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The effects of different fluid resuscitations in the acute phase of combined traumatic brain injury and hemorrhagic shock in an experimental rat model

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Abstract

It was aimed to investigate the utility of different fluid replacement therapies in the acute phase of a combined experimental traumatic brain injury and hemorrhagic shock model in terms of biochemical, hemostatic and pathological changes in the brain. 48 rats were divided up into 6 groups (n=8). Control group (S) rats were subjected to sham experimental hemorrhagic shock after which they underwent a sham operation, while trauma group (T) rats were subjected to hemorrhagic shock and subsequent head trauma with no treatment. Among the rats subjected to hemorrhagic shock and subsequent head trauma, those given 3% NaCl after this were named as HS group; those given HyperHeas [7.2% NaCl / 6% poly (O-2-Hydroxyethyl) starch] were named as HyperHS group; those given 0.9% NaCl were determined as NS group and those given ringer lactate as RL group. 24-hours later, the fluids' effects were evaluated. The brain fluid content and INR levels were significantly higher in all the experimental groups when set against those in the NS group (p ranged <0.01 to <0.001). aPTT was significantly longer in the T and HS groups than in that seen in the NS group (p<0.001 for each). Rats in the HS and RL groups showed significantly more bleeding than those in the NS group (p<0.05). Of the treatment groups, the HyperHS group had more brain edema when compared to the NS and RL groups (p<0.05). The proportion of red neuron and necrosis was partially decreased in the treatment groups, with no significant difference determined between the HS, HyperHS, NS and RL groups (p>0.05). In conclusion, study findings support the safely use of normal saline and Ringer lactate solutions in prompt fluid resuscitation where both traumatic brain injury and hemorrhagic shock have occurred, based on the overall advantages pertaining to each critical prognostic parameter.

Keywords: Traumatic brain injury, hemorrhagic shock, rats, fluid resuscitation

Introduction

Traumatic brain injury (TBI) is held to be a notable global public health issue associated with significant morbidity and mortality in respect of subsequent cortical contusion, vascular injury, hemorrhage and ischemia as well as delayed widespread neurodegeneration [1]. TBI is accompanied by hemorrhagic shock in 20% of cases and associated with earlier and higher rates of mortality than traumatic brain injury per se [2]. Hemorrhagic hypotension leads to secondary brain injury even in a very short period, associated with intracranial hypertension increasing the risk of reduced cerebral blood flow, cerebral ischemia and poor prognostic outcomes [3]. Thus, fluid replacement is also considered challenging in such patients, given the crucial role of early aggressive fluid replacement in the prevention of hypotension-related secondary damage in brain or other organs, and the necessity of treating hypotension without increasing intracranial pressure and edema [3]. However, early and aggressive fluid resuscitation may have detrimental effects such as bleeding due to clotting factor dilution, the removal of clots, and decreased blood viscosity [4]. Therefore, small volume resuscitation with more hypertonic solutions, such as 7.5% NaCl, may have a therapeutic advantage to prevent hemodilution [5].

Although the Advanced Trauma Line Support (ALTS) guidelines support the use of isotonic crystalloid solutions for early fluid

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resuscitation in patients who have experienced hemorrhagic shock, the optimal resuscitation approach and the optimal choice of fluids have not yet been clearly defined in patients with hemorrhagic shock associated with traumatic brain injury (HSTBI) [6]. Recently, several studies have encouraged the use of hypertonic fluid replacement to maintain hemodynamic stability without the risk of intracranial pressure increase although this may not improve survival or neurological outcomes [2,7]. Although maintenance of general physiology and avoidance of secondary brain injury are the key objectives of TBI management, currently, there is a lack of robust evidence on optimal strategies for general management, with evident divergences in practice at both institutional and individual levels [8-9].

Better understanding of the advantages and disadvantages of different fluid replacement therapies is crucial in the management of HSTBI, and prevention of mortality. Therefore, this study was designed to examine the utility of different fluid replacement therapies in the acute phase of a combined experimental traumatic brain injury and hemorrhagic shock model in terms of hemostatic parameters and pathological changes in the brain.

Material and Methods

Animals

The study was performed at the Inonu University Laboratory for Experimental Studies in compliance with the European Convention on Animal Care subsequent to the study's design being approved by Inonu University Animal Ethics Committee. A total of 48 adult male Sprague-Dawley rats (≥12 weeks of age and weighing 180–210 g) were kept in a room in which both light and temperature were controlled with 14- and 10-hour light and dark cycles respectively, temperature was 22˚C and relative humidity 30-70%. The animals were provided with both rat pellets as well as water ad libitum.

Study protocol

Before the experiment, the rats were separated randomly into 6 experimental groups: (1) sham, no treatment (S) (the rats underwent sham experience of hemorrhagic shock followed by sham operation with no head trauma and no blood loss); (2) hemorrhagic shock and then head trauma, absent treatment (T); (3) head trauma and hypertonic saline treatment [3% sodium chloride] subsequent to hemorrhagic shock (HS); (4) hemorrhagic shock and then head trauma with hyperHaes [7.2% NaCl/6% poly(O-2-Hydroxyethyl) starch] solution treatment (hyperHS); (5) head trauma and normal saline treatment [0.9% sodium chloride] (NS), subsequent to hemorrhagic shock; and (6) hemorrhagic shock with subsequent head trauma and Ringer lactate treatment (RL).

All surgical procedures were performed under 75 mg/kg ketamine (Parke Davis, Istanbul, Turkey) and 10 mg/kg xylazine (Rompun flacon, Bayer Inc, Germany) anesthesia. All the rats were sacrificed 24 hours after the induction of head trauma and hemorrhagic shock via intra-cardiac saline infusion under 50mg/kg propofol (Abbott, Istanbul, Turkey) deep anesthesia for wet-dry brain weight, blood biochemistry and brain tissue histopathological analyses. Data on brain water content, hemostasis parameters [platelet count, activated partial thromboplastin time (aPTT; s), and international normalized ratio (INR); %)], blood biochemistry findings [serum Na (mEq/L), plasma aldosterone (pg/mL), plasma antidiuretic hormone (ADH; ng/mL)] and histopathological findings (inflammation, hemorrhage, edema, necrosis and red neuron) were recorded in each experimental group.

Traumatic brain injury model

The TBI rat model used in this study was based on Feeney's weight-drop model as noted previously with some modifications [10], which employs a free-falling weight's gravitational forces to deliver a brain injury that is primarily focal. The tested rats were anesthetized and then placed on a stereotaxic frame. A 6x9 mm craniotomy was made with a dental drill from bregma to midline scalp at ± 3.5 mm with the parietal cortex forming the center and keeping the dura intact. Intermittent saline flush was applied to the area to protect brain tissue from heat exposure, while oxidized cellulose (Surgicel, Ethicon, Istanbul, Turkey) and bone-wax (Ethicon, Istanbul, Turkey) were used for dura and bone bleedings, respectively, when necessary. The rats were then put on a bed of foam in the prone position and traumatic injury was induced by striking the dura with an impactor (500 gr/cm force) based on the use of a flat-ended 10-g steel weight dropped from a height of 50-cm (through a cylindrical tube 7 mm in diameter, with a mobile flat surface) to strike the dura and cause a cortical contusion. After impact, the scalp was sutured closed, and the animals given time to recover from the anesthesia. All the operations were carried out with aseptic techniques by the same surgeon who was blinded to the study protocol.

Hemorrhagic shock model and fluid resuscitations

After the traumatic brain injury, anesthesia was maintained using additional doses of ketamine as necessitated by the awakening prior to the hemorrhagic shock. The rats with traumatic brain injury were placed on a thermal pad in the prone position and hemorrhagic shock was induced using a mini laparotomy intervention for inferior vena cava (IVC) catheterization with a 22-gauge needle (Introcan-W, Melsungen, Germany). After vena cava catheterization, bleeding was begun and over a period of 30-60 seconds, 30% of total blood volume was gradually extracted as per previously attested hemorrhagic shock models based on volume adjustment [7]. The model utilized was derived from an estimate that 7% of body weight would most closely approximate to the total circulating blood volume [11,12]. After 15 minutes, fluid resuscitation was initiated using normal saline (1/3 blood to fluid ratio), hypertonic saline (3%, 10 ml/kg), Ringer lactate or hyperHaes (4 ml/kg) infusions at a single dose and for 5 minutes. During fluid resuscitation, the IVC catheter was removed and compression was applied to the vein for bleeding control. The abdomen was sutured closed. All the operations were carried out with aseptic techniques by the same surgeon who was blinded to the study protocol.

Blood sample analyses

At 24 hours after the induction of HSTBI, intra-cardiac blood samples were collected into tubes appropriate for biochemical and hematological analyses. While platelet count, aPTT and INR were immediately analyzed after the euthanasia, blood samples for biochemical analysis were immediately centrifuged at 3500 rpm

for 10 minutes at +2 to+8 ºC to obtain plasma samples which were kept at -80°C until analysis. Serum sodium, plasma aldosterone, and plasma ADH levels were determined for each animal.

Histopathological analysis

10% buffered formalin was employed as a fixative for the traumatic hemisphere brain tissue samples from the trauma focus and periphery for a week – trimming and processing were then undertaken for routine histopathological examination. Paraffin, into which the issue samples were then embedded, was used for serial sectioning. 5-µm horizontal sections stained with hematoxylin and eosin (HE) were examined under a light microscope (BX50, Olympus Corp., Tokyo, Japan) and micro-images were also taken using the attached camera. Histopathological analysis was conducted by a pathologist, who was unaware of the experimental groups. The effects of traumatic brain injury and hemorrhagic shock on brain tissue were assessed semi-quantitatively based on scorings (0: none, 1: minimal; 2: moderate; 3: extensive) of inflammation, hemorrhage, edema, necrosis and red neurons.

Brain water content

Dissected contralateral hemispheres were transferred in aluminum foil and ice and weighed to determine the average wet weight. The brain samples were then dried at 105°C for 48 hours and weighed once more to record the dry weight values. Brain water content (%) was determined using the formula: [wet weight (gr) – dry weight (gr)] /wet weight (gr) x100 [13,14].

Statistical Analysis

SPSS for Windows version 17.0 (SPSS, Chicago, IL) was used for statistical analysis. Descriptive statistics were stated as mean, standard deviation, and median values. Median scores for inflammation, bleeding, edema, necrosis, and acute neuronal injury (red neurons) were compared via the Kruskal–Wallis test and comparisons between the groups were made using the Conover method. Mean plasma ADH, plasma aldosterone, serum sodium levels, mean platelet count, mean aPTT and mean INR were compared using the unpaired t test or the Mann–Whitney U test, according to the distribution of the data. A $p<0.05$ was accepted as statistically significant.

Results

Brain water content

Brain water content in all the experimental groups was significantly higher compared to the S group (p ranged <0.01 to <0.001). There was no significant difference between T, HS and hyperHS groups with respect to brain water content, while the brain water content in these three groups was also significantly higher compared to the NS and RL groups ($p<0.001$ for each). The brain water content of the NS group was significantly lower than that of the RL group ($p=0.007$) (Table I).

Table 1. The effects of hemorrhagic shock and the efficacy of different fluid therapies in terms of brain water content, biochemical and hemostatic parameters

Parameters	Study groups (n=8, for each)	Results	p*	p**	p***	p****	p*****
Wet-dry weight [(%), mean±SD] (Brain water content)	Sham-injured (S)	1.04±0.2	-	-	-	-	-
	Trauma-shock (T)	7.5±0.5	<0.001	-	-	-	-
	Hypertonic saline (HS)	6.7±0.6	<0.001	NA	-	-	-
	HyperHaes (HyperHS)	7.3±0.5	<0.001	NA	NA	-	-
	Normal saline (NS)	1.5±0.3	0.002	<0.001	<0.001	<0.001	-
	Ringer lactate (RL)	2.6±1.2	<0.001	<0.001	<0.001	<0.001	0.007
Sodium [mmol/L, mean±SD]	Sham-injured (S)	133±1.3	-	-	-	-	-
	Trauma-shock (T)	134±2.4	NA	-	-	-	-
	Hypertonic saline (HS)	136±3.2	NA	NA	-	-	-
	HyperHaes (HyperHS)	133±4.7	NA	NA	NA	-	-
	Normal saline (NS)	133±2.4	NA	NA	NA	NA	-
	Ringer lactate (RL)	133±1.8	NA	NA	NA	NA	NA
Aldosterone [pg/mL, mean±SD]	Sham-injured (S)	11.8±2.5	-	-	-	-	-
	Trauma-shock (T)	37.1±12.2	<0.001	-	-	-	-
	Hypertonic saline (HS)	20.2±13.2	NA	0.028	-	-	-
	HyperHaes (HyperHS)	21.3±12.9	NA	0.005	NA	-	-
	Normal saline (NS)	11.9±8.8	NA	0.001	NA	NA	-
	Ringer lactate (RL)	12.8±5.1	NA	0.001	NA	NA	NA
ADH [pg/mL, mean±SD]	Sham-injured (S)	0.8±0.4	-	-	-	-	-
	Trauma-shock (T)	1.7±0.6	0.002	-	-	-	-
	Hypertonic saline (HS)	0.9±0.9	NA	NA	-	-	-
	HyperHaes (HyperHS)	1.4±0.6	NA	NA	NA	-	-
	Normal saline (NS)	1.1±0.4	NA	NA	NA	NA	-
	Ringer lactate (RL)	0.7±0.5	NA	0.005	NA	NA	NA

Platelet count [(103/mm3), mean±SD]	Sham-injured (S)	755±125	-	-	-	-	-
	Trauma-shock (T)	702±103	NA	-	-	-	-
	Hypertonic saline (HS)	706±127	NA	NA	-	-	-
	HyperHaes (HyperHS)	669±125	NA	NA	NA	-	-
	Normal saline (NS)	643±177	NA	NA	NA	NA	-
	Ringer lactate (RL)	682±193	NA	NA	NA	NA	NA
aPTT [(s), mean±SD]	Sham-injured (S)	29.5±2.4	-	-	-	-	-
	Trauma-shock (T)	73±7.1	<0.001	-	-	-	-
	Hypertonic saline (HS)	67.9±15.2	<0.001	NA	-	-	-
	HyperHaes (HyperHS)	50.8±16.4	NA	0.003	NA	-	-
	Normal saline (NS)	38.7±11.5	NA	<0.001	0.001	NA	-
	Ringer lactate (RL)	53.7±19.5	NA	0.028	NA	NA	NA
INR [(%), mean±SD]	Sham-injured (S)	0.9±0.1	-	-	-	-	-
	Trauma-shock (T)	1.3±0.1	<0.001	-	-	-	-
	Hypertonic saline (HS)	1.3±0.2	0.001	NA	-	-	-
	HyperHaes (HyperHS)	1.3±0.4	0.003	NA	NA	-	-
	Normal saline (NS)	0.9±0.1	NA	<0.001	<0.001	0.005	-
	Ringer lactate (RL)	1.2±0.2	0.005	NA	NA	NA	0.007

p*: comparison of trauma and treatment groups with sham group; p**: comparison of the trauma group with treatment groups; p***: comparison of the hyperHS, NS, RL group with HS; p****: comparison of the NS and RL groups with HyperHS; p*****: comparison of the NS group with RL group

Table 2. Histopathological findings in experimental groups

Groups (n=8)	**Histopathological scores, median (IQR)**				
	Inflammation	**Red Neurons**	**Edema**	**Bleeding**	**Necrosis**
Sham (S)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
Trauma-shock (T)	0 (0-3)	3 (2-3)	3 (2-3)	2 (1-3)	2 (1-3)
Hypertonic saline (HS)	0 (0-2)	2 (1-3)	1 (1-2)	1 (0-2)	1.5 (0-2)
HyperHaes (HyperHS)	0 (0-2)	2 (1-3)	2 (1-2)	0.5 (0-2)	1 (0-2)
Normal saline (NS)	0 (0-2)	2 (1-3)	1 (1-2)	0 (0-2)	0 (0-2)
Ringer lactate (RL)	0 (0-2)	2 (1-3)	1 (1-2)	1 (0-2)	1 (1-2)
p	0.954	<0.001	<0.001	<0.001	<0.001

Blood biochemistry findings and hemostatic parameters

The blood biochemistry findings and hemostatic parameters are shown in Table I. With respect to serum Na levels, no significant difference was evident between the experimental groups. Plasma aldosterone levels were significantly higher in the T group compared to all the other experimental groups (p ranged <0.05 to <0.001). The plasma ADH levels were significantly higher in the T group than in the S group (p=0.002 for each). The mean plasma ADH level was significantly higher in the T group than in the RL group (p=0.005).

The difference between experimental groups in terms of platelet count was not significant. The aPTT value was significantly longer in the T and HS groups when compared to that in the NS group (p<0.001 for each). aPTT in the T group was significantly longer than aPTT in the hyperHS (p<0.01) group. INR levels in all the experimental groups were significantly higher when compared to those in the S and NS groups (p ranged <0.01 to <0.001).

Histopathological findings

The histopathological findings of all the groups are shown in Table II. Leukocyte accumulation (inflammation) was observed in one or two rats in all the study groups [Figure 1].

Rats in the T (B) group showed significantly more bleeding than was seen in those in the HS (C), HyperHS (D), NS (E), and RL (F) groups (p<0.05), and rats in the HS and RL groups showed significantly more bleeding than the NS group (p<0.05) [Figure 2].

Edema content was greater in the T (B) group when set against the treatment groups [Group HS (C), Group HyperHS (D), Group NS (E) and Group RL (F)] (p<0.05). Of the treatment groups, the HyperHS group had more brain edema compared to the NS and RL groups (p<0.05) [Figure 3]. The number of red neurons was significantly higher following traumatic brain injury related to hemorrhagic shock and the difference between the T (B) group and the treatment groups was significant [Group HS (C), Group HyperHS (D), Group NS (E) and Group RL (F)] (p<0.05) [Figure 4]. An increase in necrosis was observed in Group T compared to

Group HS, Group HyperHS, Group NS, and Group RL ($p<0.05$) [Figure 5].

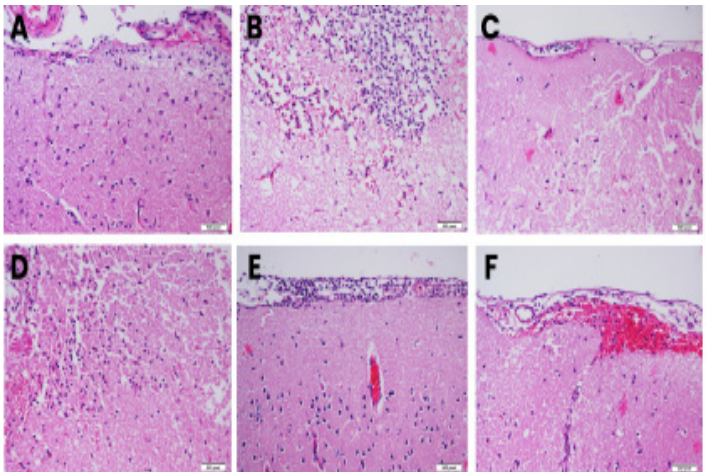

Figure 1. HxE stained images of inflammation of the study groups X200. A: Sham group B: Trauma-shock group C: Hypertonic saline group D: Hyperheas group E: Normal saline group F: Ringer lactate group

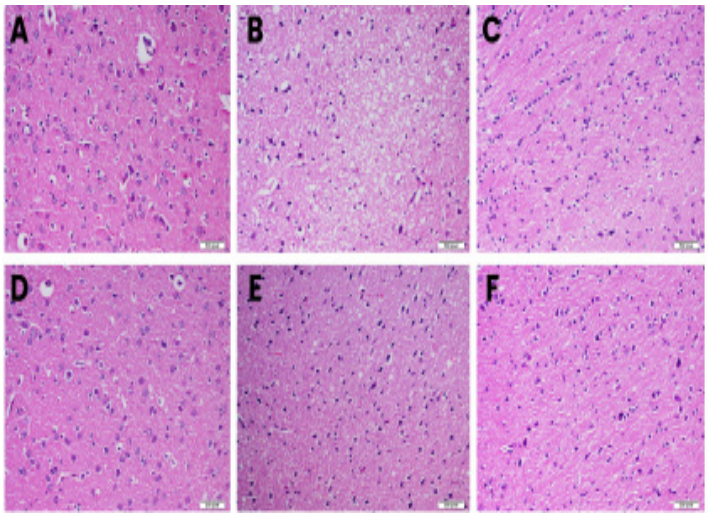

Figure 2. HxE stained red neuron images of study groups X200. A: Sham group B: Trauma-shock group C: Hypertonic saline group D: Hyperheas group E: Normal saline group F: Ringer lactate group

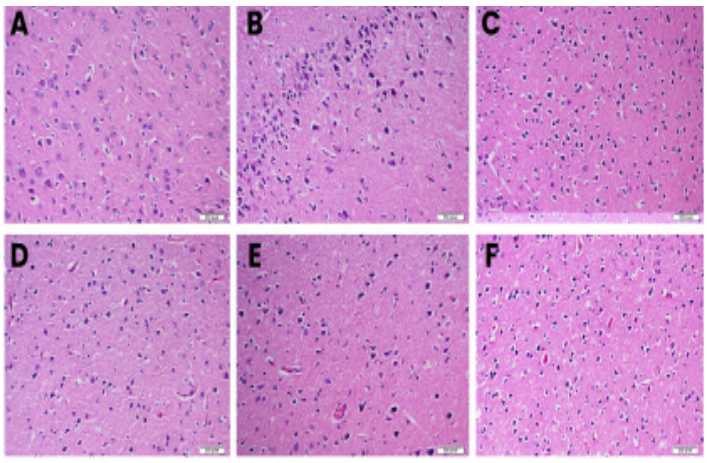

Figure 3. Edema images of study groups stained with HxE X200. A: Sham group B: Trauma-shock group C: Hypertonic saline group D: Hyperheas group E: Normal saline group F: Ringer lactate group

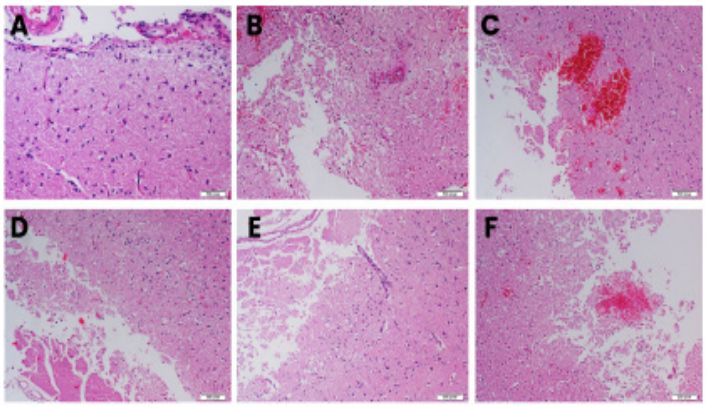

Figure 4. Bleeding appearances of study groups stained with HxE X100. A: Sham group B: Trauma-shock group C: Hypertonic saline group D: Hyperheas group E: Normal saline group F: Ringer lactate group

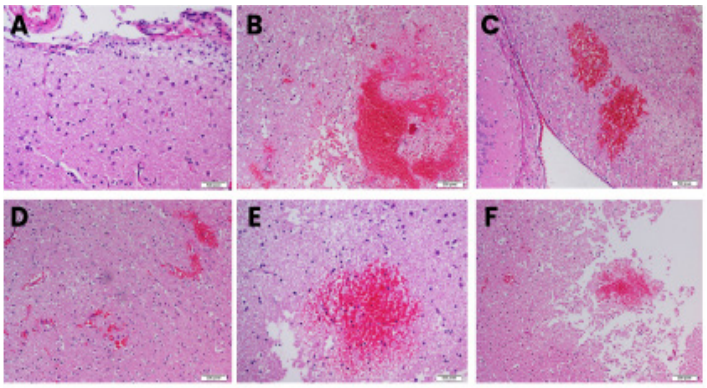

Figure 5. HxE stained necrosis images of study groups X100. A: Sham group B: Trauma-shock group C: Hypertonic saline group D: Hyperheas group E: Normal saline group F: Ringer lactate group

Discussion

The composition of fluids and the timing of administration may have a significant effect on intracranial pressure following HSTBI. Hyperosmolar fluids are used to reduce intracranial pressure elevated as a result of TBI [15- 16]. Although the optimal dose and concentration for HS are unknown, it is mostly administered at the lowest possible dose until side-effects are encountered [16,17]. The present study was performed to ascertain the effects of different resuscitation fluids administered in the early stages after HSTBI.

Although the efficacy on improved survival or neurological outcomes remains inconclusive, the use of hypertonic fluid replacement has been addressed in both clinical and experimental studies and reported to maintain hemodynamic stability without risk of increasing intracranial pressure [2,3,7,18]. Rapid replacement through the utilization of hypertonic saline has been considered to enable control of hypovolemic shock without the risk of increased intracranial pressure in the management of patients with massive intracranial hemorrhage and brain edema [19-20]. Replacement with 3% HS compared to NS [21] and RL [22] and replacement with 7.5% HS compared to RL [7, 23] have also been reported to be associated with better control of intracranial pressure in hemorrhagic shock models. The use of hypertonic fluid replacement has been reported to be advantageous in terms of the risk of intracranial pressure increase [23] and better than RL in terms of a reduction of brain edema [2] in combined traumatic

brain injury and hemorrhagic shock models. The findings of the current study revealed that the brain water content level was significantly lower in the normal saline group (in comparison with all other fluid groups), and in the Ringer lactate group (in comparison with the hypertonic saline and HyperHaes groups). Likewise, a higher brain tissue edema-score was observed in histopathological analysis with hyperHS replacements than in both NS and RL replacements. This seems to be consistent with current ATLS (Advanced Trauma Life Support) guidelines that recommend isotonic fluid replacement with RL or NS in the early pre-hospital or emergency management of traumatic hemorrhagic shock [6]. However, in a comparison between the treatment groups and the untreated group, the scores for red neuron intensity (an indicator of ischemic damage) and necrosis (an indicator of cell death) were lowest in the former.

HS has been used in fluid resuscitation in both hemorrhagic shock and intracranial mass models, and these studies have demonstrated its efficacy [19-21]. The short-term and long-term effects of 7.5% NaCl and Ringer's lactate (RL) in rapid fluid resuscitation were compared in respect of hypernatremia and hyperosmolarity, which are the principal side-effects of HS. In experimental and human studies of TBI, blood sodium levels after bolus HS infusion have been shown to be generally 140 and 150 mmol/l, 150 and 160 mmol/l in a few studies [24,25], and >160 mmol/l in one study [26]. The blood sodium level is the most important factor contributing to serum osmolarity. Increased serum osmolarity may contribute to the risk of kidney injury [27]. Therefore, blood osmolarity should not be raised above 320 mOsm/L especially in hypovolemic patients as there is a elevated risk of acute tubular necrosis and renal failure [27]. In the current study, all the fluid replacement groups had similar serum sodium levels.

Head injury may lead to an inappropriate increase in ADH level [28]. In the present study, it was seen that with respect to the treatment groups, plasma ADH levels were lower than those in the trauma group. ADH level increases in response to renin-angiotensin system activation by hemorrhage induced hypotension [29,30]. Although an increase in ADH level has been reported in hemorrhagic shock models [29,31] and in traumatic brain injury [32], the current study findings revealed no increase in ADH levels in the fluid replacement groups compared to the S and T groups, although a higher ADH level was seen in the hyperHS group compared to the RL group. No superiority of HS or HyperHS was determined in respect of reducing ADH, compared to the NS and RL groups. This finding supported the previously reported result that the administration of crystalloid or hypertonic solutions will reduce ADH levels by restoring plasma volume [33].

Previous reports of high aldosterone values in patients with acute head injury and increased intracranial pressure have been recored [34]. Therefore, from the standpoint of of the severity of increased intracranial pressure and head injury, aldosterone serves as a useful biochemical indicator. In the current study, although no significant difference was observed between the treatment groups, higher blood aldosterone levels were seen in the untreated group than in the treatment groups. This finding suggested that NS or HS treatment will restore plasma volume, which in turn may prevent increases in blood aldosterone levels as well as in ADH.

As it is identified based on INR, aPTT, fibrinogen levels and thrombocyte count [35], coagulopathy is considered to already be present in approximately 33% of trauma patients suffering from blood loss at the time of admission and is linked with an heightened risk of multiple organ failure and death [35,36]. The findings of the current study showed that normal saline replacement and hyperHS were the two replacement fluids associated with better aPTT levels, and NS was superior to HS in terms of aPTT, and to all other fluid replacement therapies in terms of INR levels. This seems to indicate the activation of both pro-thrombotic and thrombolytic processes in early acute coagulopathy linked to traumatic injury, which of late has been evaluated as a multifactorial primary condition resulting from the conjunction of bleeding-induced shock, tissue injury-related thrombin-thrombomodulin-complex generation and anticoagulant and fibrinolytic pathway activation [36]. Likewise, in an experimental study of a TBI and hemorrhagic shock model in pigs, NS resuscitation was reported to be linked to with early activation of coagulation, natural anticoagulation, and endothelial systems, compared with colloids and fresh frozen plasma resuscitation [37].

The level of bleeding was significantly lower in the treatment groups than that in in the trauma group and among the treatment groups, bleeding was more remarkable in the HS and RL groups when compared to the NS and HyperHS groups. Elliot et al. reported increased tissue damage from HS treatment administered immediately following traumatic brain injury, but the extent of tissue damage was not increased when administered at 6 hours [38] Increased permeability of the blood-brain barrier immediately following injury compared to after 1 hour has been previously demonstrated [39,40]. Increased hemorrhage by HS may be secondary to the aggravation of tissue damage when administered immediately after HSTBI when the level of permeability of the blood-brain barrier is greater.

There were some limitations in this study. First, the use of rat models for controlled HSTBI may not fully mirror the actual clinical conditions applicable to humans. Second,delays in fluid resuscitation were likewise not evaluated. Third, the samples were collected from the animals after 24 hours, and the data did not address long-term complications of fluids. Therefore, there is a need for further experimental studies with longer periods of observation to evaluate specimens for the late effects of different fluids.

Conclusion

In conclusion, the use of different fluid replacement therapies in this rat model of HSTBI revealed the likelihood of unique advantages of each replacement fluid in terms of histopathological, biochemical and hemostatic parameters, challenging the recommendation of a single replacement therapy over others. Nonetheless, these findings support the use of normal saline and Ringer lactate solutions in prompt fluid resuscitation of HSTBI, based on the overall advantages pertaining to each critical prognostic parameter (brain water content, hemostatic parameters, and histopathological changes). Therefore, crystalloid solutions can be used safely in the initial stages of HSTBI in the Emergency Department.

Conflict of interests
The authors declare that they have no competing interests.

Financial Disclosure
This study was supported by Inonu University Research Project Unit.

Ethical approval
Animals employed in the present study were kept in the Inonu University, Faculty of Medicine, Animal Research Laboratory and approval for all experimental procedures was obtained from the Animal Research Ethics Committee of Inönü University (approval no. protocol no:2013/104)

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