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Original Article

How to treat hemorrhagic shock and head trauma in the emergency department: isotonic or hypertonic saline?

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Abstract: Aims: This study was performed to compare the effects of physiological saline and hypertonic saline administered at an early stage in a model of traumatic brain injury associated with hemorrhagic shock. Material and methods: Twenty-eight male Sprague-Dawley rats were divided into four groups. The rats in the control group (S) underwent a sham experimental hemorrhagic shock followed by a sham operation. The rats in the trauma group (T) underwent a hemorrhagic shock followed by head trauma and no treatment. The rats in the NS group underwent a hemorrhagic shock followed by head trauma and received 0.9% NaCl. The rats in the HS group underwent a hemorrhagic shock followed by head trauma and received a 7.5% NaCl solution. The weight-drop method was used for achieving head trauma. After the head trauma, hypovolemia was induced by the controlled hemorrhage of 30% of the blood volume. The animals were exposed to hypovolemic shock for a further 30 min prior to fluid resuscitation. Each animal received a single volume infusion of their assigned fluid within few minutes. The effects of different fluids were evaluated after 24-hours by their brain water contents, and histological, and biochemical tests. Results: Group T had a significantly higher mean value for brain water content than did the NS group ($P < 0.0001$). Also, the HS group had significantly higher mean values for brain water content than the NS group ($P = 0.003$). Edema and bleeding were more marked in the HS group compared to the NS group (both, $P < 0.001$) in the histopathological evaluation. Leukocyte accumulation was significantly increased in the untreated rats compared to the HS and NS groups (both, $P < 0.001$). More red neurons were observed in the rats in the T group than in the NS and HS groups (both, $P < 0.001$). The mean serum osmolarity was higher in the T group compared to NS, HS, and S groups (all, $P < 0.001$). The mean plasma ADH levels and the mean plasma aldosterone levels were significantly higher in the T group than in the S, NS, and HS groups (all, $P < 0.0001$). Conclusion: Although not significantly different in biochemistry, animals treated with HS early in hemorrhagic shock secondary to head injury had more brain water than those the received NS as defined histopathologically. Therefore, in emergency settings, NS should be used safely in the early stage of hemorrhagic shock secondary to head trauma.

Keywords: Fluid resuscitation, head trauma, hemorrhagic shock, hypertonic saline, isotonic saline

Introduction

Injury-related death ranks sixth among the ten leading causes of mortality in humans between the ages of 5 and 44 years old [1]. The severity of injury and additional complications, including hemorrhagic shock and concomitant injury to other organs, are among the essential factors for both morbidity and mortality in patients with trauma. Head injury is the major cause of death secondary to trauma.

Hemorrhagic shock is recognized in up to one fifth of patients with head injuries. Shock is an acknowledged cause of secondary brain injury. Therefore, early and effective fluid resuscitation is necessary to prevent adverse outcomes. Early aggressive fluid resuscitation with various fluids, including crystalloids, colloids, and mannitol, is used for primary volume replacement in hemorrhagic shock associated with traumatic brain injury (HSTBI) in emergency and pre-hospital settings [2, 3]. The type of volume replace-

ment fluids may have a noticeable impact on intracranial pressure [4-6]. Hyperosmolar fluids, including hypertonic saline (HS) solution (3% NaCl), have been proposed to resolve elevated intracranial pressure (ICP) from traumatic brain injuries [4-6]. However, early and aggressive fluid resuscitation may have detrimental effects. Recent animal studies have shown that early aggressive fluid resuscitation is related to increased bleeding due to the dilution of clotting factors, the removal of clots, and decreased blood viscosity [7, 8]. Increased mortality and prolonged coagulation times were noted in hypotensive trauma patients with penetrating torso injuries who were resuscitated aggressively with fluids [9]. Therefore, small volume resuscitation with more hypertonic solutions, such as 7.5% NaCl, may have a therapeutic advantage to prevent hemodilution [10]. Fluid volume in the intercellular compartment may be mobilized more rapidly into circulation. Cardiovascular effects, including the restoration of cardiac output, systemic blood pressure, and regional blood flow, were elicited with small volume resuscitation without resultant fluid overload and an enhanced risk of bleeding [11, 12].

The combination of traumatic brain injury (TBI) and hemorrhagic shock (HS) has been associated with increased morbidity and mortality [13]. Hypotension is a major cause of secondary brain injury even in a very short period and contributes to worse outcomes [6]. Fluid resuscitation plays a critical role in restoring and maintaining systemic and cerebral circulations in patients with HS [14]. Although Advanced Trauma Life Support (ATLS) recommends 1-2 liters of crystalloid solutions in patients with HS, the optimal resuscitation approach and the optimal choice of fluids have not yet been clearly defined in patients with HSTBI [15]. Various treatment modalities have been proposed, such as osmotherapy agents, especially hypertonic saline solutions (HTS), and are currently used in the treatment of patients with posttraumatic cerebral edema and raised ICP resulting from TBI [16]. Such treatment is believed to be particularly useful in the treatment of ICP with small volume fluid resuscitation [17, 18].

This experimental study was performed to compare the effects of physiological (normal) saline (0.9%) and hypertonic (7.5%) saline administered at the 30th minute in a model of HSTBI. Histological and biochemical evaluations were performed to determine the effects of fluid treatment.

Materials and methods

The study was performed at the Yeditepe University Laboratory for Experimental Studies in compliance with the European Convention on Animal Care following approval of the design by the Yeditepe University Animal Ethics Committee. Twenty-eight male Sprague-Dawley rats (aged between 10 and 12 weeks, weighing between 200 and 230 g) were divided into four groups, each consisting of seven rats. The rats in each group were kept in separate cages in rooms with controlled light and temperature (24°C and a 14-h light/10-h dark cycle) and were given access to standard chow and water ad libitum.

Study design

Before the experiment, the rats were randomly divided into the following 4 experimental groups: (1) sham, no treatment (S) (the rats underwent sham experiencing hemorrhagic shock followed by a sham operation with no head trauma and no blood loss); (2) hemorrhagic shock followed by head trauma, no treatment (T); (3) hemorrhagic shock followed by head trauma and normal saline treatment [0.9% sodium chloride] (NS); (4) hemorrhagic shock followed by head trauma and 7.5% hypertonic saline solution treatment (HS).

All rats in the HS and TBI groups were weighed before the procedure and 7% of the weight was taken as total blood volume. Under general anesthesia, the weight drop technique was employed to induce TBI. After TBI, the tail vein was cannulated and bleeding was initiated.

Traumatic brain injury

The weight drop technique described by Marklund et al. [19] and Feeney et al. [20] was used to induce TBI. Prior to TBI, the rats were anesthetized with ketamine at a dose of 80 mg/kg and xylazine at a dose of 5 mg/kg administered intraperitoneally. The rats were immobilized in the supine position on a rough surface by taping the extremities and were placed on a thermal pad to maintain a constant body temperature of 37°C after surgery. The operations were performed by the same surgeon, who was

blinded to the groups. For the rats in the T, NS, and HS groups, a craniotomy (6 × 9 mm) centered over the right parietal cortex at bregma -3.5 and 3.5 mm lateral to the midline was performed using a dental drill. A 10-g weight was dropped from a height of 50 cm onto a piston 5 mm in diameter resting on the exposed dura, without bouncing.

Experimental protocol, induction of hemorrhagic shock and resuscitation process

After TBI, anesthesia was maintained using additional doses of ketamine as necessary prior to the hemorrhagic shock. The subsequent steps were performed after each animal was placed on a thermal pad to maintain a constant body temperature of 37°C throughout the rest of the procedure. A rectal thermometer was used to monitor temperature. After tail vein cannulation, bleeding was initiated and 35% of the rat's total blood volume was gradually withdrawn over 30-60 s, in accordance with previ-ously described volume-adjusted hemorrhagic shock models [21, 22]. This model was used based on the estimate that 7% of body weight can best approximate the total circulating blood volume [21-23]. The animals were exposed to hypovolemic shock for a further 30 minutes prior to fluid resuscitation. Thereafter, each animal received a single volume infusion of the assigned fluid within a few minutes. The venous line was removed after resuscitation.

The rats in the T, NS, and HS groups underwent HSTBI. Subsequently, the rats in the HS group were administered 7.5% hypertonic saline at a dose of 4 mL/kg and 3 mL NS for each milliliter of blood loss within a few minutes via the catheter after the development of hemorrhagic shock.

Euthanasia

The rats were euthanized 24 h after HSTBI. Prior to euthanasia, the rats were anesthetized by the intraperitoneal administration of propofol (Abbott Laboratory Inc. Co., Istanbul, Turkey) at a dose of 50 mg/kg. The euthanasia was performed by transcardiac perfusion using 0.9% sodium chloride. Intracardiac blood samples were taken after the induction of deep anesthesia for the biochemical analysis. The brains were removed rapidly after the euthanasia. The right and left hemispheres were separated; the right hemisphere was used for histopathological evaluation, and the left hemisphere was used for the analysis of brain water content.

Determination of brain water content

The brain water content was evaluated using the wet-dry weight technique [24]. Briefly, the brains were weighed to assess the wet weight (WW) and then dried for 24 hours at 104°C to determine the dry weight (DW). Based on the wet and dry weight, the brain water content was calculated as: brain water content (%): (WW) (DW)/WW x 100) [24].

Biochemical evaluation

Twenty-four hours after the induction of HSTBI, blood samples were collected from each rat by intracardiac puncture and placed into biochemical tubes for serum sodium and osmolarity, and ethylene diamine tetraacetic acid (EDTA) tubes for aldosterone and anti-diuretic hormone extraction. Serum sodium, plasma aldosterone, and plasma anti-diuretic hormone (ADH) levels were determined in each animal.

Histopathological evaluation

After euthanasia, the brain was removed immediately, and the right and left hemispheres were separated. The right hemisphere containing the contusion epicenter was immersed in 10% formaldehyde for 1 week, embedded in paraffin, and cut into serial horizontal sections 5 μm thick. The sections were stained with hematoxylin and eosin (H-E) and visualized by light microscopy (BX50; Olympus Corp., Tokyo, Japan) to determine the structural changes. A quantitative histopathological evaluation of the brain lesion was conducted for each sample under the lighted microscope. Photographs of brain specimens were taken under the lighted microscope. Among the effects of HSTBI, inflammation (defined as the presence of vascular dilatation with interstitial fluid and leukocyte accumulation), bleeding, edema, and acute neuronal injury (i.e., red neurons) were scored semi-quantitatively as not increased (0 points), mildly increased (1 point), moderately increased (2 points), or significantly increased (3 points).

Statistical analysis

SPSS for Windows version 16.0 (SPSS, Chicago, IL) was used for the statistical analysis.

Table 1. Impact of different fluid therapies on groups of rats with hemorrhagic shock

Parameters	Study groups		Results (± SD)
Wet-dry weight (%) (Brain water content, brain edema)	Sham-injured	S	55 ± 1.7
	Untreated	T	63 ± 1.1
	0.9% NaCl	NS	60 ± 1.5
	7.5% NaCl	HS	62 ± 1.1
Osmolarity (mOsm/l)	Sham-injured	S	296 ± 3
	Untreated	T	311 ± 5
	0.9% NaCl	NS	293 ± 4
	7.5% NaCl	HS	293 ± 5
Sodium (mmol/l)	Sham-injured	S	143 ± 1
	Untreated	T	146 ± 5
	0.9% NaCl	NS	143 ± 2
	7.5% NaCl	HS	143 ± 2
Aldosterone (ng/ml)	Sham-injured	S	44 ± 10
	Untreated	T	151 ± 26
	0.9% NaCl	NS	56 ± 10
	7.5% NaCl	HS	52 ± 17
ADH (pg/ml)	Sham-injured	S	3.9 ± 0.9
	Untreated	T	9.7 ± 1.1
	0.9% NaCl	NS	2.7 ± 1.1
	7.5% NaCl	HS	2.7 ± 1.7

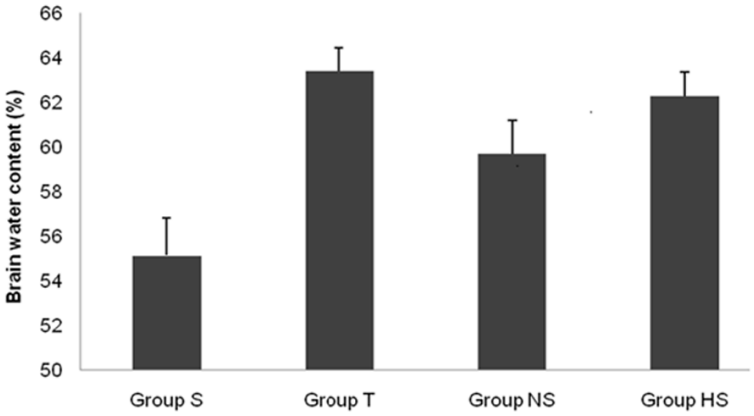

Figure 1. Brain water content values. Rats in group sham-injured (S) showed significantly decreased water content in the brain hemispheres compared to the no treatment (T), normal saline treatment (NS), and hypertonic saline treatment (HS) groups (all, $P < 0.0001$). The T group had significantly elevated brain water content compared to the S and NS groups (both, $P < 0.0001$), but there is no difference compared to the HS group. There was also a significant difference between NS and HS groups ($P = 0.003$).

Descriptive statistics are reported, including the mean, standard deviation, and median. Median scores for inflammation, bleeding, edema, and acute neuronal injury (red neurons) were compared using the Kruskal-Wallis test and comparisons among the groups were performed with the Conover method. Mean plasma ADH, plasma aldosterone, and serum sodium levels, and serum osmolarity were compared using the unpaired *t* test or the Mann-Whitney U test, depending on whether the data were normally distributed. In all analyses, $P < 0.05$ was taken to indicate statistical significance.

Results

A total of 28 rats were included in the analysis. The animals were assigned randomly to the experimental groups. None of the rats died immediately following trauma, including rats in the brain-injured, sham, treated, and untreated groups.

Brain water content

Table 1 and **Figure 1** show the brain water content for all groups. Rats in the sham injury (S) group showed significantly decreased water content in the brain hemispheres compared to the no treatment (T), normal saline treatment (NS), and hypertonic saline treatment (HS) groups (all, $P < 0.0001$). The T group had significantly elevated water content compared to the S and NS groups (both, $P < 0.0001$), but there was no difference compared to the HS group. There was also a significant difference between NS and HS groups ($P = 0.003$).

Serum osmolarity, serum sodium, plasma ADH, and plasma aldosterone

Table 1 and **Figure 2** show the mean plasma ADH levels for all groups. The mean plasma

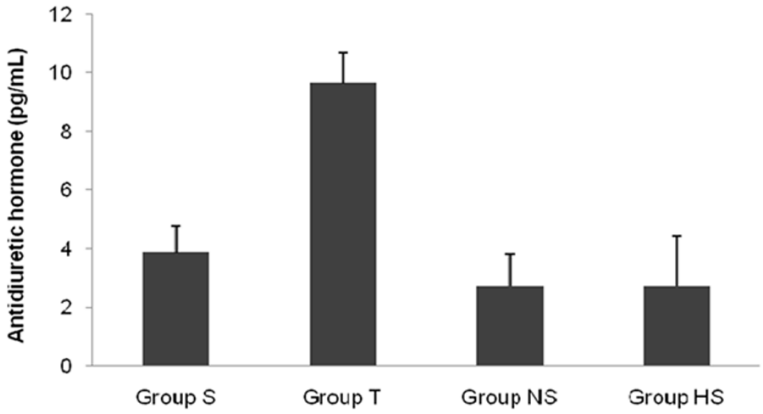

Figure 2. Levels of plasma ADH. The mean plasma ADH levels were significantly higher in the T group than the S, NS, and HS groups (all, P < 0.0001). There were no significant differences between the S, NS, and HS groups (all, P > 0.05).

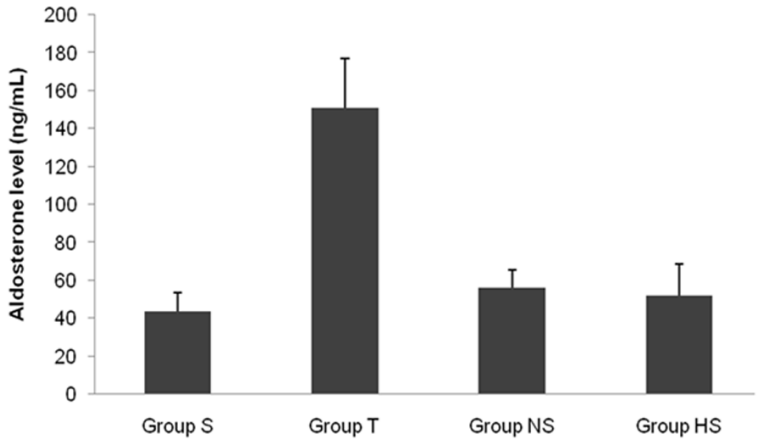

Figure 3. Levels of plasma aldosterone. The mean plasma aldosterone levels were greater in the T group than in the S, NS, and HS groups (all, *P* < 0.0001). There was a significant difference between the S group and the NS group (*P* = 0.032), but no significant difference between the NS and HS groups (*P* > 0.05).

ADH levels were significantly higher in the T group than the S, NS, and HS groups (all, *P* < 0.0001). There were no significant differences between the S, NS, and HS groups (all, P > 0.05).

The plasma aldosterone levels are presented in **Table 1** and **Figure 3**. The mean plasma aldosterone levels were greater in the T group than in the S, NS, and HS groups (all, *P* < 0.0001). There was a significant difference between the S group and the NS group (*P* = 0.032), but no significant difference between the NS and HS groups (*P* > 0.05).

The serum sodium levels are shown in **Table 1** and **Figure 4**. The mean serum sodium level was higher in the T group (146 mmol/L) than in the S, NS, and HS groups, but the differences between all the groups were not significant (all, *P* > 0.05).

Table 1 and **Figure 5** present the serum osmolarity values for all the groups. The mean serum osmolarity was significantly elevated in the T group compared to the NS, HS, and S groups (all, *P* < 0.001). There were no statistically significant differences in mean serum osmolarity between the S, NS, and HS groups (all, *P* > 0.05).

Histopathological findings

Leukocyte accumulation (inflammation) was not observed in the S and HS groups (**Table 2**; **Figure 6**). In the T group, leukocyte accumulation was significantly increased compared to the NS and HS groups (both, *P* < 001). Inflammation was partially depressed in the NS (C) group, and, in this group, leukocyte accumulation was significantly increased compared to the S and HS groups (both, *P* = 0.001). There was no significant difference in leukocyte accumulation between the S group and HS group (all, *P* > 0.05).

Bleeding was not observed in the S group (**Table 2**; **Figure 7**). Rats in the HS group showed significantly more bleeding than T and NS groups (*P* = 0.033 and *P* < 0.001, respectively), and rats in the T group showed more bleeding than in the NS group (*P* = 0.002).

Table 2 and **Figure 8** present the edema contents. The edema content was greater in the T, NS, and HS groups compared to the S group (*P* < 0.001, P < 0.005, and P < 0.001, respective-

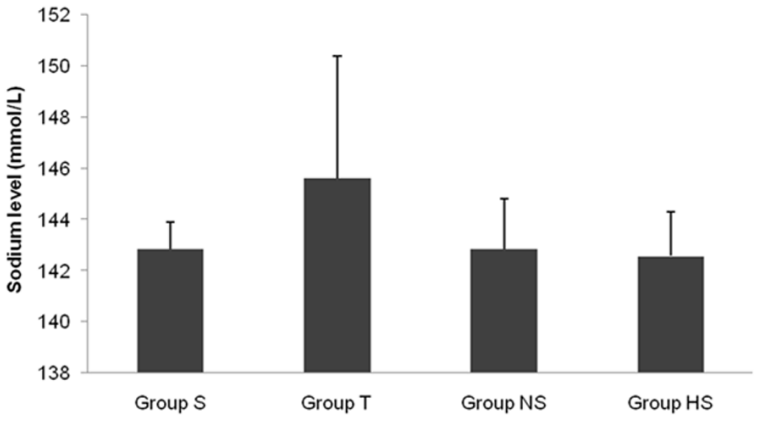

Figure 4. Levels of serum sodium. The mean serum sodium level was higher in the T group (146 mmol/L) than in the S, NS and HS groups, but the differences between all groups were not significant (all, P > 0.05).

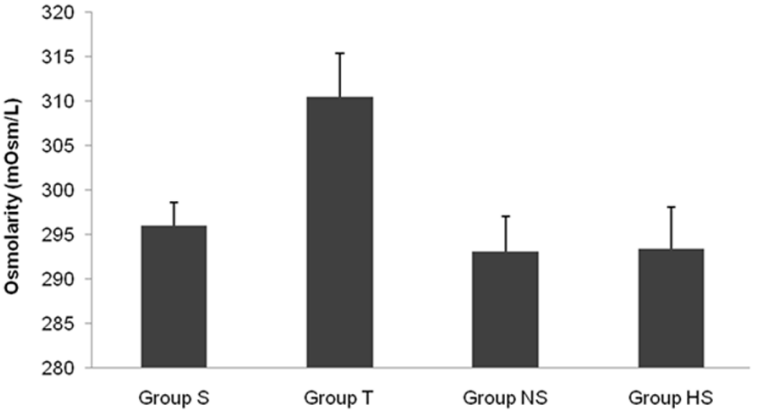

Figure 5. Levels of serum osmolarity. The mean serum osmolarity was significantly elevated in the T group compared to the NS, HS, and S groups (all, P < 0.001). There were no statistically significant differences in mean serum osmolarity between the S, NS, and HS groups (all, P > 0.05).

ly), and the T and HS groups had more brain edema compared to the NS group (P = 0.006 and *P* < 0.001, respectively). There was no significant difference in brain edema between the T group and HS group (*P* > 0.05).

Red neurons were observed only in the T, NS, and HS groups (**Table 2**; **Figure 9**), and there was a significant difference between the T group and the treatment groups (NS and HS groups) (both, *P* < 0.001). There was no significant difference in red neurons between the NS and HS groups (*P* > 0.05).

Discussion

The type of fluid used for volume replacement may have a significant effect on intracranial pressure. Hyperosmolar fluids were used to reduce the intracranial pressure elevated due to TBI [4-6, 25]. The present study was performed to investigate the effects of resuscitation fluids administered in the early stages after HSTBI.

The composition of fluids and the timing of administration would have an impact on edema following HSTBI. Although the optimal dose and concentration for HS are unknown, it is mostly administered at the lowest possible dose until side effects are encountered or target serum sodium levels are achieved [26]. The available HS formulations range from 3 to 20% in Turkey. Similar efficacies of different concentrations with no apparent harmful metabolic side effects have been reported [27]. HS may be administered as an infusion over a period of hours in lower concentrations, such as 3%, or as rapid boluses over 15 or 20 minutes at concentrations higher than 3% [26]. Similar effects on reducing ICP were demonstrated by both routes [28]. In the present study, HS was administered intravenously to the treatment groups at a dose of 4 mL/kg and NS was administered intravenously to the treatment groups at dose of 3 mL for each milliliter of blood loss in a rapid bolus.

The morbidity and mortality in HSTBI may be reduced using rapid fluid resuscitation [15]. Pre-hospital volume resuscitation with HS may be beneficial in these patients. The beneficial effects of fluid resuscitation with HS have been demonstrated in both experimental and clinical investigations [29, 30]. In a clinical retrospective study, Qureshi et al. [31] reported that 3% NaCl reduced intracranial pressure in patients with head trauma and edema. HS has been used in fluid resuscitation in models of both hemorrhagic shock and intracranial mass, and these studies demonstrated its efficacy [32-

Table 2. The histopathological evaluation of the groups

Median items score	Group S	Group T	Group NS	Group HS	*p*
Inflammation/leukocyte accumulation	0 (0 to 0)	2 (1 to 2)	1 (0 to 1)	0 (0 to 0)	< 0.001
Bleeding	0 (0 to 0)	2 (1 to 3)	1 (1 to 2)	3 (2 to 3)	< 0.001
Edema	0 (0 to 0)	1 (1 to 2)	1 (0 to 1)	2 (1 to 2)	< 0.001
Red neuron	0 (0 to 0)	2 (1 to 3)	1 (0 to 1)	1 (0 to 1)	< 0.001

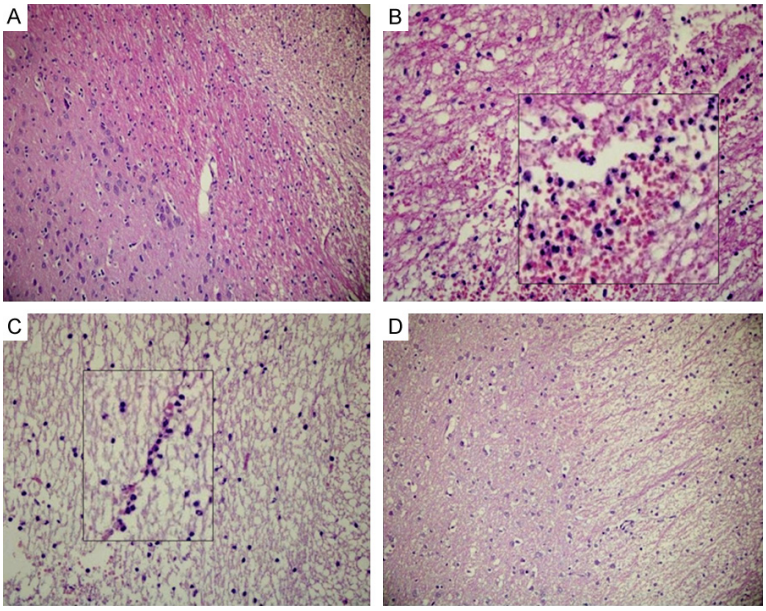

Figure 6. Inflammation for all groups in histopathological evaluation. In the T (B) group, inflammation was significantly pronounced following traumatic brain injury associated with hemorrhagic shock, and leukocyte accumulation was significantly increased compared to the NS and HS groups (both, P < 001). Inflammation was not observed in the S (A) and HS (D) groups. In the NS (C) group, inflammation was partially depressed, and in this group, leukocyte accumulation was significantly increased compared to the S and HS groups (both, P = 0.001). There was no significant difference for leukocyte accumulation between the S and NS groups as well (P > 0.05) (hematoxylin and eosin, 400x).

34]. The short-term and long-term effects of 7.5% NaCl and Ringer's lactate (RL) in rapid fluid resuscitation were compared by Anderson et al. [33] in a sheep model of cryogenic injury and hemorrhagic shock. The ICP was lower in the first 1.5 hours in animals that received 7.5% NaCl bolus compared to those administered RL. Cerebral water content was not different between the two groups at 24 hours. In a porcine model with cryogenic injury and hemorrhagic shock, a bolus followed by infusion of 7.5% NaCl with dextran was more effective than a bolus followed by infusion of RL in restoring the mean arterial pressure (MAP) and cardiac output [35]. ICP and cerebral white matter water content were lowest in the entire 24-hour observation period in this study. Continuous infusion after the initial bolus of 7.5% NaCl may prolong its beneficial hemodynamic and cerebral effects.

HS administration may cause some changes in the posttraumatic brain tissue when given immediately following injury, including increased vascular permeability, enhanced edema formation, and increased tissue injury due to the increased brain water content [36]. The presence of edema and edema content may be useful to estimate the actual amount of damage following head trauma [37]. Although sodium may accumulate in the injured brain tissue and rebound cerebral edema may occur as water is drawn into the injured tissue [38], cerebral edema and tissue damage were not worsened by HS when administered at 6 hours after head injury [39, 40]. These findings may be secondary to decreased blood-brain barrier permeability after 1 hour following head injury and the reducing effects of HS on injury volume and edema formation at time points later than 1 hour. It has been suggested that blood-brain barrier permeability plays an important role in edema and injury volume in cerebral injury. The findings of the present study indicated that edema content following head injury was influenced primarily by the timing of fluid treatment administration. Immediate fluid treatment with HS seemed to contribute to worsening of edema content following head trauma. The type of resuscitation fluid is also an important factor in edema content, with a greater increase seen in rats treat-

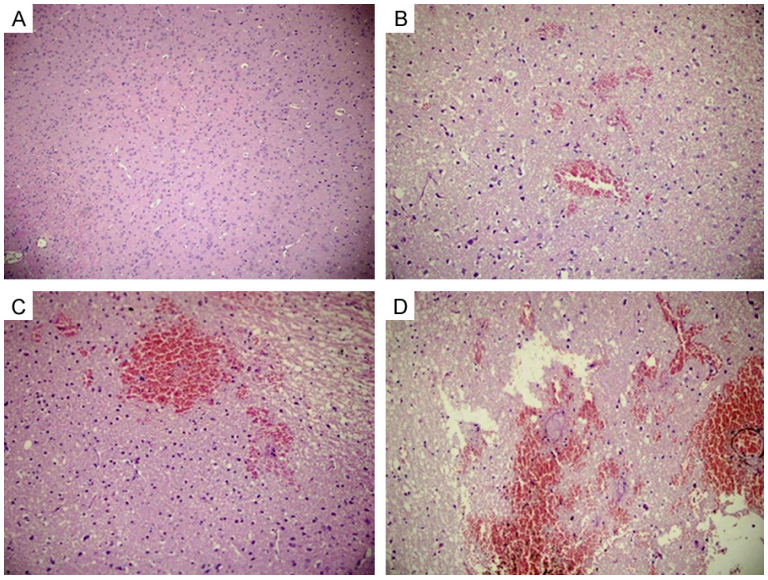

Figure 7. Bleeding for all groups in histopathological evaluation. Bleeding was not observed in the S (A) group. Rats in the HS (D) group showed significantly more bleeding than the T (B) and NS (C) groups (P = 0.033 and P < 0.001, respectively), and rats in the T (B) group showed more bleeding than those in the NS (C) group (P = 0.002) (hematoxylin and eosin, 100x).

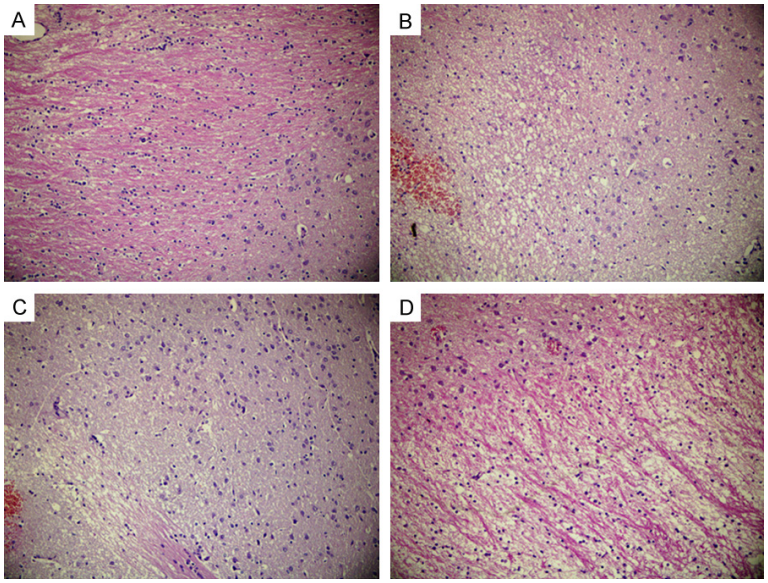

Figure 8. Cerebral edema for all groups in histopathological evaluation. Cerebral edema was not observed in the Group S (A). Edema content was greater in the T (B), NS (C), and HS (D) groups compared to the S (A) group (P < 0.001, P < 0.005, and P < 0.001, respectively), and the T (B) and HS (D) groups had more brain edema compared to the NS (C) group (P = 0.006 and P < 0.001, respectively). There was no significant difference in brain edema between the T (B) group and the HS (D) group (P > 0.05) (hematoxylin and eosin, 200x).

ed with 7.5% NaCl in the present study. Elliott et al. [36] reported that edema content was actually greater following immediate fluid treatment with hypertonic saline immediately after head injury, although the difference was not significant. In animals treated with HS 6 hours or more following head trauma, edema content was significantly smaller than in those treated immediately, suggesting a time-dependent effect.

The amount of bleeding was significantly higher in the HS group than in the NS and other groups and was similar in untreated animals and in animals treated with isotonic saline. Elliot et al. reported increased tissue damage by HS treatment when given immediately after traumatic brain injury, but the extent of tissue damage was not increased when administered at 6 hours [36]. Increased permeability of the blood-brain barrier immediately after injury compared to after 1 hour was demonstrated previously [39, 40]. Increased hemorrhage by HS may be secondary to the aggravation of tissue damage when given immediately after HSTBI when the blood-brain barrier is more permeable.

The principal side effects of HS are hypernatremia and hyperosmolarity. HS may increase serum osmolarity above 300 mOsm/L, which may increase the risk of kidney injury [41]. Therefore, blood osmolarity should not be raised above 320 mOsm/L especially in hypovolemic patients due to the increased risk of acute tubular necrosis and renal failure [41, 42]. The blood sodium level is the most important factor contributing to serum osmolarity. In experimental and human studies on TBI, blood sodium levels after bolus HS infusion were generally 140 and

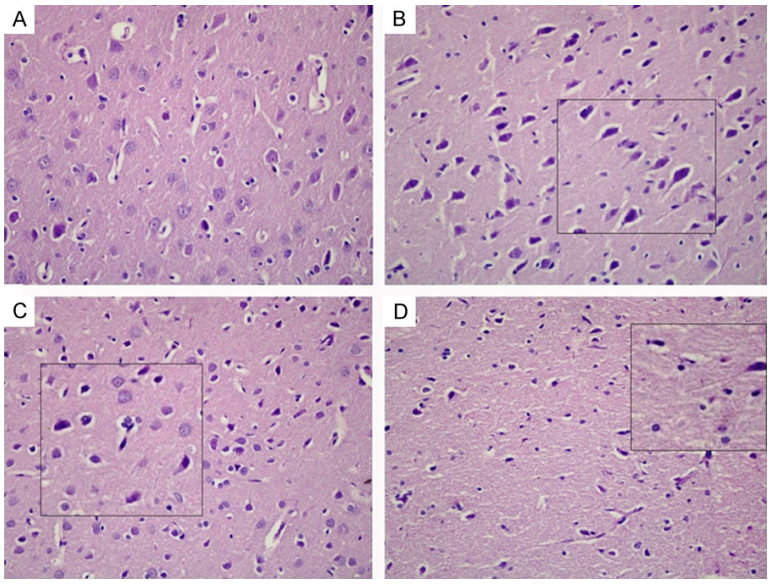

Figure 9. Red neuron for all groups in histopathological evaluation. Red neuron was not observed in Group S (A). In group T (B), red neuron was significantly increased following traumatic brain injury associated with hemorrhagic shock and there was significant different between the T (B) group and the treatment groups [Group NS (C) and Group HS (D)] (both, $P < 0.001$). In the treatment groups (C and D), red neuron was partially decreased and there was no significant difference in the red neurons between the NS (C) and HS (D) groups ($P > 0.05$) (hematoxylin and eosin, 400x).

150 mmol/l, 150 and 160 mmol/l in a few studies [43, 44], and higher than 160 mmol/l in one study [45]. Blood sodium levels and osmolarity were increased to a greater extent in trauma groups. In this study, blood sodium levels in the HS group were lower than those in the NS group. Although blood sodium levels and osmolarity were expected to be higher in animals treated with 7.5% NaCl, these parameters were not increased in this group. This observation may be interpreted as supportive of HS use in HSTBI.

The immunomodulatory effects of HS were demonstrated both in vivo and in vitro [42, 46, 47]. HS was shown to decrease the adherence of leukocytes by expression of the adhesion molecule L-selectin in mice [48], and it may also reduce the activation and migration of leukocytes [36]. The lower leukocyte accumulation, i.e., lower inflammation in the treated group than the untreated group in this study, supported the possible immunomodulatory or anti-inflammatory effects of HS. Although there were no significant differences in leukocyte accumulation between the NS and HS groups, the HS group showed less accumulation of leukocytes than the NS group. Red neurons were also fewer in the treated groups than in the untreated group. Although no significant distinctions with regard to the anti-inflammatory properties of HS were observed, the findings suggest that a sodium content of at least 0.9% in resuscitation fluid may alleviate inflammation in injured brain tissue.

Head injury may lead to an inappropriate increase in ADH level [49]. Blood ADH levels in the untreated group were higher than those in the treatment groups. The results of the present study indicated that HS was not superior with regard to reducing ADH, as compared to NS. This finding supported the result that the administration of NS or HS will reduce ADH levels by restoring plasma volume [50, 51]. In our study, blood osmolarity was lower in the treatment groups than the untreated group. There was no supporting evidence in the study about the superiority of HS to NS in reducing osmolarity. This observation suggests that blood osmolarity for ADH release may be preserved by NS and HS infusion.

High aldosterone values were reported previously in patients with acute head injury and increased intracranial pressure [25]. Therefore, aldosterone is a useful biochemical indicator of the severity of increased intracranial pressure and head injury. Although there was no significant difference between the NS and HS groups, blood aldosterone levels were higher in the untreated group than in the treatment groups in this study. 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 ADH.

Limitations of the study

There were some limitations in this study. First, the use of rat models for controlled HSTBI may not completely reflect the true clinical situation in humans. Second, the effects of delayed fluid resuscitation were not evaluated. HS was administered at an early stage after HSTBI

because the objective was to compare the effects of different fluids at an early period after injury. Further studies are required to evaluate the delayed effects of resuscitation fluids; in particular, the effects of HS on hemorrhaging should be analyzed in the late post-injury period. Third, the samples were collected from animals after 24 hours, and our data did not address long-term complications of fluids. Therefore, future experimental studies with longer periods of observation, to evaluate specimens for late effects of different fluids are required.

Conclusion

The results of the present study indicate that early fluid resuscitation with hypertonic or isotonic fluid in the treatmenst of HSTBI is associated with lower levels of inflammatory reactions, and normal biochemical variables, including osmolarity, sodium, aldosterone, and ADH levels, with the exception of brain water content. Treatment with HS results in a greater brain water content than NS. The lower degree of leukocyte accumulation may suggest decreased inflammation in fluid-resuscitated animals. However, the median scores for bleeding and edema were significantly higher in the HS group than in the NS group. Red neurons were observed in the T, HS, and NS groups. Although the difference was not significant biochemically, histopathological findings and brain water content were significantly more favorable with early administration of HS than NS in HSTBI. Therefore, NS can be used safely in the early stages of HSTBI in emergency settings.

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Disclosure of conflict of interest

None.

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References

[1] How can injuries be prevented? World Health Organization: How can injuries be prevented? 2006. http://www.who.int/features/qa/36/en/index.html. 2015. World Health Organization.

[2] Hatoum OA, Bashenko Y, Hirsh M, Krausz MM, Abu-Hatum O. Continuous fluid resuscitation for treatment of uncontrolled hemorrhagic shock following massive splenic injury in rats. Shock 2002; 18: 574-9.

[3] Dawidson I. Fluid resuscitation of shock: current controversies. Crit Care Med 1989; 17: 1078-1080.

[4] Holcroft J, Vassar M. 3% NaCl and 7.5% NaCl/dextran 70 in the resuscitation of severely injured patients. Ann Surg 1987; 206: 279-288.

[5] Velanovich V. Crystalloid vs. Colloid resuscitation: a meta-analysis of mortality. Surgery 1989; 105: 65-70.

[6] Doyle JA, Davis DP, Hoyt DB. The use of hypertonic saline in the treatment of traumatic brain injury. J Trauma 2001; 50: 367-383.

[7] Stern SA, Dronen SC, Birrer P, Wang X. Effect of blood pressure on hemorrhage volume and survival in a near-fatal hemorrhage model incorporating a vascular injury. Ann Emerg Med 1993; 22: 155-163.

[8] Sakles JC, Sena MJ, Knight DA, Davis JM. Effect of immediate fluid resuscitation on the rate, volume, and duration of pulmonary vascular hemorrhage in a sheep model of penetrating thoracic trauma. Ann Emerg Med 1997; 29: 392-399.

[9] Holmes JF, Sakles JC, Lewis G, Wisner DH. Effects of delaying fluid resuscitation on an injury to the systemic arterial vasculature. Acad Emerg Med 2002; 9: 267-74.

[10] Turner J, Nicholl J, Webber L, Cox H, Dixon S, Yates D. A randomised controlled trial of prehospital intravenous fluid replacement therapy in serious trauma. Health Technol Assess 2000; 4: 1-57.

[11] Kreimeier U, Messmer K. Small-volume resuscitation with hypertonic sodium colloid solution-what does clinical routine tell us? Wien Klin Wochenschr 2002; 114: 76-7.

[12] Kreimeier U, Messmer K. Small-volume resuscitation: from experimental evidence to clinical routine. Advantages and disadvantages of hypertonic solutions. Acta Anaesthesiol Scand 2002; 46: 625-38.

[13] Jeremitsky E, Omert L, Dunham CM, Protetch J, Rodriguez A. Harbingers of poor outcome the day after severe brain injury: hypothermia, hypoxia, and hypoperfusion. J Trauma 2003; 54: 312-319.
[14] Myburgh J, Cooper DJ, Finfer S, Bellomo R, Norton R, Bishop N, Kai Lo S, Vallance S. Saline or albumin for fluid resuscitation in patients with traumatic brain injury. N Engl J Med 2007; 357: 874-884.
[15] American College Of Surgeons Committee on trauma. advanced trauma life support (atls) student course manual, 9th edition, American College of Surgeons, Chicago: 2012. pp. 72-p75.
[16] Elliott MB, Jallo JJ, Barbe MF, Tuma RF. Hypertonic saline attenuates tissue loss and astrocyte hypertrophy in a model of traumatic brain injury. Brain Res 2009; 1305: 183-91.
[17] Hashiguchi N, Lum L, Romeril E, Chen Y, Yip L, Hoyt DB, Junger WG. Hypertonic saline resuscitation: efficacy may require early treatment in severely injured patients. J Trauma 2007; 62: 299-306.
[18] Rockswold GL, Solid CA, Paredes- Andrade E, Rockswold SB, Jancik JT, Quickel RR. Hypertonic saline and its effect on intracranial pressure, cerebral perfusion pressure, and brain tissue oxygen. Neurosurgery 2009; 65: 1035-41.
[19] Marklund N, Salci K, Lewen A, Hillered L. Glycerol as a marker for post-traumatic membrane phospholipid degradation in rat brain. Neuroreport 1997; 717: 109-117.
[20] Feeney DM, Boyeson MG, Linn RT, Murray HM, Dail WG. Responses to cortical injury: I. Methodology and local effects of contusions in the rat. Brain Res 1981; 211: 67-77.
[21] Riddez L, Drobin D, Sjostrand F, Svensen C, Hahn RG. Lower dose of hypertonic saline dextran reduces the risk of lethal rebleeding in uncontrolled hemorrhage. Shock 2002; 17: 377-82.
[22] Cabrales P, Nacharaju P, Manjula BN, Tsai AG, Acharya SA, Intaglietta M. Early difference in tissue pH and microvascular hemodynamics in hemorrhagic shock resuscitation using polyethylene glycol-albumin- and hydroxyethyl starch-based plasma expanders. Shock 2005; 24: 66-73.
[23] Lee CC, Chang IJ, Yen ZS, Hsu CY, Chen SY, Su CP, Chiang WC, Chen WJ. Effect of different resuscitation fluids on cytokine response in a rat model of hemorrhagic shock. Shock 2005; 24: 177-181.
[24] Okiyama K, Smith DH, Gennarelli TA, Simon RP, Leach M, McIntosh TK. The sodium channel blocker and glutamate release inhibitor BW1003C87 and magnesium attenuate regional cerebral edema following experimental brain injury in the rat. J Neurochem 1995; 64: 802-809.
[25] Takahashi H, Sato H, Tsuji Y. Biochemical markers in the acute stage of head injury--aldosterone and CK-BB. Neurol Med Chir (Tokyo) 1989; 29: 192-195.
[26] Kheirbek T, Pascual JL. Hypertonic saline for the treatment of intracranial hypertension. Curr Neurol Neurosci Rep 2014; 14: 482.
[27] Major EH, O'Connor P, Mullan B. Single bolus 30% hypertonic saline for refractory intracranial hypertension. Ir J Med Sci 2015; 184: 159-165.
[28] Mortazavi MM, Romeo AK, Deep A, Griessenauer CJ, Shoia MM, Tubbs RS, Fisher W. Hypertonic saline for treating raised intracranial pressure: literature review with meta-analysis. J Neurosurg 2012; 116: 210-221.
[29] Rocha-e-Silva M, Poli-de-Figueiredo LF. Small volume hypertonic resuscitation of circulatory shock. Clinics 2005; 60: 159-172.
[30] Shackford S, Norton C, Todd M. Renal, cerebral and pulmonary effects of hypertonic resuscitation in a porcine model of hemorrhagic shock. Surgery 1988; 104: 553-560.
[31] Qureshi AI, Suarez JI, Bhardwaj A, Mirski M, Schnitzer MS, Hanley DF, Ulatowski JA. Use of hypertonic (3%) saline/acetate infusion in the treatment of cerebral edema: effect on intracranial pressure and lateral displacement of the brain. Crit Care Med 1998; 26: 440-446.
[32] Gunnar W, Jonasson O, Merlotti G, Stone J, Barret J. Head injury and hemorrhagic shock: studies of the blood brain barrier and intracranial pressure after resuscitation with normal saline solution, 3% saline solution, and dextran-40. Surgery 1988; 103: 398-407.
[33] Anderson JT, Wisner DH, Sullivan PE, Matteucci M, Freshman S, Hildreth J, Wagner FC Jr. Initial small-volume hypertonic resuscitation of shock and brain injury short- and long-term effects. J Trauma 1997; 42: 592-600.
[34] Battistella FD, Wisner DH. Combined hemorrhagic shock and head injury: effects of hypertonic saline (7.5%) resuscitation. J Trauma 1991; 31: 182-188.
[35] Walsh JC, Zhuang J, Shackford SR. A comparison of hypertonic to isotonic fluid in the resuscitation of brain injury and hemorrhagic shock. J Surg Res 1991; 50: 284-292.
[36] Elliott MB, Jallo JJ, Gaughan JP, Tuma RF. Effects of crystalloid-colloid solutions on traumatic brain injury. J Neurotrauma 2007; 24: 195-202.
[37] Başkaya MK, Doğan A, Temiz C, Dempsey RJ. Application of 2,3,5-triphenyltetrazolium chloride staining to evaluate injury volume after

controlled cortical impact brain injury: role of brain edema in evolution of injury volume. J Neurotrauma 2000; 17: 93-99.
[38] McManus ML, Soriano SG. Rebound swelling of astroglial cells exposed to hypertonic mannitol. Anesthesiology 1998; 88: 1589-1591.
[39] Baldwin SA, Fugaccia I, Brown DR, Brown LV, Scheff SW. Blood-brain barrier breach following cortical contusion in the rat. J Surg Res 1996; 84: 476-481.
[40] Whalen MJ, Carlos TM, Kochanek PM, Clark RS, Heineman S, Schiding JK, Franicola D, Memarzadeh F, Lo W, Marion DW, Dekosky ST. Neutrophils do not mediate blood-brain barrier permeability early after controlled cortical impact in rats. J Neurotrauma 1999; 16: 583-594.
[41] Froelich M, Ni Q, Wess C, Ougorets I, Härtl R. Continuous hypertonic saline therapy and the occurrence of complications in neurocritically ill patients. Crit Care Med 2009; 37: 1433-1441.
[42] Pascual JL, Ferri LE, Seely AJ, Campisi G, Chaudhury P, Giannias B, Evans CD, Razek T, Michel RP, Christou NV. Hypertonic saline resuscitation of hemorrhagic shock diminishes neutrophil rolling and adherence to endothelium and reduces in vivo vascular leakage. Ann Surg 2002; 236: 634-642.
[43] Al-Rawi PG, Tseng MY, Richards HK, Nortie J, Timofeey I, Matta BF, Hutchinson PJ, Kirkpatrick PJ. Hypertonic saline in patients with poor-grade subarachnoid hemorrhage improves cerebral blood flow, brain tissue oxygen, and pH. Stroke 2010; 41: 122-128.
[44] Battison C, Andrews PJ, Graham C, Petty T. Randomized controlled trial on the effect of a 20% mannitol solution and a 7.5% saline/6% dextran solution on increased intracranial pressure after brain injury. Crit Care Med 2005; 33: 196-202.
[45] Rockswold GL, Solid CA, Paredes-Andrade E, Rockswold SB, Jancik JT, Quickel RR. Hypertonic saline and its effect on intracranial pressure, cerebral perfusion pressure, and brain. Neurosurgery 2009; 65: 1035-1042.
[46] Junger WG, Hoyt DB, Davis RE, Herdon-Remelius C, Namiki S, Junger H, Loomis W, Altman A. Hypertonicity regulates the function of human neutrophils by modulating chemoattractant receptor signaling and activating mitogen-activated protein kinase p38. J Clin Invest 1998; 101: 2768-2779.
[47] Rotstein OD. Novel strategies for immunomodulation after trauma: revisiting hypertonic saline as a resuscitation strategy for hemorrhagic shock. J Trauma 2000; 49: 580-583.
[48] Angle N, Hoyt DB, Cabello-Passini R, Herdon-Remelius C, Loomis W, Junger WG. Hypertonic saline resuscitation reduces neutrophil margination by suppressing neutrophil L selectin expression. J Trauma 1998; 45: 7-12.
[49] Moses AM, Streeten DHP. Disorders of the neurohypophysis. In: Wilson JD, Braunwald E, Isselbacher KJ, editors. Harrison's principles of internal medicine. New York: McGraw-Hill Inc; 1998. pp. 2003.
[50] Rocha e Silva M, Velasco IT, Nogueira da Silva RI, Oliveira MA, Negraes GA, Oliveira MA. Hyperosmotic sodium salts reverse hemorrhagic shock: other solutes do not. Am J Physiol 1987; 253: H751-762.
[51] Velasco IT, Pontieri V, Rocha e Silva M Jr, Lopes OU. Hyperosmotic NaCl and severe hemo rrhagic shock. Am J Physiol 1980; 239: 664-673.