Comparison of warm fluid and cold fluid resuscitation during uncontrolled hemorrhagic shock model in rats

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ABSTRACT

BACKGROUND: This study was designed to compare the effects of resuscitation with cold and warm fluid on survival time, rate and volume of hemorrhage, hemodynamics, hypothermia, coagulopathy, acid-base balance, hematocrit, lactate, and base deficit during uncontrolled hemorrhagic shock (HS) model in rats.

METHODS: HS model was created with splenic vascular and parenchymal injury in 29 rats under ketamine and xylazine anesthesia. Thirty minutes after the hemorrhage, the rats were randomized to receive 14.5 mL/kg 0.9% sodium chloride solution at either 24ºC (Group 1; n=9) or 4ºC (Group 2; n=10) for 20 minutes. Groups 1 and 2 were compared with group that did not receive fluid (Group 3; n=10). Statistical data were represented as mean±SD. SPSS for Windows, Version 15.0 (SPSS, Inc., Chicago, IL, USA) software, Bonferroni-adjusted Mann-Whitney U test and Kaplan-Meier procedure were used to perform statistical data analysis. P value of ≤0.05 was considered statistically significant.

RESULTS: Cold fluid resuscitation decreased survival time due to increased rate and volume of hemorrhage, acidosis, hypothermia, lactate, and base deficit.

CONCLUSION: There is a great need for further experimental and clinical trials on fluid resuscitation in trauma in order to define which fluid should be administered, temperature of the fluid, quantity to be delivered, and duration.

Keywords: Fluid resuscitation; hemorrhagic shock; intravenous cold fluid; rat.

INTRODUCTION

The most common cause of death under the age of 40 years is trauma.[1] The most frequent cause of early death due to trauma is bleeding and shock.[2] In developed and developing countries, 50% to 80% of deaths due to trauma occur in the prehospital field.[3] Recent studies investigating means to reduce mortality related to trauma have targeted the prehospital field, and have focused on fluid resuscitation as a way to reduce prehospital mortality.[4,5] The temperature of the fluid used in such resuscitation is a new area of discussion. There are numerous studies about hypothermic fluid resuscitation in non-trauma patients. This type of fluid resuscitation is included in the guidelines for management of non-trauma related cardiac arrest, but there are no clinical studies about the use of this treatment in trauma patients. Available limited experimental studies are inconclusive. In this study, it was hypothesized that cold fluid resuscitation, which has had beneficial effects in cardiac arrest patients, would also prolong survival in trauma.

MATERIALS AND METHODS

The study protocol was approved by the Gülhane Military Medical Academy animal experimentation ethics commit-
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tee, on April 1, 2011 (file number 11/10). The study sub-
jects were 30 male Sprague Dawley rats weighing between
217 and 376 g. The animals were fed commercial rat chow
and provided with tap water until surgery. They were kept
in cages at room temperature for more than 1 week. All of
the animals were sedated with intraperitoneal injection of xy-
lazine (4 mg/kg) and ketamine (40 mg/kg); additional doses
were administered if necessary. One animal died and was
excluded from the study. After sedation, the animals were
placed in supine position for spontaneous breathing. After
the tail was heated with an external heater to between 45ºC
and 50ºC for 1 minute, the tail artery was cannulated with
26-gauge intracath (Mediflon; Eastern Medikit Ltd., Gurgaon,
India) for hemodynamic monitoring and blood sampling, and
the lateral tail vein was cannulated with 26-gauge intracath
(Mediflon; Eastern Medikit Ltd., Gurgaon, India) for fluid re-
suscitation (Figure 1). Cardiocap/5 monitor (Model F-FML;
Datex-Ohmeda, Inc., GE Healthcare Finland Oy, Helsinki,
Finland) with temperature probe, and pressure monitor set
(Zyron, Point Medikal San. Ve Tic. Ltd. Şti., Ankara, Turkey)
were used for hemodynamic and rectal temperature (RT)
monitoring (Figures 1, 2). Preparations were completed with-
in 45 minutes, and the rats were allowed 10 minutes to be-
come stable. Mean arterial blood pressure (MAP), heart rate
(HR), RT, arterial blood gas (ABG) parameters [pH, hema-
tocrit (HCT), lactate, base deficit], and D-dimer levels were
recorded before hemorrhage was initiated. Stat Profile Criti-
cal Care Xpress blood gas analyzer (Nova Biomedical, Inc.,
Waltham, MA, USA) and Amax CS-190 coagulation analyzer
(Trinity Biotec, Lemgo, Germany) were used for these mea-
surements. Blood samples (0.5 mL) were collected from tail
artery at 0 minute (before starting hemorrhage), 60 minutes,
and every 30 minutes subsequently using heparinized syringe
(BD Preset Eclipse, Belliver Industrial Estate, Plymouth, UK).
After blood samples were collected, intravenous (IV) saline
solution (0.5 mL) was infused in 3 minutes to replace these
samples. Five minutes after recording the data, the abdomi-
nal wall of the rat was sterilized, and the abdomen was entered
via a left subcostal incision. Splenic parenchymal incision
was made in transverse direction at 1 level, and 1 of the 3 main
branches of the splenic artery was cut (Figure 3). After allow-
ing free flow of blood into the peritoneal cavity, abdominal
incision was sutured. Thirty minutes after hemorrhage, rats
were randomized to receive either 24ºC (Group 1; n=9) or
4ºC (Group 2; n=10) 0.9% sodium chloride solution (14.5
mL/kg) via infusion pump (Injectomat Agilia; Fresenius Kabi,
Brezins, France) for 20 minutes (Figure 2). Quantity of fluid
was chosen to simulate 1 liter of fluid for 70 kg patient since
healthcare personnel can carry and administer 1 liter of fluid
easily to a bleeding patient in the field. Twenty minutes was
selected to simulate the time for transportation of patient
from the field to the hospital. Groups 1 and 2 were compared
with the control group, which did not receive fluid treatment.
The rats were observed at room temperature without any other treatment. Apnea and lack of pulse were defined as signs of death. Once death occurred, the peritoneal cavity was opened immediately, and the volume of hemorrhage was measured on a scale (Precisa Gravimetrics A.G., Dietikon, Switzerland) by calculating the difference in weight between dry gauze and gauze soaked with blood clots. MAP, HR, and RT were evaluated at 0, 30, 40, 60 minutes, and subsequently every 30 minutes until the animal died. Afterward, volume and rate of hemorrhage, and survival time were recorded. Volume of hemorrhage was calculated as percentage of total blood volume (60 mL/kg). Rate of hemorrhage was calculated as mL/kg/h.

SPSS for Windows, Version 15.0 (SPSS, Inc., Chicago, IL, USA) was used to perform statistical analysis. Statistical data are presented as mean±SD. Differences between groups were analyzed using Bonferroni-adjusted Mann-Whitney U test. Differences between groups in terms of survival time were assessed using Kaplan-Meier survival analysis. In determining the number of subjects in each group, effect size of 1.52 was estimated based on a previous study (2). Sample size of 8 subjects in each group would allow us to detect statistically significant difference with 5% Type I error risk and 80% power. P value of ≤0.05 was considered statistically significant.

RESULTS

There were no statistically significant differences between groups in terms of weight of rats, MAP, HR, RT, ABG parameters, or D-dimer level at the beginning of the experiment (Table 1). There were statistically significant differences between groups in survival time and volume and rate of hemorrhage (Table 2) (Figure 4).

### Hemodynamic Changes

Thirty minutes after hemorrhage, MAP, HR, and RT decreased without significant difference in all groups. Final analysis revealed that Group 3 had significantly lower MAP and HR than Groups 1 and 2 (p<0.05). Group 1 had significantly higher MAP than Group 2 (p<0.05). There were no significant differences between Groups 1 and 2 in terms of HR. Group 3 had sig-

### Table 1. Weight of rats, MAP, HR, RT, ABG parameters, and D-dimer level in the groups at the beginning of the experiment

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>Mean±SD</td>
<td>n</td>
<td>Mean±SD</td>
<td>n</td>
<td>Mean±SD</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>9</td>
<td>279.56±33.52</td>
<td>10</td>
<td>280.26±36.45</td>
<td>10</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>9</td>
<td>97.00±5.17</td>
<td>10</td>
<td>98.00±12.11</td>
<td>10</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>9</td>
<td>343.67±48.89</td>
<td>10</td>
<td>365.08±35.15</td>
<td>10</td>
</tr>
<tr>
<td>RT (ºC)</td>
<td>9</td>
<td>37.18±0.20</td>
<td>10</td>
<td>37.01±0.07</td>
<td>10</td>
</tr>
<tr>
<td>Base deficit (mEq/L)</td>
<td>9</td>
<td>-1.44±0.65</td>
<td>10</td>
<td>-1.00±1.04</td>
<td>10</td>
</tr>
<tr>
<td>D-dimer (ng/mL)</td>
<td>9</td>
<td>141.78±10.93</td>
<td>10</td>
<td>141.80±28.05</td>
<td>10</td>
</tr>
<tr>
<td>pH</td>
<td>9</td>
<td>7.41±0.03</td>
<td>10</td>
<td>7.40±0.02</td>
<td>10</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>9</td>
<td>50.56±2.07</td>
<td>10</td>
<td>50.70±2.91</td>
<td>10</td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td>9</td>
<td>1.77±0.52</td>
<td>10</td>
<td>1.85±0.38</td>
<td>10</td>
</tr>
</tbody>
</table>


### Table 2. Survival time, volume of hemorrhage, and rate of hemorrhage in the groups

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>Mean±SD</td>
<td>n</td>
<td>Mean±SD</td>
<td>n</td>
<td>Mean±SD</td>
</tr>
<tr>
<td>Survival time (min)</td>
<td>9</td>
<td>268.00±50.71</td>
<td>10</td>
<td>215.92±54.37</td>
<td>10</td>
</tr>
<tr>
<td>Volume of Hemorrhage (%)</td>
<td>9</td>
<td>24.37±8.43</td>
<td>10</td>
<td>36.51±8.47</td>
<td>10</td>
</tr>
<tr>
<td>Rate of hemorrhage (mL/kg/h)</td>
<td>9</td>
<td>3.50±1.45</td>
<td>10</td>
<td>6.58±3.23</td>
<td>10</td>
</tr>
</tbody>
</table>

significantly higher RT than Group 2 (p<0.05). Group 3 also had lower RT than Group 1 without significant difference. Group 1 had significantly higher RT than Group 2 (p<0.05) (Table 3).

Table 3. Comparison of the groups in terms of MAP, HR, RT, HCT, and D-dimer at timed intervals after hemorrhagic shock

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Comparison of fluid therapies between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Warm* &gt; Cold+</td>
</tr>
<tr>
<td>MAP</td>
<td>180</td>
</tr>
<tr>
<td></td>
<td>Time (minute) after HS with p&lt;0.05</td>
</tr>
<tr>
<td>HR</td>
<td>No difference</td>
</tr>
<tr>
<td>RT</td>
<td>40, 50, 60, 90, 120, 150, 180, 210</td>
</tr>
<tr>
<td>HCT</td>
<td>90, 120</td>
</tr>
<tr>
<td>D-dimer</td>
<td>No difference</td>
</tr>
</tbody>
</table>

*Warm (24 °C) Fluid Therapy in Group 1. +Cold (4 °C) Fluid Therapy in Group 2. °Control: Group 3. MAP: Mean arterial blood pressure; HR: Heart rate; RT: Rectal temperature; HCT: Hematocrit.

ABG Parameters
During the study, base deficit/lactate levels increased, and pH/HCT levels decreased in all groups. Group 3 had significantly higher base deficit/lactate level and lower pH/HCT level than Groups 1 and 2 (Table 3 and Figures 5–7). Group 2 had significantly higher base deficit/lactate level and lower pH/HCT levels compared to Group 1. Group 1 had significantly higher pH levels than Group 2 (p<0.001) and Group 3 (p<0.05) (Figure 5). Group 3 had significantly lower pH levels compared to Group 1 (p<0.042) and Group 2 (p<0.001) (Figure 5).

Figure 4. Cumulative survival (Kaplan-Meier) during hemorrhagic shock.

Figure 5. PH level during hemorrhagic shock. Group 1: Warm fluid therapy. Group 2: Cold fluid therapy. Group 3: Control.

Figure 6. Base deficit level during hemorrhagic shock. Group 1: Warm fluid therapy. Group 2: Cold fluid therapy. Group 3: Control.

Figure 7. Lactate level during hemorrhagic shock. Group 1: Warm fluid therapy. Group 2: Cold fluid therapy. Group 3: Control.
level than Group 1 (Table 3 and Figures 5–7).

D-dimer: No statistically significant difference was determined between groups in D-dimer level (Table 3).

DISCUSSION
The primary finding of the present study was that warm fluid resuscitation had more beneficial effects on survival than cold fluid resuscitation. Many clinical and experimental studies focusing on prehospital cardiac arrest have demonstrated that cold fluid resuscitation positively contributes to survival time, hemodynamics, acid-base balance, and neurological and renal functions.\(^{[6–9]}\) Conversely, we observed deleterious effects of this treatment in trauma-related hemorrhagic shock (HS). In the literature, there are only 2 studies that compare fluid resuscitation at different temperatures in HS model, and both of these were pig models.\(^{[2,10]}\) Our study is the first to be conducted using rats. Norio et al.\(^{[2]}\) reported that cold fluid infusion increased survival time. They thought this was a result of therapeutic hypothermia, which decreased HR and stroke volume index, and thus reduced oxygen demand of the heart. In contrast, we found that cold fluid infusion decreased survival time. Cold fluid infusion may have increased volume and rate of hemorrhage, hypothermia, and acidosis, which might be related to poor tissue perfusion by causing vasoconstriction. As we did, Wu et al.\(^{[10]}\) found that cold fluid infusion decreased survival time compared with warm fluid infusion, but they also detected that 2°C and 24°C fluid infusions increased survival time compared with 38°C fluid infusion due to therapeutic hypothermia (34°C).\(^{[10]}\) In our study, increasing volume and rate of hemorrhage in cold fluid infusion group might be due to hypothermic coagulopathy. This coagulopathy might be explained by hypothermia below 34°C, which results in platelet dysfunction, inhibition of protein kinase D, and increased prostacyclin synthesis.\(^{[2,11]}\) Norio et al.\(^{[2]}\) did not find significant differences between groups in terms of volume of hemorrhage. They attributed this finding to stable body temperature of 35.5°C in cold fluid infusion group, and thus, did not encounter any hypothermic coagulopathy. While Norio et al. and Wu et al.\(^{[2,10]}\) didn’t indicate significant differences between groups in terms of MAP, we found that cold fluid infusion decreased MAP. Cold fluid infusion may have increased hypothermia and acidosis, and thus decreased MAP due to cardiac arrhythmia and low cardiac output. While these studies determined lower HR in cold fluid infusion groups, we didn’t find a significant difference between treatment groups in terms of HR. They considered low HR to be result of protective effect of hypothermia on heart.\(^{[2,10]}\) Unlike those studies, we observed that HR after treatment didn’t remain at stable level, and always fell. This result can be explained by HR decreases in rats based on parasympathetic activation, instead of sympathetic activation in the heart, when blood pressure falls.\(^{[11,13]}\) Norio et al.\(^{[2]}\) found cold fluid infusion increased HCT, and attributed this finding to hypothermia-induced hemococoncentration generated by plasma leakage into extravascular spaces. We observed that cold fluid infusion decreased HCT. We think this finding may have been due to greater volume and rate of hemorrhage in cold fluid infusion group. While Norio and Wu et al.\(^{[2,10]}\) didn’t report significant differences between groups in terms of pH and base deficit, we found that cold fluid infusion decreased pH and increased lactate and base deficit levels as result of acidosis. As in our study, Wu et al.\(^{[10]}\) found that cold fluid infusion increased lactate levels with acidosis. However, there was no correlation between lactate and base deficit levels in their study. Normally, base deficit increases when lactate increases. After treatment of HS, lactate and base deficit are expected to decrease.\(^{[14]}\) Conversely, the previous authors indicated base deficit and lactate levels increased after treatment. There were also other differences in materials and methods used in our study in comparison with these 2 studies. They included volume-controlled hemorrhagic stage, which did not totally represent an uncontrolled HS model, and thus did not simulate trauma very well.\(^{[15,16]}\) These studies simulated hospital phase more than prehospital phase, as they incorporated mechanical ventilation and intensive care. We excluded this stage to simulate prehospital phase for trauma patients. There was also no control group in those studies. Furthermore, coagulopathy, one of the parameters of the “lethal triad” in HS, was not evaluated.\(^{[2,10]}\) In a previous study conducted by Zhang et al.,\(^{[17]}\) it was reported that D-dimer might be prognostic factor in a rat model of HS. We used D-dimer, a fibrin degradation product, to assess coagulopathy; however, there was no significant difference in D-dimer level between groups. We think D-dimer may not be a suitable parameter for evaluation coagulopathy since there are some differences between humans and rats in terms of coagulation system.\(^{[18]}\) We believe that further studies are required to demonstrate relationship between D-dimer and coagulopathy during HS.

In addition to our main findings, interestingly, we found that compared with the control group, both treatment groups had smaller volume and lower rate of hemorrhage, and thus had higher MAP, HR, HCT, and pH, as well as lower lactate and base deficit. This result could be explained by the fact that infusions were colder than body temperature and therefore caused vasoconstriction, and/or volume of fluid used in resuscitation was less than has been used in other studies.\(^{[19–23]}\) Other studies found aggressive fluid therapy at dose of 35-70 mL/kg increased volume and rate of hemorrhage because it caused dilutional coagulopathy, hypothermia, disruption of thrombus formation, higher blood pressure, and inhibition of vasoconstriction.\(^{[19–23]}\) Low dose (14.5 mL/kg) of fluid may decrease volume and rate of hemorrhage. As a result, we think that crystalloid fluid resuscitation at dose of 14.5 mL/kg for 20 minutes, even at different temperatures, can have beneficial effects on survival.

Limitations
The limitations of the present study are lack of large animal...
model and of hot fluid resuscitation group. We may not have simulated real clinical situations using small animal model. For example, rats are more prone to hypothermia than pigs. We observed deep hypothermia (25°C) instead of therapeutic hypothermia (34°C) during the experiment. Anesthetic agents, fluids that were colder than body temperature, exposure to room temperature, HS, and laparotomy might have also triggered deep hypothermia in rats. In theory, HS patients should receive fluid resuscitation at body temperature to prevent hypothermia. If we could have added 37°C hot fluid infusion group to our study, we would have been able to better compare efficiency of fluid therapies at different temperature.

Conclusion
Cold fluid resuscitation decreased survival time because it increased rate and volume hemorrhage, acidosis, and hypothermia. During pre-hospital phase (the time before patient arrival to hospital), also known as the “golden hour,” fluid resuscitation should be administered immediately in order to decrease mortality. Additional experimental studies, especially including large animal models and clinical studies, are needed to further examine fluid resuscitation, including temperature and type of fluid, quantity of fluid to be provided, and duration of administration by evaluating effects on acidosis, coagulopathy, and hypothermia.

Conflict of interest: None declared.

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OLGU SUNUMU

Sıçanlarda oluşturulan kontrolsüz hemorajik şok modelinde ılık sıvı ile soğuk sıvı resüsitasyonlarının karşılaştırılması

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AMAÇ: Bu çalışma, sıçanlarda oluşturulan kontrolsüz hemorajik şok (HŞ) modelinde, soğuk sıvı resüsitasyonunun yaşam süresi, kanama miktarı, kanama hızı, koagülopati, asit-baz dengesi, hematokrit, laktat ve baz defisit üzerine etkilerini, ılık sıvı resüsitasyonunun etkileriyle karşılaştırmak için planlandı.

GEREÇ VE YÖNTEM: Çalışmada ketamin ve ksilazin ile anestezi altına alınan 29 sıçanın dalağında, vasküler ve parankim yaralanması ile HŞ modeli oluşturuldu. Hemorajik şokun 30. dakikasında, sıçanlara randomize olarak, 14.5 ml/kg dozunda %0.9 NaCl solüsyonu, 24 ºC sıcaklıkta (Grup 1, n=9) ve 4 ºC sıcaklıkta (Grup 2, n=10) 20 dakika süreyle intravenöz infüze edildi. Grup 1 ve 2’nin tedavi etkileri, sıvı tedavisi uygulananlar grubuna (Grup 3, n=10) karşılaştırıldı. İstatistiksel olarak veriler, ortalama±standart sapma olarak hesaplandı. İstatistiksel analiz için SPSS for Windows 15.0 programı, Bonferroni düzeltmeli Mann-Whitney U-testi ve Kaplan-Meier prosedürü kullanıldı. P≤0.05 olduğunda, istatistiksel olarak anlamlı kabul edildi.

BULGULAR: Soğuk sıvı resüsitasyonu kanama miktarını, kanama hızını, asidozu, hipotermi, laktatı, baz defisitini artırarak ve de kan basınıncını, hematokriti düşürerek yaşam süresini kısaltmıştır.

TARTIŞMA: Travmada sıvı resüsitasyonu ile ilgili olarak; hangi sıvının, hangi sıcaklıkta, hangi miktarda ve ne kadar sürede verileceğini inceleyen birçok deneySEL ve klinik çalışmalardan planlanması büyük ihtiyaç olduğu değerlendirilmiştir.

Anahtar sözcükler: Hemorajik şok; intravenöz soğuk sıvı; sıçan; sıvı resüsitasyonu.