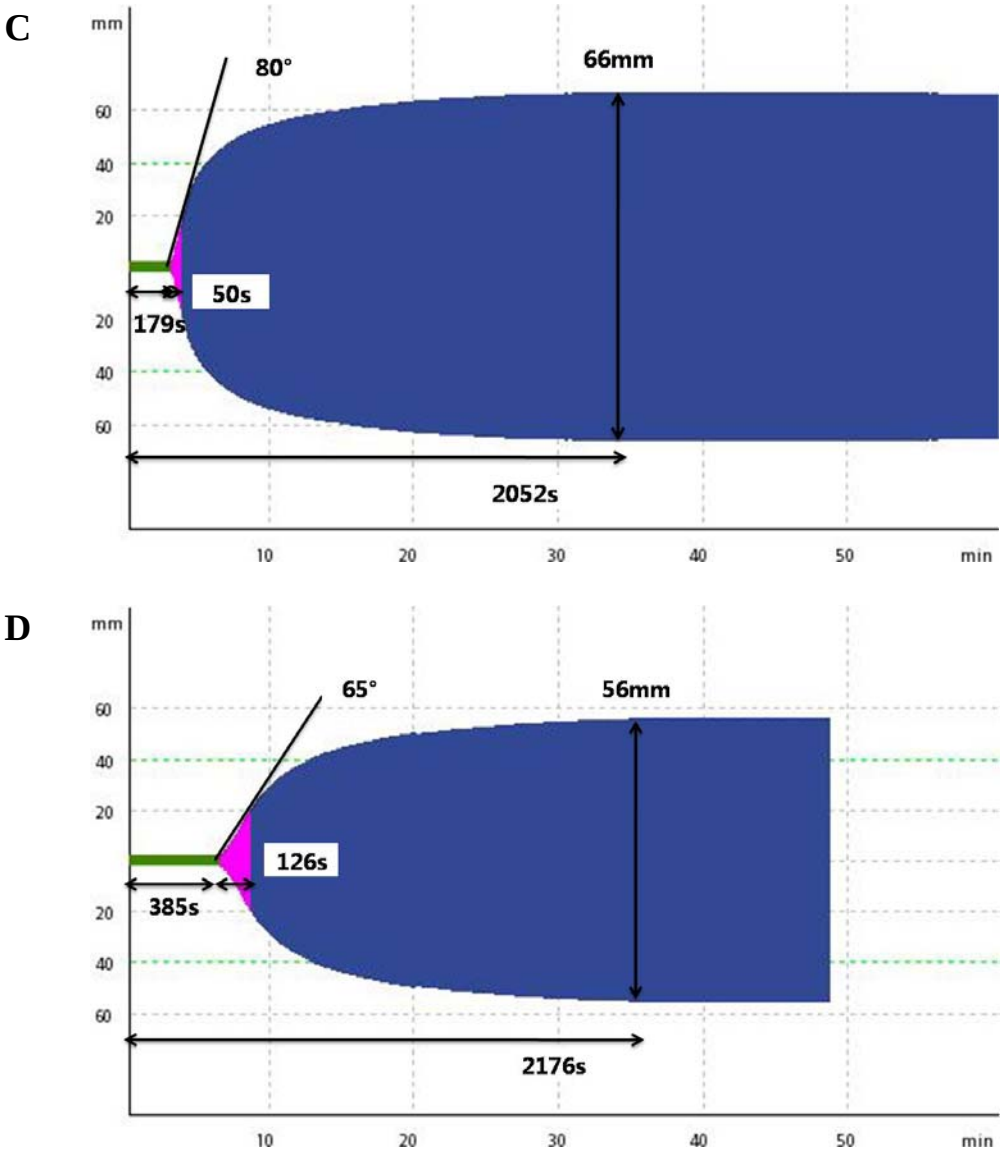

Fig. 4. Representative ROTEM® in the hypothermic hemorrhagic shock. **A.** Normal hep-TEM result of a rat in the control group. **B.** Baseline hep-TEM result. **C.** Result at the end of the shock period. MCF decrease and MCF time was prolonged. **D.** Result at the end of the resuscitation period. CT and CFT was prolonged, α-angle and MCF decreased.

(MCF; maximal clot firmness, CT; clotting time, CFT; clot formation time)

3. Fluid resuscitation and transfusion

Normal saline was infused during resuscitation period in all groups. Three blood samplings were conducted at one animal, and 1 cc of blood was withdrawn at each laboratory test. The same volume of normal saline was infused in non-shock groups. The suitable volumes of normal saline and blood were given for shock groups to restore MAP to >90 mmHg. There were no differences in the volume of normal saline and transfused blood between group NS and HS (Table 3).

Table 3. Infused normal saline and transfused blood during resuscitation period

	Group N	Group H	Group HS	Group NS	P
Infused normal saline (cc)	3.0 (3.0, 3.0)* †	3.0 (3.0, 3.0)* †	23.0 (9.0, 36.0)	18.8 (12.0, 33.0)	<0.001
Transfused blood (cc)	0 (0.0, 0.0)* †	0 (0.0, 0.0)* †	4.0 (3.0, 5.0)	5.0 (2.0, 6.0)	<0.001

Data are presented as median and range.

Group N; normothermia without shock, Group H; hypothermia without shock, Group HS; hypothermia with shock, Group NS; normothermia with shock

* $p<0.0083$ when compared with Group NS, †$p<0.0083$ when compared with Group HS

4. Survival

All sixteen rats in groups N and H survived for 48 hours, whereas four of eight rats in group HS died within the observation period (p = 0.026). Two of eight rats in group NS additionally died during observation; this effect was non-significant in comparison with groups N and H (p = 0.143). No survival differences were observed between groups HS and NS (p = 0.325) (Fig. 3). Ischemic bowel changes and swelling were found after gross necropsy of the four rats in group HS and two in group NS that died during observation. No intra-abdominal bleeding was detected after splenectomy.

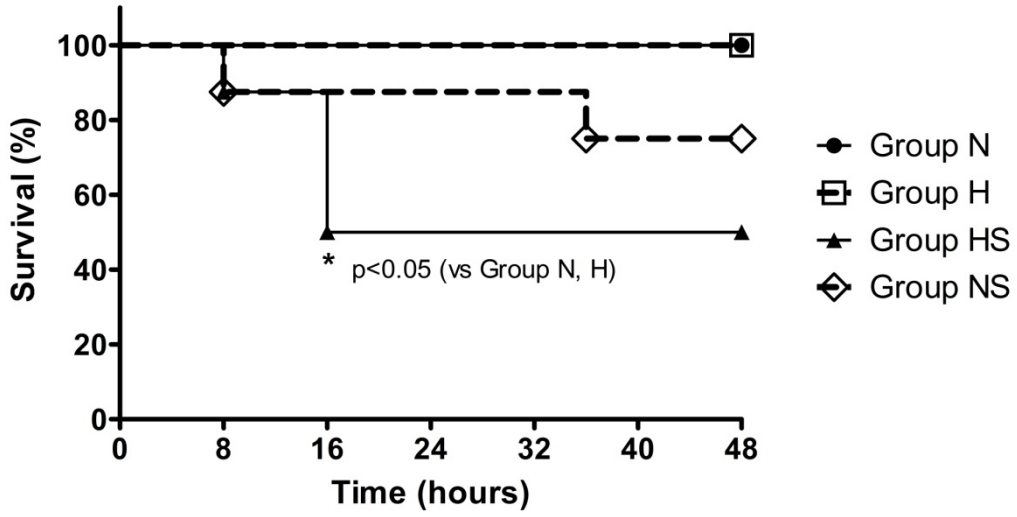

Fig. 5. Kaplan–Meier survival curve of the four groups.

Group N, normothermia without shock; group H, hypothermia without shock; group HS, hypothermia with shock; group NS, normothermia with shock.

IV. Discussion

The author investigated the effect of mild hypothermia on coagulation and survival in rats subjected to uncontrolled hemorrhagic shock. Rat subjected to mild hypothermia alone had no alteration in coagulatory function compared to the control group. Coagulopathy was observed only in rats subjected to hemorrhagic shock, and mild hypothermia significantly extended the time to maximal clot firmness. Survival was no different between groups HS and NS, and only group HS demonstrated poor survival compared with non-shock groups.

Hypothermia with controlled bleeding has ameliorated survival without significant coagulopathy in several animal studies (11, 12, 16). Controlled hemorrhagic shock develops when blood is withdrawn at a constant rate via catheter in the absence of traumatic injury and targeted low blood pressure is maintained for some time. Nevertheless, the mechanisms for improved survival during hemorrhagic shock with mild hypothermia remain unclear. Lowered heart rates and higher MAP in hypothermic groups may account for better survival, indicating that better perfusion protects vital organs from ischemia (12). Additionally, hypothermia groups have lower levels of cellular stress including base deficiency and lactate (11). However, these experimental protocols are dissimilar to clinical traumatic shock due to control of hemorrhage and administration of protective hypothermia. Acute traumatic coagulopathy is a response developing from the combined processes of tissue damage and hemorrhagic shock (17). Recent hemorrhagic shock modelling has been conducted using spontaneous uncontrolled hemorrhage, including liver injury, spleen injury, and femur fracture, which closely recapitulate

clinical situations (4, 18, 19). Hypothermia of 34°C or 30°C prolongs survival time during lethal hemorrhagic shock induced by tail amputation in rats (20-22). However, these studies evoked hemorrhagic shock experimentally by blood withdrawal through a catheter in the inferior vena cava. In other words, shock was induced by volume-controlled hemorrhage, whereupon tails were amputated. In the present study, hemorrhagic shock was induced by spleen trauma; the hypothermic shock group exhibited poor survival compared with non-shock groups. No difference was observed upon survival analysis between hypothermic and normothermic shock groups.

In animal experiments, hypothermia is artificially induced by external cooling or cold fluid infusion. Cellular energy sources are relatively preserved, metabolism is reduced, and shivering is abolished chemically during induced hypothermia, leading the American Heart Association to recommend hypothermic therapy (32–34°C for 24 hours) for all cardiac arrest victims remaining comatose. Cold intravenous fluid infusion and surface or endovascular cooling are proposed as cooling methods (13). In contrast, unintentional hypothermia, such as that seen in trauma patients, is a failure of homeostatic mechanisms and a sign of metabolic exhaustion (23, 24). The author demonstrated hemorrhagic shock through the major trauma of splenic laceration; venous blood was withdrawn via femoral catheter and preserved for transfusion during resuscitation. In addition, hypothermia was induced not only by external cooling and cold saline infusion but also by laparotomy and cold-water intraperitoneal irrigation. Therefore, hypothermia and hemorrhagic shock were more consistent with a clinical situation.

In the present study, mild hypothermia alone did not result in acidosis or serious coagulopathy. Hypothermia and hemorrhagic shock are risk factors for acute traumatic

coagulopathy (25). Lau et al. reported that rewarming patients alone dramatically improves coagulatory function (26). Hypothermia combined with exsanguination definitely causes fibrinogen breakdown or coagulopathy (27). In this study, there were no changes of the time to start initial fibrin formation. Shortened CFT and increase α-angles in Group NS means that speed of fibrin building and cross-linking are accelerated, but no significant difference was found in Group HS compared with non-shock groups. Platelet function significantly decreased within Group HS as the experiment progressed. MCFt, the time to form maximal platelet and fibrinogen was significantly prolonged in Group H and HS during shock and resuscitation period. It reflects that more bleeding might continue until clotting reach to MCFt.

These results are consistent with experimentation in a pig model (28). Hypothermia prolonged clotting time and clot-formation time, and clotting rapidity decreased. Clot strength also decreased in the hemorrhage with resuscitation group, and all thromboelastography parameters were abnormal in the hypothermic hemorrhage with resuscitation group (28). Traumatic hypothermia is different to protective hypothermia. Protective hypothermia during hemorrhagic shock improves survival without altered coagulatory function (12); in trauma patients, consumptive and dilutional coagulopathy changed haemostatic function, and hypothermia also attenuated coagulation capacity. In the present experiments, clotting components consumed by traumatic spleen laceration and exposure of the intraperitoneal cavity induced hypothermia. Trauma-related unintentional hypothermia was caused by insufficient heat production.

Massive transfusion and fluid resuscitation dilute blood of trauma patients (5). Blood dilution could weaken MCF of shock groups during resuscitation period. There were no

differences in the volumes of transfused blood and resuscitation fluid, and coagulation parameters measured by ROTEM® had no differences between shock period and resuscitation period in shock groups. Dilution had little effects on coagulation functions in this study.

The values of ROTEM® in this study were not pathologic when compared with human references, since rat blood coagulates with greater facility than human blood. Thrombelastometry in a non-activated TEM test normally demonstrated shorter CT, CFT and longer MCF than human parameters (29).

This study had a few limitations. Although the author elicited mild hypothermia through intraperitoneal organ exposure and cold saline infusion and irrigation in group HS, only protective hypothermia was induced in group H. Secondly, the author induced uncontrolled hemorrhagic shock using a spleen injury to make the model clinically realistic, but, of course, it did not perfectly simulate multiple injuries. Additionally, the exact causes of death in the experimental rats were unknown, because gross necropsy was done without pathologic analysis. The authors conducted the experiment with only mild hypothermia. Severe hypothermia (30℃) would show definite differences on coagulation function in hemorrhagic shock.

V. Conclusion

When hypothermia combined with traumatic hemorrhagic shock, coagulatory function deteriorated; platelet function decreased and it took longer time to form maximal clot during the shock and resuscitation period. Although clot firmness is weak in hemorrhagic shock with normothermia, it needs a shorter time to reach MCF. Hemorrhage might continue for a longer time in hypothermic hemorrhagic shock than in normothermic hemorrrhagic shock. The animals were randomized using a free online calculator offering simple random allocation into equal-sized groups.

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Abstract in Korean

출혈성 쇼크를 유발한 쥐에서 저체온이 혈액 응고 기능과 생존에 미치는 영향

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박경혜

배경과 목적: 급성 응고장애, 저체온, 산증은 중증 외상 환자에서 치명적인 세 가지 요소이다. 최근 저체온이 조절된 출혈성 쇼크에서 생존율을 향상시킨다는 동물 실험 보고가 있었다. 이 연구의 목적은 실제 중증 외상 환자와 유사한 조절되지 않는 출혈성 쇼크에서 경도의 저체온이 응고 기능과 생존에 미치는 영향을 알아보고자 한다.

대상 및 방법: 서른두 마리의 Sprague-Dawley 쥐를 무작위로 네 군으로 배정하여 실험하였다. 대조군인 정상체온 군 (N), 저체온 군 (H), 출혈성 쇼크를 유발한 저체온 군 (HS), 출혈성 쇼크를 유발한 정상체온 군 (NS)으로 나뉘었다. 비장 열상을 일으켜 출혈성 쇼크를 유발하였고, 응고기능은 회전성 혈전탄성묘사기 (Rotation thrombelastometry, ROTEM®)를 이용하였다.

응고기능과 혈액가스분석을 실험 시작 시, 쇼크기간 종료 시, 소생술 종료 시에 측정하였다. 48 시간 동안 생존 여부를 관찰하였다.

결과: 모든 실험군에서 기본적인 피검사와 응고검사는 차이가 없었다. H 군은 대조군과 비교하여 응고기능에 차이가 없었다. HS 군의 혈전형성시간 (Clot formation time, CFT)과 혈전강도최대치 (Maximal clot firmness, MCF) 는 실험과정에서 유의하게 감소하였다. H 군과 HS 군의 혈전강도최대 도달시간 (Maximal clot firmness time, MCFt)은 NS 군과 비교하여 쇼크 후, 소생 후에 유의하게 연장되었다. NS 군에서 MCF 는 유의하게 변하지 않았지만, MCFt 는 처음과 비교하여 단축되었다. HS 군의 생존율은 쇼크를 일으키지 않은 군에 비해 저조하였지만, NS 군과는 차이가 없었다.

결론: 외상성 출혈성 쇼크에서 혈액은 덜 단단하게 응고되며, 저체온은 응고작용을 지연시켰다. 그러나 정상체온을 유지한 출혈성 쇼크에서는 응고작용이 가속화되었다. 출혈성 쇼크에서 저체온이 동반되면 응고는 견고하지 않고 느리게 진행되므로 출혈이 더 많이 일어날 수 있다. 저체온이 동반된 출혈에서는 정상체온 출혈과 비교해 생존에는 의미있는 차이가 없었다.

핵심되는 말: Hemorrhagic shock, Traumatic shock, Thromboelastography, Hypothermia, Coagulopathy