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REVIEW

Hypertonic saline for treatment of shock: have we looked for everything?

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This review is dedicated to the memory of Luis Poli de Figueiredo, who is responsible for much of what we know about small volume resuscitation. Small volume hypertonic resuscitation was originally based on the concept that a physiologically significant blood volume expansion follows the administration of a comparatively small volume of hypertonic fluid. Because hypertonicity is a physiological vasodilator, its use after severe blood loss induces reperfusion. Previously reported research disclosed a number of additional properties of hypertonic NaCl, amongst them (i) the correction of endothelial and red cell edema with significant consequences in terms of capillary blood flow; (ii) modulation of the immune system, with applications repercussions in the field of sepsis. This review covers developments in this field that occurred from 2005 through 2013. We discuss the discrepancy between animal experiments and human trials, and argue that the basic objective of most multicenter clinical trials may have been based upon a poor strategy.

KEYWORDS: Shock; Hemorrhage; Sepsis Hypertonic saline; Immune circulation.

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INTRODUCTION

This review is dedicated to the memory of Luis Poli de Figueiredo, who is responsible for much of what we know about small volume resuscitation. Hypertonic salt solutions (osmolality > 300 mOsm/L) have been viewed with interest since 1919, when Penfield reported that a small volume of 1.8% NaCl (600 mOsm/L) induced a transient recovery of the hypotension caused by blood loss in dogs.[1] Fifty years later, Baue et al. and Messmer et al. reported equally transient effects of 5% NaCl in severely hypotensive animals.[2,3] Gazitua et al. from Arthur Baue's laboratory, showed that these same formulations induced vasodilation when selectively infused into the renal, coronary, and limb circulations.[4] Enhanced interest in the field was induced when Velasco et al[5] demonstrated that 4 ml/kg of a 7.5% NaCl solution induced a highly significant hemodynamic recovery and a high percentage of long term survival compared to no treatment. By the end of 2013, this paper had been cited 433 times in journals in the Thomson Reuters Journal of Citation Reports. Over the past 20 years, i.e., 14 to 34 years after publication, it collected 253 citations (12.5 per year) and apparently counting. Second generation citations total 12,444.[6]

A preliminary human study by de Felippe et al showed that 7.5% NaCl solutions given in successive 50 ml aliquots to patients in hemorrhagic shock induced beneficial hemodynamic effects.[7] Soon afterwards, in the laboratory of George Kramer, it was shown that the association of dextran to hypertonic saline combined the hyperosmotic effects of NaCl to the hyperoncotic effects of dextran and added new conceptual and practical possibilities.[8,9] This review is a follow-up of our previously published coverage of the subject and endeavors to follow developments over the past eight years.[10,11]

METHOD

This review is based upon a systematic search performed in the Medline databases using the following search parameters: ((hypertonic saline) AND (NaCl)) AND ((shock) OR (hemorrhage) OR (sepsis) OR (Traumatic brain injury)). A publication date [2005-2013] was added as a filter. The search brought up 114 articles. They were then manually short listed to 59 based on pertinence. Thirteen references relating to the previous history of the topic were added. This study is designed as a sequel to a previously published review on the same topic.[11]

PHYSICAL PROPERTIES OF HS AND HSD

The initial effect of hypertonic saline is significant plasma volume expansion. Performing a very simple set of experiments, it was possible to work out the theoretical distribution of space evolving through the first minutes following its intravenous administration.[11] Given that 4 mL of 7.5% NaCl contains 5.12 mEq. Na^+, its simple dilution into 40 ml of water produces a concentration of 128 mEq. Na^+/L. In theory, if added instantaneously to the plasma volume of a normovolemic dog (approximately equal to 40 mL/kg), this Na^+ load should increase plasma Na^+ to

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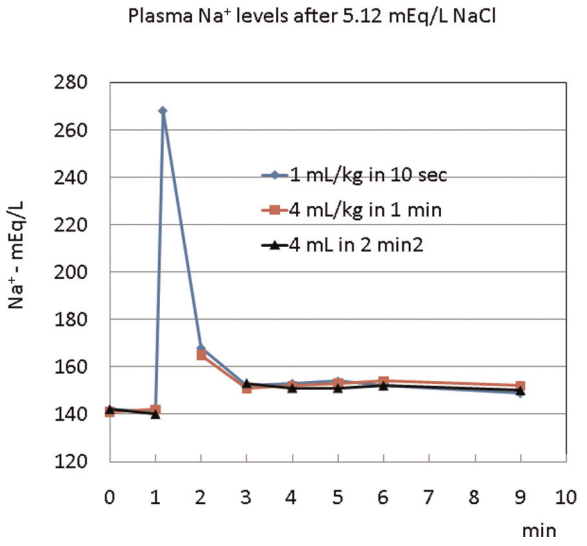

Figure 1 - Plasma concentrations of Na^+ (mEq/L) after three different administrations of hypertonic saline (Adapted from Rocha e Silva & Figueiredo, 2005, by permission of CLINICS).

approximately 268 mEq/L at time zero. (128 mEq/L above the basal level of 140 mEq/L). Figure 1 shows that this is achieved if the salt load is administered intravenously over 10 seconds, and plasma Na^+ read immediately thereafter.[11] After 1 min, Na^+ concentration is reduced to 165 mEq/L. This value is practically identical to that observed at the end of a slower, 1 min infusion of the same load. The 25 mEq/L Na^+ increase over the normal level of 140 mEq/L is the correct theoretical value for the dilution of 5.12 mEq/kg across the entire 200 ml/kg of extracellular space (5.12 mEq/0.2L = 25.6 mEq/L). At the end of the 2nd minute Na^+ concentration is down to 152 mEq/L regardless of whether the load is delivered in 10 sec, or 1 or 2 min. This concentration remains essentially constant over the following 8 minutes. In summary, these three decay curves of Na^+ indicate that the theoretical distribution space is the intravascular compartment at zero time, the extracellular space at 1 min. and the resulting volume expansion after 2-10 minutes. In the normovolemic dog, the plasma expansion is 8% up to 216 ml/kg. After severe blood loss, extracellular expansion would theoretically be greater. The original Velasco study[5] suggests an initial volume expansion of 11 ml/kg, a 40% increase over post-hemorrhage levels. The expansion of the extravascular compartment can only be accounted for by assuming that water has been osmotically drawn out of the intracellular compartment by hypertonic saline. This is a consequence of the fact that the endothelial barrier is highly permeable to both Na^+ and Cl^-, whereas the cell membrane is virtually impermeable to the cation.

Thus, the 4 ml/kg infusion of 7.5% NaCl expands plasma volume by 11 ml/kg, or 2.75 mL of plasma for each mL of the injected solution. In contrast, standard isotonic solutions only induce an expansion of 0.33 mL for each mL injected, as a consequence of its isotonic distribution throughout the extravascular compartment.

The association of 6% dextran-70 with a 7.5% NaCl solution (HSD) introduced by Kramer et al.[8,9] enhances the initial plasma expansion. However, it's most important effect is the maintenance of intravascular expansion for longer periods, thus prolonging the hemodynamic benefit of hypertonic solutions. The addition of HSD alters the dynamics of fluid shift by generating an oncotic gradient across the endothelial barrier. This further expands the intravascular compartment, but does it at the expense of removing fluid from the interstitial space. The 6% Dextran-70 solution exerts an oncotic pressure of 70 mm Hg, greater than that observed with a similar concentration of human albumin. As might be expected, no significant data have been added to these basic physical concepts after 2005.

PHYSIOLOGICAL PROPERTIES OF HS/HSD

Cardiovascular system

Work reported before 2006 and reviewed in our previous publication[11] showed that hypertonic resuscitation induced cardiovascular improvement consisting of a partial recovery of mean arterial pressure and cardiac output. This was variously attributed to plasma volume expansion, vasodilation in several vascular beds, and to a direct cardiac inotropic effect. Vasodilation of the renal and mesenteric territories was also reported. An early development suggested that the central nervous system was important in the development of the cardiovascular response to hypertonic saline,[12] but this concept has been revised. Two papers from the laboratory of Sergio Cravo indicate (i) that the effect of hypertonic saline in the restoration of cardiovascular function is attenuated by denervation of the carotid sinus, but not of the aortic arch;[13] and (ii) that this response is virtually suppressed by selectively knocking out the carotid chemoreceptor bodies.[14]

Hypertonic saline has been reported to act directly or indirectly on cardiac function. Frithiof et al.[15] showed that increased Na^+ concentration at the Paraventricular Nucleus combined with its effects on the cerebral angiotensin receptor AT(1) contribute to induce a positive inotropic effect, thus enhancing cardiac performance. Letson et al[16] reported an interesting difference between the effects of simple hypertonic saline (HS), on one hand, and the same combined with either dextran (HSD) or hetastarch (HSHES). In a rat model with 40% blood loss, none of the treatments induced arrhythmias or led to mortality. Simple HS suppressed arrhythmias and mortality; HSD had no effect on arrhythmias but suppressed mortality; HSHES had no effect on arrhythmias or mortality

Microcirculation was the object of a number of reports. In a pig model of severe hemorrhage, an evaluation of hypertonic saline-hespan, hespan alone and Gelatin by Maier et al[17] revealed restoration of baseline parameters in all three groups, with significantly higher filling pressures and cardiac output after the isotonic colloids. Sublingual microcirculation blood flow and flow quality were also equivalent with the three treatments, but again, only the isotonic colloid improved microvascular hemoglobin oxygen content. The use of polymerized hemoglobin to enhance O_2 carrying capacity as an adjunct to small volume resuscitation has been revisited by Cabrales et al[18] in a hamster dorsal window chamber model. Severe hemorrhagic shock was resuscitated by hypertonic saline plus polymerized bovine hemoglobin, 4 or 23 g/dL. Interestingly, only the smaller dose restored perfusion, functional capillary density, and tissue PO_2. In a sequel study using the same model, Palmer et al[19] claimed that hypertonic saline resuscitation followed by the administration of high molecular weight tense-state polymerized human or bovine hemoglobin. Human

hemoglobin induced vasoconstriction and decreased perfusion when compared to bovine hemoglobin or albumin. Bovine hemoglobin produced a pronounced recovery in blood gas parameters and tissue PO_2 vs. albumin or human hemoglobin. The authors of this study suggested that appropriately engineered polymerized hemoglobin may enhance/reinstate oxygenation, without hypertension or vasoconstriction, and could be useful in situations where blood transfusion is not logistically feasible. Gong et al[20] examined the microcirculation of the piamater and found that hypertonic saline does not inhibit neutrophil – endothelial interactions induced by severe blood loss. This is an interesting observation in that it sharply contrasts with many reports indicating that this inhibition effectively occurs in the systemic circulation. Apparently the piamater has another specific property not shared by other blood – tissue barriers.

The immune system

A series of papers added to previously established facts about the interaction of hypertonicity with the immune system. For convenience, this discussion will separate its effects on lymphocytes and polymorphonuclear neutrophils.

Lymphocyte Function

In vitro hypertonic saline interferes beneficially with lymphocyte function after severe blood loss. It has been shown to restore T cell function. Two mechanisms have been proposed. (a) The Migratory Inhibitory Factor (MIF) has been reported by Yoon et al[21] to be depressed in response to hemorrhagic shock, but restored by hypertonic saline treatment. (b) The release of lymphocyte cell ATP is induced by hypertonicity, and, thus, restores its function. This effect appears to be mediated through pannexin-1 hemichannels and P2X receptors, as reported by Woerhle et al.[22] This same report suggests that hypertonic saline could be used to improve T cell function in trauma patients. These concepts have been confirmed in vivo by Lu et al[23] in a rat model of severe hemorrhagic shock, where resuscitation with hypertonic saline attenuated the rise of CD4 T lymphocytes, and CD4/CD8 induced by the previous blood loss. In a separate report, the same protocol showed that hemorrhage induced intense apoptosis in the intestinal mucosa; and that this is partially reduced by hypertonic saline resuscitation, but not by resuscitation with normal saline.[24]

Neutrophil function

It has been previously established that neutrophil function is enhanced by hemorrhage leading to organ and tissue damage, but attenuated by hypertonic saline resuscitation. Lee et al[25] have shown that in shock, neutrophils are primed for an increased production of superoxide, which is implicated in acute lung injury. Neutrophils were isolated from healthy donors, primed with arachidonic acid and incubated with hypertonic saline. The well established activation of neutrophils by arachidonic acid was inhibited. Neutrophil degranulation and proinflammatory mediator synthesis, also implicated in pulmonary inflammation, is likewise enhanced by hemorrhage as shown by Deree et al[26] in a murine model of severe hemorrhagic shock. Resuscitation with a combination of hypertonic saline and pentoxifylline drastically reduced this response; whereas, lactated Ringer's was ineffective. As described above, hypertonic saline effectively decreases the interaction of neutrophils with endothelial cells in the systemic circulation, but not in the blood brain microcirculation.[20] In spite of an inhibitory effect on neutrophil function, Bahrami et al[27] report that in a murine model of very severe hemorrhagic shock, with arterial blood pressure reduced to and kept at 30-35 mm Hg for two hours, no reduction in mortality was observed following hypertonic saline, or hypertonic saline plus hydroxyl ethyl starch. Theobaldo et al,[28] employed a mouse model of cecal ligation and puncture and showed that hypertonic saline drives neutrophils from bystander organs to an infectious site in polymicrobial sepsis: a cecal ligation and puncture model.

Other immune effects

In a rat model of severe hemorrhage over-resuscitated with either 81 ml/kg isotonic or 9.7 ml/kg hypertonic saline, H. Chen et al found that toll-like receptors were activated by hypertonic resuscitation.[29] More recently, L.W. Chen et al[30] report that in rats submitted to burns and resuscitated as described above,[29] hypertonic saline enhances host defenses and reduces apoptosis by increasing the level of toll-like receptors. Fernandes et al[31] reported that hypertonic saline solution increases the expression of heat shock protein 70 and improves lung inflammation early after reperfusion in a rodent model of controlled hemorrhage.

The Central Nervous System

The importance of the central nervous system in the resuscitative properties of systemically infused hypertonic saline had been suggested soon after the Velasco paper;[12] however, during the period under study, this observation was once again revised this time by Frithiof et al[32] Using a sheep model of hemorrhage, with or without anesthesia, they report that an intact autonomic cardiovascular control system is crucial for the effect of intracerebralventricular hypertonic saline and indicate that intravenous hypertonic saline exerts some of its action through the central nervous system. Xiao et al[33] used 7.5% NaCl (alone or in combination with hydroxyethyl starch-40) to treat a two hit condition (acute intracranial hypertension plus hemorrhage). The hypertonic starch combination exhibited the fastest mean arterial pressure recovery. Cerebral perfusion pressure was increased after resuscitation with both solutions, but intracranial pressure was decreased only by the simple hypertonic saline solution. Other reports used the a 3% NaCl solution to treat subdural hematoma,[34] traumatic brain injury,[35,36] or subdural hematoma[37] with beneficial results. Balbino et al,[38] in a canine model of traumatic brain injury associated with hemorrhage, report hypertonic saline prevents intraneural calcium influx. Noppens et al[39] employed a rat model of global cerebral ischemia. Treatment with a single bolus of 7.5% NaCl + 6% hydroxyethyl starch resulted in an important restoration of regional cerebral blood flow, reduced post-ischemic mortality, and ameliorated neurologic symptoms 10 days after insult, Nout et al[40] employed repeated injections of 5% NaCl to successfully attenuate cord swelling and edema in experimental spinal cord injury animal model.

ANIMAL CLINICAL TRIAL MODELING

In this section, an animal clinical trial modeling is defined as any trial performed in a live animal model, in which a

Table 1 - Summary of animal clinical trials for hypertonic saline

Author (ref)	Species	Test Agent	CTR	Target
Superiority				
Coimbra[43]	rat	HS* + PTX‡	NS†	Immune function
Constantini[44]	rat	HS + PTX	LR§	Immune function
Cruz[45]	rat	HS + PTX	NS	Immune function
Lu[23]	rat	HS	NS	Immune function
Lu[46]	rat	HS	NS	Apoptosis; Organ damage
Hoppen[47]	rat	HS	NS	Liver function
Sharma[48]	rat	HS	LR	renal function
Equivalence				
Younes[49]	rat	HS	NS	Survival
Nascimento[50]	dog	HS	LR	renal function
Watters[51]	swine	HS	NS	renal function
Legrand[52]	rat	HS	NS	renal function
Garcia-Martinez[53]	dog	HSD + naloxone	blood	Hemodynamics

*: Hypertonic Saline; ‡: Pentoxiphyline; †: Normal saline; §: Lactated Ringer's.

realistic human clinical condition is simulated. I will restrict myself to discussing experiments in which a reasonable approximation to standard of care was used as a control, with two exceptions, both of which are included as Sepsis trials.[41,42] This discussion shall be divided into two sections, namely **hemorrhage** and **sepsis** trials.

Hemorrhage trials

We shall subdivide reports on these hemorrhage trials into groups showing (a) superiority or (b) equivalence of small volume resuscitation vs. conventional isotonic treatment. Table 1 summarizes the distribution.

Superiority

In a simple hemorrhage/resuscitation model, Coimbra et al[43] report that a combination of small volume hypertonic saline combined with pentoxifylline, compared with conventional lactated Ringers resuscitation, decreases organ injury. They conclude that this strategy with marked immunomodulatory potential may be an attractive alternative to conventional hemorrhagic shock resuscitation. They later showed that the hypertonic saline-pentoxifylline (vs. conventional lactated Ringers resuscitation) modulates pulmonary transcription factor activation and reduces lung injury.[44] An equivalent conclusion, indicating superiority of the hypertonic saline-pentoxifylline strategy, was reached by Cruz et al,[45] regarding cardiovascular performance and gastric mucosal oxygenation. As previously noted, Lu et al[23] examined the effects of small volume resuscitation and report that it showed itself to be more effective than normal saline in reducing immunologic disorders and promoting a more balanced profile of T-lymphocyte subpopulations regulating networks. More recently, they reported on the effects of standard isotonic saline vs. small volume hypertonic resuscitation on the expression of heme oxygenase-1 mRNA and apoptosis in various organs of rats submitted to hemorrhagic shock. They found a significant up-regulation of heme oxygenase-1 in the small intestinal mucosa, liver and kidney following small volume vs. normal saline resuscitation, and conclude that this may be one of the mechanisms alleviating organ damage.[46] Liver function and integrity was the object of a report by Hoppen et al:[47] in a rat model of hemorrhage and resuscitation, hypertonic saline restored bile secretion and liver integrity better than conventional lactated Ringer's up to 6 hours after resuscitation. Sharma et al[48] report that rats submitted to a hemorrhagic shock procedure with 100% lethality if untreated, were effectively resuscitated by normal saline, lactated Ringers solution, or small volume. However, they claim that hypertonic saline was more effective in preventing kidney damage than isotonic fluid administration. This contrast with equivalence reports on renal function, as described below.

Equivalence

Younes et al[49] performed a very extensive study with a rat model of hemorrhage, including 180 animals. Standard of care isotonic saline was compared to hypertonic saline and total blood retransfusion in a hemodilution model: they report that hypertonic saline is an effective and safe initial therapy for hemodiluted rats undergoing hemorrhagic shock, with an overall outcome comparable to blood replacement or high volume isotonic saline administration. Nascimento et al[50] submitted anesthetized dogs to a 40% blood volume hemorrhage and treated them with lactated Ringers, hypertonic saline, hypertonic saline-dextran or hydroxyethyl starch. In spite of initial differences in hemodynamic responses, all treatments were equally effective in restoring renal performance 120 min after treatment. A similar equivalence of response with regard to systemic hemodynamics and renal function was described for a swine model by Watters et al.[51] In a rat model, Legrand et al[52] also reported an equivalence of response between isotonic vs. hypertonic saline vs. blood retransfusion: none of the regimens restored renal O_2 delivery or PO_2. In a canine model of severe hemorrhage, Garcia-Martinez[53] reported that the addition of naloxone to small volume hypertonic-hyperoncotic resuscitation (vs. retransfusion of shed blood) does not alter the vascular response or the acid-base equilibrium.

In a diagnostic-oriented protocol, Reynolds et al[54] evaluated lactate profiles as a resuscitation assessment tool in a rat model of battlefield hemorrhage resuscitation A comparison was made between 7.5% hypertonic saline and 6% hetastarch for low volume resuscitation. They conclude that survivors exhibited a decline in lactate levels following resuscitation.

Sepsis trials

Reports on animal sepsis trials will likewise be divided into a group showing (a) superiority or (b) equivalence of small volume resuscitation vs conventional treatment. A total of nine such reports were found, eight of which indicate superiority in the use of small volume vs. conventional treatment with respect to surrogate endpoints. It must be noted that in only one of these studies was survival shown to be improved. A single report showed equivalence. Table 2 summarizes these findings.

Superiority

In a rat model of cecal ligation puncture, Shih et al[55] reported that hypertonic saline prevented circulatory failure and alleviated multiple organ dysfunction syndromewhen compared to. conventional Lactated Ringer's treatment. These effects were attributed to decreased lung neutrophil

Table 2 - Summary of animal trials for hypertonic saline in sepsis

Author (ref)	Species	Test Agent	CTR	Model/Target
Superiority				
Shih[55]	rat	HS	LR	CLP/immune response
Shih[56]	rat	HS	LR	CLP/immune/survival
Yu[41]	rat	HS or HSH	-	Endotoxin/lung injury
Somell[57]	swine	HS + tezosentan	NS	Endotoxin/Cardiac function
Kim[58]	rat	HS + PTX	LR	CLP/immune/lung injury
Liu[42]	rat	HS	-	CLP/survival/lung injury
Gao[59]	rat	HS or HSS	LR	Hemorrhage + sepsis/lung
Luiz Zanoni[60]	rat	HS	LR	Intestinal obstruction/ immune & gut damage
Equivalence				
Wan[61]	sheep	HS	NS	Gram-negative sepsis/ renal function

Table 3 - Summary of human clinical trials for hypertonic saline

Author (ref)	patients	Test Agent	CTR	Model/Target
Superiority				
Rizoli[62]	27	HSD	NS	Hemorrhage/immune response
Junger[63]	34	HS/HSD	NS	Hemorrhage/immune response
Bulger[64]	62	HSD	NS	Hemorrhage/immune response
Yildizdas[65]	67	HS	mannitol	pediatric cerebral edema/intracranial pressure
Schroth[66]	50	HSH	NS	pediatric open heart surgery
Equivalence				
Bulger[67]	209	HSD	LR	Blunt trauma & hypotension/ARDS-free at 28 days
Bulger[68]	1331	HS/HSD	NS	Head Injury/Brain deficit at 6 months
Bulger[69]	853	HS/HSD	NS	Hemorrhage/Survival at 28 days
Fang[70]	94	HS/HCO_3	NS	Sepsis & Hypotension/ Survival

infiltration and liver necrosis. Mortality rate was not improved. However, in a sequel report, the same authors report that a two-dose hypertonic saline administration (3 and 9 hr after cecal ligation) significantly reduced mortality and improved hyponatremia, hypocalcemia, metabolic acidosis, and electrolyte imbalance.[56] In a rat model of endotoxin administration, Yu et al[41] report that small volume resuscitation with hypertonic saline or hypertonic sodium chloride + hydroxyethyl starch 40 reduced lung damage when compared to isotonic saline. In a porcine model of endotoxemic sepsis, Somell et al[57] claim that the combination of tezosentan and hypertonic saline-dextran improved cardiac index and arterial oxygenation. In a two hit model (hemorrhage + cecal ligation puncture), Kim et al[58] showed that the combination of hypertonic saline and pentoxifylline when compared to lactated Ringers reduced lung injury score and neutrophil count. The combination of hypertonic saline with valproic acid worked synergistically to attenuate inflammation and improve survival. In this report, by Liu et al,[42] no control standard of care treatment was used. In a hemorrhage + lipopolysaccharide model, Gao et al[59] report that hypertonic saline alone, and especially when combined with hydroxyethylstarch, reduces lung tissue damage and pulmonary edema. In rats subjected to intestinal obstruction and ischemia, lactated Ringer's or hypertonic saline was used by Luiz Zanoni et al[60] as initial treatment, followed by resection of necrotized small bowel. Treatment with hypertonic saline reduced leukocyte-endothelial interactions and expression of neutrophil chemoattractants 1 and 2 to values attained in sham rats. Hypertonic saline-treated rats exhibited significant reductions in bacterial translocation, bacteremia, and local intestinal damage when compared to lactated Ringer's.

Equivalence

In a single report, by Wan et al,[61] equivalence between small volume and conventional resuscitation was reported. In a gram-negative sepsis sheep model, bolus resuscitation with hypertonic saline or normal saline were reported to induce similar and transient systemic and regional hemodynamic effect, No effects on renal perfusion, and only short-lived effects on renal function were reported.

In summary, it can be argued that hypertonic saline is an effective pharmacological agent. Under conditions of hemorrhage or sepsis in tightly controlled animal experiments, the described pharmacological actions of hypertonic saline appear to be mainly beneficial as treatment of shock.

CLINICAL TRIALS

In clinical trials, it is impossible to attain the tight homogeneity normally present in animal experiments. Also, in contrast to animal experiments, human clinical trials necessarily aim for survival as a primary endpoint. All of the reports to be discussed below targeted superiority of small volume resuscitation as compared to standard of care as the trial objective. It a well known fact that none of the standard of care procedures used today have ever been put to the formal test of efficacy or safety, but the extensive medical experience attached to their use warrants the view that they are generally effective and usually safe. Over the period covered by this review, we found nine reports in which small volume resuscitation was compared to standard of care in double blinded prospective trials. Five of them report superiority of small volume resuscitation over standard of care in surrogate or secondary objectives, but none showed survival superiority. In terms of survival, all trials pointed toward equivalence, even though they did not have sufficient statistical power to warrant the conclusion. Table 3 summarizes the main points of these reports.

Superiority

Rizoli et al[62] compared hypertonic saline dextran to normal saline in a study including 27 traumatic hemorrhage patients. They found no differences in clinical measurements, but report that small volume resuscitation blunted neutrophil activation by abolishing shock-induced CD11b up-regulation. Consequently, it altered the shock-induced monocyte redistribution pattern by reducing the drop in ''classic'' CD14 and the expansion of the

''pro-inflammatory'' CD14/CD16 subsets. In parallel, HSD significantly reduced pro-inflammatory tumor necrosis factor (TNF)-alpha production while increasing anti-inflammatory IL-1ra and IL-10. HSD prevented shock-induced norepinephrine surge with no effect on adrenal steroids. More recently, the same group, headed by Junger et al,[63] studied a different group of traumatic hemorrhage patients and reported that initial resuscitation with hypertonic saline, but not normal saline or hypertonic saline dextran, attenuate posttraumatic neutrophil and endothelial cell activation. A report by Bulger et al,[64] on 62 patients with traumatic hemorrhage, claims that CD11b expression, 12 hours after injury, was increased 1.5-fold in patients resuscitated with lactated Ringer's compared with controls. In contrast, those resuscitated with hypertonic saline dextran had a significant reduction in CD11b expression compared with Ringer's.

Two studies were reported on pediatric patients. Yildizdas et al[65] claim that hypertonic saline is superior to mannitol in reducing cerebral edema and increasing cerebral blood blow. Schroth et al[66] reported on the use of hypertonic saline dextran in the immediate post-operative phase of pediatric open heart cardiac surgery and concluded that it might be a useful and safe treatment and the amelioration of contractility, inotropy, and the possible treatment of early-onset capillary leakage.

Equivalence

Four studies report equivalence. A prospective double blind prospective trial was conducted by Bulger et al[67] with 209 blunt trauma hypotensive patients. No significant difference in ARDS-free survival at 28 days was demonstrated overall, but the use of hypertonic saline dextran may have offered maximum benefit in patients at highest risk for ARDS. A recent large scale multicenter trial produced two reports. (a) In a population of 1,282 traumatic brain injury patients with no hemorrhage, Bulger et al[68] reported that a three arm study (with a planned enrollment of 2,122 patients) was interrupted for futility. The conclusion was that initial resuscitation with either hypertonic saline or hypertonic saline dextran, compared with normal saline, did not result in superior 6-month neurologic outcome or survival. (b) In a population of 853 traumatic hypovolemic shock patients, a similarly planned study by Bulger et al,[69] was also interrupted very early (after 23% of the planned population had been included) for futility, but also because of a potential safety concern. There was no difference in 28-day survival between the three arms, but a higher mortality for the post-randomization subgroup of patients who did not receive blood transfusions in the first 24 hours. They received instead hypertonic fluids as compared to normal saline. Fang et al[70] investigated the effects of normal saline, hypertonic saline, and hypertonic bicarbonate on 94 severe sepsis patients with hypotension. No differences were detected among the three groups in cardiopulmonary parameters during the 120 min trial or the 8 hour follow-up, or in the observed mortality rate after 28 days. Authors conclude that all three crystalloid solutions may be used for initial volume loading in severe sepsis, but that sodium bicarbonate confers a limited benefit in humans with severe sepsis.

Some comments on the trials resulting in equivalence are warranted.

1. In a feasibility study for a pre-hospital hypertonic resuscitation-head injury and multi organ dysfunction, Morrison et al[71] noted that although previous clinical trials suggest no survival superiority of small volume over normal saline, subgroup analyses suggest there may be a reduction in the inflammatory response and multi-organ failure which may lead to better survival and enhanced neurocognitive function with small volume resuscitation.
2. In a recently published review, Dubick et al[72] note that in spite of a huge body of literature showing that HSD and HS improve hemodynamic and metabolic responses, modulate immune function, and reduce brain edema, translating these results into improved survival in clinical trials in hemorrhagic shock and traumatic brain injury has been difficult. This is not altogether surprising because clinical trials have several limitations, such as being too underpowered to show improved survival, or not having been focused on the trauma population most likely to benefit. When compared to animal experiments, patients may have received significantly less than 4 ml/kg; the total volume of coded solution, 250 ml/kg, corresponds to giving 4 ml/kg to a person with a body weight of 62.5 kg, which is far less than the average weight of the predominantly male young adult population included in the trials.
3. This reviewer will add a third point, namely that in all previously conducted clinical trials, a poor strategy regarding objective may have been systematically embarked upon: all trials focused on a quest for superiority of small volume vs. standard of care resuscitation. However, the demonstration of medical ***equivalence*** would have brought out a truly significant logistical advantage, especially in military and mass civilian injury scenarios: the volume of pre-hospital administration employing small volume would be reduced by nearly 90%; the simplicity of ministration would likewise be enhanced. Accessory advantages might include the facts that simple hypertonic saline is sterile per se and only freezes below -4°C. Not surprisingly, the single segment of the population receiving initial small volume resuscitation is to found in military scenarios, where civilian restrictions may be overlooked and where the logistic advantages would be maximal.
4. Another point that should be made concerns the concept of diluting NaCl to 3% or 5% using a larger infused volume. This would partly defeat the main logistic advantage and might not trigger the beneficial immune responses described for 7.5% NaCl.

In conclusion, it is the contention of one of its discoverers, that proving equivalence is the most viable future for the use of simple 7.5% NaCl as a clinically useful tool, even if this has had to wait for more than 30 years after its discovery.[5]

RESUMO

O procedimento de ressuscitação hipertônica a pequeno volume foi originalmente baseado no conceito de que uma expansão fisiologicamente significativa de volume sanguíneo resulta da administração de um pequeno volume de fluido hipertônico. A hipertonia é reconhecidamente um vasodilatador fisiológico, de modo que seu uso após uma importante perda

de sangue induz reperfusão. Pesquisas anteriormente divulgadas descrevem uma série de propriedades da solução hipertônica de NaCl, entre eles (i) a correção de endotelial e edema de células vermelhas com consequências significativas em termos de fluxo sanguíneo capilar, (ii) a modulação do sistema imunológico. Esta avaliação abrange a evolução neste campo entre 2005 e 2013. Como conclusão, discutimos a discrepância entre achados experimentais e clínicos; argumentamos que em sua essência, são possivelmente falhos os objetivos dos ensaios clínicos multicêntricos recentemente realizados.

REFERENCES

1. Penfield WG. The treatment of severe and progressive hemorrhage by intravenous injections. Am J Physiol. 1919;48:121–8.
2. Messmer K, Mokry G, Jesch F. The protective effect of hypertonic solutions in shock. Br J Surg. 1969;56(8):626.
3. Baue AE, Tragus ET, Parkins WM. Effects of sodium chloride and bicarbonate in shock with metabolic acidosis. Am J Physiol. 1967; 212(1):54–60.
4. Gazitua S, Scott JB, Chou CC, Haddy FJ. Effect of osmolarity on canine renal vascular resistance. Am J Physiol. 1969;217(4):1216–23.
5. Velasco IT, Pontieri V, Rocha e Silva Jr M, Lopes OU. Hyperosmotic NaCl and severe hemorrhagic shock. Am J Physiol. 1980;239(5):H664–73.
6. ThomsonReuters. Web of Science. 2013 [cited 2013]; Available from: www.isiknowledge.com.
7. de Felippe Jr. J, Timoner J, Velasco IT, Lopes OU, Rocha-e-Silva Jr M. Treatment of refractory hypovolaemic shock by 7.5% sodium chloride injections. Lancet. 1980;2(8202):1002–4.
8. Smith GJ, Kramer GC, Perron P, Nakayama S, Gunther RA, Holcroft JW. A comparison of several hypertonic solutions for resuscitation of bled sheep. J Surg Res. 1985;39(6):517–28.
9. Kramer GC, Perron PR, Lindsey DC, Ho HS, Gunther RA, Boyle WA, et al. Small-volume resuscitation with hypertonic saline dextran solution. Surgery. 1986;100(2):239–47.
10. Rocha e Silva M. Evolving concepts in small volume resuscitation: the experimental basis. In: Kreimeier U, Christ, F, Messmer, K., editor. Small-volume hyperosmolar volume resuscitation. Heidelberg: Springer-Verlag; 1999.
11. Rocha-e-Silva M, Poli de Figueiredo LF. Small volume hypertonic resuscitation of circulatory shock. Clinics (Sao Paulo). 2005;60(2):159–72.
12. Lopes OU, Pontieri V, Rocha e Silva Jr M, Velasco IT. Hyperosmotic NaCl and severe hemorrhagic shock: role of the innervated lung. Am J Physiol. 1981;241(6):H883–90.
13. de Almeida Costa EF, Pedrino GR, Lopes OU, Cravo SL. Afferent pathways involved in cardiovascular adjustments induced by hypertonic saline resuscitation in rats submitted to hemorrhagic shock. Shock. 2009; 32(2):190–3.
14. Pedrino GR, Rossi MV, Schoorlemmer GH, Lopes OU, Cravo SL. Cardiovascular adjustments induced by hypertonic saline in hemorrhagic rats: Involvement of carotid body chemoreceptors. Auton Neurosci. 2011;160(1-2):37–41.
15. Frithiof R, Eriksson S, Bayard F, Svensson T, Rundgren M. Intravenous hypertonic NaCl acts via cerebral sodium-sensitive and angiotensinergic mechanisms to improve cardiac function in haemorrhaged conscious sheep. J Physiol. 2007;583(Pt 3):1129–43.
16. Letson HL, Dobson GP. Small volume 7.5% NaCl with 6% Dextran-70 or 6% and 10% hetastarch are associated with arrhythmias and death after 60 minutes of severe hemorrhagic shock in the rat in vivo. J Trauma. 2011;70(6):1444–52.
17. Maier S, Holz-Holzl C, Pajk W, Ulmer H, Hengl C, Dunser M, et al. Microcirculatory parameters after isotonic and hypertonic colloidal fluid resuscitation in acute hemorrhagic shock. J Trauma. 2009;66(2):337–45.
18. Cabrales P, Tsai AG, Intaglietta M. Polymerized bovine hemoglobin can improve small-volume resuscitation from hemorrhagic shock in hamsters. Shock. 2009;31(3):300–7.
19. Palmer AF, Zhang N, Zhou Y, Harris DR, Cabrales P. Small-volume resuscitation from hemorrhagic shock using high-molecular-weight tense-state polymerized hemoglobins. J Trauma. 2011;71(4):798–807.
20. Gong W, Marks JA, Sanati P, Sims C, Sarani B, Smith DH, et al. Hypertonic saline resuscitation of hemorrhagic shock does not decrease in vivo neutrophil interactions with endothelium in the blood-brain microcirculation. J Trauma. 2011;71(2):275–81, discussion 81-2.
21. Yoon YH, Choi SH, Hong YS, Lee SW, Moon SW, Cho HJ, et al. Effect of hypertonic saline and macrophage migration inhibitory factor in restoration of T cell dysfunction. J Korean Surg Soc. 2011;81(4):229–34.
22. Woehrle T, Yip L, Manohar M, Sumi Y, Yao Y, Chen Y, et al. Hypertonic stress regulates T cell function via pannexin-1 hemichannels and P2X receptors. J Leukoc Biol. 2010;88(6):1181–9.
23. Lu YQ, Cai XJ, Gu LH, Mu HZ, Huang WD. Hypertonic saline resuscitation maintains a more balanced profile of T-lymphocyte subpopulations in a rat model of hemorrhagic shock. J Zhejiang Univ Sci B. 2007; 8(1):70–5.
24. Lu YQ, Huang WD, Cai XJ, Gu LH, Mou HZ. Hypertonic saline resuscitation reduces apoptosis of intestinal mucosa in a rat model of hemorrhagic shock. J Zhejiang Univ Sci B. 2008;9(11):879–84.
25. Lee L, Kelher MR, Moore EE, Banerjee A, Silliman CC. Hypertonic saline inhibits arachidonic acid priming of the human neutrophil oxidase. J Surg Res. 2012;174(1):24–8.
26. Deree J, Martins JO, Leedom A, Lamon B, Putnam J, de Campos T, et al. Hypertonic saline and pentoxifylline reduces hemorrhagic shock resuscitation-induced pulmonary inflammation through attenuation of neutrophil degranulation and proinflammatory mediator synthesis. J Trauma. 2007;62(1):104–11.
27. Bahrami S, Zimmermann K, Szelenyi Z, Hamar J, Scheiflinger F, Redl H, et al. Small-volume fluid resuscitation with hypertonic saline prevents inflammation but not mortality in a rat model of hemorrhagic shock. Shock. 2006;25(3):283–9.
28. Theobaldo MC, Llimona F, Petroni RC, Rios EC, Velasco IT, Soriano FG. Hypertonic saline solution drives neutrophil from bystander organ to infectious site in polymicrobial sepsis: a cecal ligation and puncture model. PLoS One. 2013;8(9):e74369.
29. Chen H, Koustova E, Shults C, Sailhamer EA, Alam HB. Differential effect of resuscitation on Toll-like receptors in a model of hemorrhagic shock without a septic challenge. Resuscitation. 2007;74(3):526–37.
30. Chen LW, Su MT, Chen PH, Liu WC, Hsu CM. Hypertonic saline enhances host defense and reduces apoptosis in burn mice by increasing toll-like receptors. Shock. 2011;35(1):59–66.
31. Fernandes TR, Pontieri V, Moretti AI, Teixeira DO, Abatepaulo F, Soriano FG, et al. Hypertonic saline solution increases the expression of heat shock protein 70 and improves lung inflammation early after reperfusion in a rodent model of controlled hemorrhage. Shock. 2007;27(2):172–8.
32. Frithiof R, Mats R, Johan U, Stefan E, Hans H. Comparison between the effects on hemodynamic responses of central and peripheral infusions of hypertonic NaCl during hemorrhage in conscious and isoflurane-anesthetized sheep. Shock. 2006;26(1):77–86.
33. Xiao HP, Gu MN, Xiao JF, Xu X, Zhao ZL. [Effects of hypertonic sodium chloride hydroxyethyl starch 40 injection in treatment of acute intracranial hypertension complicated by hemorrhagic shock in dogs]. Nan Fang Yi Ke Da Xue Xue Bao. 2008;28(3):385–8.
34. Jussen D, Papaioannou C, Heimann A, Kempski O, Alessandri B. Effects of hypertonic/hyperoncotic treatment and surgical evacuation after acute subdural hematoma in rats. Crit Care Med. 2008;36(2):543–9.
35. Exo JL, Shellington DK, Bayir H, Vagni VA, Janesco-Feldman K, Ma L, et al. Resuscitation of traumatic brain injury and hemorrhagic shock with polynitroxylated albumin, hextend, hypertonic saline, and lactated Ringer's: Effects on acute hemodynamics, survival, and neuronal death in mice. J Neurotrauma. 2009;26(12):2403–8.
36. Pinto FC, Capone-Neto A, Prist R, MR ES, Poli-de-Figueiredo LF. Volume replacement with lactated Ringer's or 3% hypertonic saline solution during combined experimental hemorrhagic shock and traumatic brain injury. J Trauma. 2006;60(4):758–63, discussion 63-4.
37. Lee S, Stier G, Marcantonio S, Lekic T, Allard M, Martin R, et al. 3% hypertonic saline following subarachnoid hemorrhage in rats. Acta Neurochir Suppl. 2008;102:405–8.
38. Balbino M, Capone Neto A, Prist R, Ferreira AT, Poli-de-Figueiredo LF. Fluid resuscitation with isotonic or hypertonic saline solution avoids intraneural calcium influx after traumatic brain injury associated with hemorrhagic shock. J Trauma. 2010;68(4):859–64.
39. Noppens RR, Christ M, Brambrink AM, Koerner IP, Heimann A, Kempski O. An early bolus of hypertonic saline hydroxyethyl starch improves long-term outcome after global cerebral ischemia. Crit Care Med. 2006;34(8):2194–200.
40. Nout YS, Mihai G, Tovar CA, Schmalbrock P, Bresnahan JC, Beattie MS. Hypertonic saline attenuates cord swelling and edema in experimental spinal cord injury: a study utilizing magnetic resonance imaging. Crit Care Med. 2009;37(7):2160–6.
41. Yu G, Chi X, Hei Z, Shen N, Chen J, Zhang W, et al. Small volume resuscitation with 7.5% hypertonic saline, hydroxyethyl starch 130/0.4 solution and hypertonic sodium chloride hydroxyethyl starch 40 injection reduced lung injury in endotoxin shock rats: comparison with saline. Pulm Pharmacol Ther. 2012;25(1):27–32.
42. Liu Z, Li Y, Liu B, Deperalta DK, Zhao T, Chong W, et al. Synergistic effects of hypertonic saline and valproic acid in a lethal rat two-hit model. J Trauma Acute Care Surg. 2013;74(4):991–7, discussion 7-8.
43. Coimbra R, Porcides R, Loomis W, Melbostad H, Lall R, Deree J, et al. HSPTX protects against hemorrhagic shock resuscitation-induced tissue injury: an attractive alternative to Ringer's lactate. J Trauma. 2006; 60(1):41–51.
44. Costantini TW, Deree J, Martins JO, Putnam JG, Campos T, Coimbra R. A novel fluid resuscitation strategy modulates pulmonary transcription factor activation in a murine model of hemorrhagic shock. Clinics (Sao Paulo). 2010;65(6):621–8.
45. Cruz Jr. RJ, Yada-Langui MM, de Figueiredo LF, Sinosaki S, Rocha e Silva M. The synergistic effects of pentoxifylline on systemic and regional perfusion after hemorrhage and hypertonic resuscitation. Anesth Analg. 2006;102(5):1518–24.

46. Lu YQ, Gu LH, Jiang JK, Mou HZ. Effect of hypertonic versus isotonic saline resuscitation on heme oxygenase-1 expression in visceral organs following hemorrhagic shock in rats. Biomed Environ Sci. 2013; 26(8):684–8.
47. Hoppen RA, Corso CO, Grezzana TJ, Severino A, Dal-Pizzol F, Ritter C. Hypertonic saline and hemorrhagic shock: hepatocellular function and integrity after six hours of treatment. Acta Cir Bras. 2005;20(6):414–7.
48. Sharma P, Benford B, Karaian JE, Keneally R. Effects of volume and composition of the resuscitative fluids in the treatment of hemorrhagic shock. J Emerg Trauma Shock. 2012;5(4):309–15.
49. Younes RN, Deutsch F, Itinoshe M, Fontes B, Poggetti R, Birolini D. Responsiveness to different volume therapies following hemodilution and hemorrhagic shock: a comparative experimental study in rats. Acta Cir Bras. 2007;22(5):355–60.
50. Nascimento Jr P, de Paiva Filho O, de Carvalho LR, Braz JR. Early hemodynamic and renal effects of hemorrhagic shock resuscitation with lactated Ringer's solution, hydroxyethyl starch, and hypertonic saline with or without 6% dextran-70. J Surg Res. 2006;136(1):98–105.
51. Watters JM, Tieu BH, Differding JA, Muller PJ, Schreiber MA. A single bolus of 3% hypertonic saline with 6% dextran provides optimal initial resuscitation after uncontrolled hemorrhagic shock. J Trauma. 2006; 61(1):75–81.
52. Legrand M, Mik EG, Balestra GM, Lutter R, Pirracchio R, Payen D, et al. Fluid resuscitation does not improve renal oxygenation during hemorrhagic shock in rats. Anesthesiology. 2010;112(1):119–27.
53. Garcia-Martinez D, Portilla-de Buen E, Leal C, Santillan P, Muniz J. The immediate response to severe shock in a canine model with a combination of hypertonic-hyperoncotic solution with naloxone. Shock. 2006;26(4):379–85.
54. Reynolds PS, Barbee RW, Ward KR. Lactate profiles as a resuscitation assessment tool in a rat model of battlefield hemorrhage resuscitation. Shock. 2008;30(1):48–54.
55. Shih CC, Chen SJ, Chen A, Wu JY, Liaw WJ, Wu CC. Therapeutic effects of hypertonic saline on peritonitis-induced septic shock with multiple organ dysfunction syndrome in rats. Crit Care Med. 2008;36(6):1864–72.
56. Shih CC, Tsai MF, Chen SJ, Tsao CM, Ka SM, Huang HC, et al. Effects of small-volume hypertonic saline on acid-base and electrolytes balance in rats with peritonitis-induced sepsis. Shock. 2012;38(6):649–55.
57. Somell A, Weitzberg E, Suneson A, Sollevi A, Hjelmqvist H. Effects of the dual endothelin receptor antagonist tezosentan and hypertonic saline/dextran on porcine endotoxin shock. Acta Physiol (Oxf). 2007; 190(4):291–302.
58. Kim HJ, Lee KH. The effectiveness of hypertonic saline and pentoxifylline (HTS-PTX) resuscitation in haemorrhagic shock and sepsis tissue injury: comparison with LR, HES, and LR-PTX treatments. Injury. 2012; 43(8):1271–6.
59. Gao J, Zhao WX, Xue FS, Zhou LJ, Yu YH, Zhou HB. Effects of different resuscitation fluids on acute lung injury in a rat model of uncontrolled hemorrhagic shock and infection. J Trauma. 2009;67(6):1213–9.
60. Luiz Zanoni F, Costa Cruz JW, Martins JO, Benabou S, Vicente Greco K, Ramos Moreno AC, et al. Hypertonic saline solution reduces mesenteric microcirculatory dysfunctions and bacterial translocation in a rat model of strangulated small bowel obstruction. Shock. 2013;40(1):35–44.
61. Wan L, Bellomo R, May CN. Bolus hypertonic or normal saline resuscitation in gram-negative sepsis: systemic and regional haemodynamic effects in sheep. Crit Care Resusc. 2011;13(4):262–70.
62. Rizoli SB, Rhind SG, Shek PN, Inaba K, Filips D, Tien H, et al. The immunomodulatory effects of hypertonic saline resuscitation in patients sustaining traumatic hemorrhagic shock: a randomized, controlled, double-blinded trial. Ann Surg. 2006;243(1):47–57.
63. Junger WG, Rhind SG, Rizoli SB, Cuschieri J, Shiu MY, Baker AJ, et al. Resuscitation of traumatic hemorrhagic shock patients with hypertonic saline-without dextran-inhibits neutrophil and endothelial cell activation. Shock. 2012;38(4):341–50.
64. Bulger EM, Cuschieri J, Warner K, Maier RV. Hypertonic resuscitation modulates the inflammatory response in patients with traumatic hemorrhagic shock. Ann Surg. 2007;245(4):635–41.
65. Yildizdas D, Altunbasak S, Celik U, Herguner O. Hypertonic saline treatment in children with cerebral edema. Indian Pediatr. 2006;43(9):771–9.
66. Schroth M, Plank C, Meissner U, Eberle KP, Weyand M, Cesnjevar R, et al. Hypertonic-hyperoncotic solutions improve cardiac function in children after open-heart surgery. Pediatrics. 2006;118(1):e76–84.
67. Bulger EM, Jurkovich GJ, Nathens AB, Copass MK, Hanson S, Cooper C, et al. Hypertonic resuscitation of hypovolemic shock after blunt trauma: a randomized controlled trial. Arch Surg. 2008;143(2):139–48, discussion 49.
68. Bulger EM, May S, Brasel KJ, Schreiber M, Kerby JD, Tisherman SA, et al. Out-of-hospital hypertonic resuscitation following severe traumatic brain injury: a randomized controlled trial. JAMA. 2010;304(13):1455–64.
69. Bulger EM, May S, Kerby JD, Emerson S, Stiell IG, Schreiber MA, et al. Out-of-hospital hypertonic resuscitation after traumatic hypovolemic shock: a randomized, placebo controlled trial. Ann Surg. 2011; 253(3):431–41.
70. Fang ZX, Li YF, Zhou XQ, Zhang Z, Zhang JS, Xia HM, et al. Effects of resuscitation with crystalloid fluids on cardiac function in patients with severe sepsis. BMC Infect Dis. 2008;8:50.
71. Morrison LJ, Rizoli SB, Schwartz B, Rhind SG, Simitciu M, Perreira T, et al. The Toronto prehospital hypertonic resuscitation-head injury and multi organ dysfunction trial (TOPHR HIT)–methods and data collection tools. Trials. 2009;10:105.
72. Dubick MA, Shek P, Wade CE. ROC trials update on prehospital hypertonic saline resuscitation in the aftermath of the US-Canadian trials. Clinics (Sao Paulo). 2013;68(6):883–6.