# Review
# Clinical review: Hypertonic saline resuscitation in sepsis

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## Abstract

The present review discusses the hemodynamic effects of hypertonic saline in experimental shock and in patients with sepsis. We comment on the mechanisms of action of hypertonic saline, calling upon data in hemorrhagic and septic shock. Specific actions of hypertonic saline in severe sepsis and septic shock are highlighted. Data are available that support potential benefits of hypertonic saline infusion in various aspects of the pathophysiology of sepsis, including tissue hypoperfusion, decreased oxygen consumption, endothelial dysfunction, cardiac depression, and the presence of a broad array of proinflammatory cytokines and various oxidant species. The goal of research in this field is to identify reliable therapies to prevent ischemia and inflammation, and to reduce mortality.

**Keywords** hemorrhagic, hypertonic saline, inflammation, sepsis, shock

The incidence of septic shock has increased during the past several decades, whereas mortality rates have remained constant or have decreased slightly [1]. Septic shock is associated with high mortality rates of 30–80% [1]. Sepsis presents with a systemic inflammatory response, peripheral vasodilatation, myocardial depression, intravascular volume depression, and increased metabolism. Despite considerable knowledge of the pathophysiology of the systemic inflammatory response syndrome, clinical trials using interventions such as immunotherapy have yielded negative results [2,3]. Global tissue hypoxia results in an imbalance between systemic oxygen delivery and demand, and is a key development preceding multiple organ failure and death [2]. Rivers and colleagues [2] demonstrated the importance of goal-directed therapy in septic shock and severe sepsis. An early resuscitation strategy, which was goal oriented with respect to manipulation of cardiac preload, afterload and contractility, reduced the incidence of multiple organ dysfunction and mortality.

Hemodynamic management in severe sepsis and septic shock includes rapid restoration of intravascular volume and adequate balance between systemic oxygen delivery and demand. Several liters of fluids (crystalloids or colloids) are usually necessary to normalize preload and filling pressures, with the objective of establishing adequate tissue perfusion and oxygen delivery [2]. The infusion of several liters of fluid is associated with the adverse effect of extravasation into the interstitial space. In sepsis in particular, this may result in pulmonary edema. Nevertheless, adequate volume repletion with hemodynamic normalization may not be sufficient to prevent persistent microcirculatory dysfunction, which may cause ischemia and tissue damage [2,4,5].

The observation reported by Velasco and colleagues in 1980 [6] of beneficial effects of 7.5% saline solutions in dogs with severe hemorrhagic shock attracted interest to this field. The short duration of the circulatory effects of hypertonic saline solution (HSS) has been attributed to a rapid equilibrium of the hyperosmotic solute between extracellular and intracellular compartments. Therefore, HSS has been combined with colloids (i.e. dextran or hetastarch) in order to achieve a longer intravascular effect. This combination has synergistic effects, by increasing plasma osmolarity and osmotic pressure [7,8]. Since the 1980s, several studies have been

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HOG = high-osmolarity glycerol response; HSS = hypertonic saline solution; MAPK = mitogen-activated protein kinase.

performed that used small volume resuscitation [6,9–13], which is defined as a rapid infusion of HSS (NaCl 7.2–7.5%), in combination with dextran or hetastarch, at a dose of 4 ml/kg into a peripheral vein [6,9–13]. Recent studies have used HSS in the treatment of sepsis [14–19] and have demonstrated some promising beneficial effects.

## Hypertonic resuscitation in experimental models of sepsis

There is a decreased susceptibility to sepsis following administration of HSS in hemorrhagic shock. After 24 hours of cecal ligation and perforation, animals that received HSS had fewer bacteria in serum, lower formation of abscesses in liver and lungs, and less pulmonary and hepatic injury [20]. Reduced organ injury in the HSS group might have been related to an improved hemodynamic profile and decreased extrapulmonary volume. Also, effects on microcirculation (i.e. reduction in ischemia and effects related to immune function) might have contributed partly to decreased organ injury.

Experimental studies in septic shock have shown beneficial effects similar to those reported in studies of hemorrhagic shock [14–19]. Studies of HSS alone or combined with hetastarch in sepsis demonstrated hemodynamic improvements, but these effects had a short period of action. However, these findings bring new possibilities to treatment of septic patients, if the treatments are instituted early in course of the disease. Hemodynamic resuscitation *per se* can reduce the inflammatory response in sepsis, reducing the phenomenon of ischemia/reperfusion [2]. On the other hand, several studies have demonstrated that HSS modulates immune function favorably (i.e. by reducing production and release of proinflammatory cytokines and augmenting interleukin-10 induction; by reducing L-selectin expression in neutrophils; and by reducing the oxidative burst) [21–29]. Together, those studies indicate that HSS has actions in two important aspects of septic shock: hemodynamics and immunomodulation. Notwithstanding the recent intense focus on immunomodulation in sepsis, Rivers and colleagues [2] showed that early hemodynamically centered therapy yielded significant benefits with respect to outcome.

Observations from several experimental studies suggest that HSS combined with a colloid solution is able to improve macrocirculation in sepsis [14,18,19,30,31]. Also, HSS prevented vascular dysfunction and restored microcirculatory blood flow by capillary reopening. This effect resulted in a beneficial redistribution of regional blood flow to heart, kidney, and splanchnic organs.

## Hypertonic resuscitation in clinical studies of sepsis

The first clinical study to evaluate the effects of small volume resuscitation in severe sepsis was conducted by Hannemann and colleagues [16]. Those authors observed increased oxygen transport, cardiac output, and pulmonary capillary wedge pressure in patients treated with HSS. Except for the increase in pulmonary capillary wedge pressure, none of the cardiovascular changes lasted for longer than 60 min. Plasma sodium levels increased and normalized within 24 hours after HSS infusion.

Oliveira and colleagues [32] studied the hemodynamic effects of a hypertonic saline/dextran solution as compared with those of a normal saline solution in severe sepsis. Patients were randomly assigned, in a blinded manner, to receive 250 ml of a solution of either normal saline ($n = 16$) or hypertonic saline (NaCl 7.5%/dextran 8%; $n = 13$). Before they received normal saline or HSS, patients had to have been stable (i.e. no requirement for vasoactive drug or volume change) for at least 60 min. Over the 180 min following infusion of normal saline or HSS (i.e. the period of study), the rate of infusion of regular fluid or vasoactive drug was not changed. The cardiac and stroke volume indices increased, and systemic vascular resistance decreased only in the HSS group, without any change in arterial pressure. The increase in plasma sodium levels lasted for 6 hours in the HSS group. Those investigators concluded that hypertonic saline/dextran solution improved cardiovascular performance and resuscitated severely septic patients through a volume effect, but may also directly improve cardiac function.

## Mechanisms of action of hypertonic saline solution

The main proposed mechanisms of action of HSS are as follows [14,27,33–49]: instantaneous mobilization of fluids from intracellular to extracellular compartments by the osmotic gradient produced by HSS; increased myocardial contractility; reduced endothelial and tissue edema, improving microcirculation; improved blood viscosity due to hemodilution; and immunomodulation.

### Intravascular volume expansion

A rapid increase in mean arterial pressure occurs following HSS infusion. Studies have shown a redistribution of fluids from the perivascular to the intravascular space, and consequent plasma expansion [6,9,50]. The hemodynamic effects of HSS infusion have been studied in sepsis [15–17,19]. Most of the studies found that HSS infusion caused a rapid and significant increase in oxygen delivery, and elevated cardiac output and increased oxygen extraction, but these effects were transient [15,16,18,19]. Therefore, despite the immunologic background of sepsis and the significant role played by immunologic mechanisms in the disease, hemodynamic resuscitation has also proved important in management of sepsis [2]. HSS may be able to resuscitate septic patients better and more rapidly, during the critical 'golden hours' of the disease.

### Cardiac contractility effects

Improvement in myocardial contractility may be related to a direct hyperosmolar effect, restoring transmembrane poten-

tials or decreasing myocardial edema [13,33]. Reported findings indicate that ventricular contractile force is enhanced by moderate degrees of hyperosmolarity and is depressed by severe hyperosmolarity both *in vivo* and *in vitro* [51]. HSS has been shown to increase left ventricular $dP/dt_{max}$, cardiac output, and stroke work at equivalent or lower atrial filling pressures than with isotonic solutions [10,43,52]. Myocardial function is depressed even in the early hyperdynamic phase of sepsis [53]. However, hypertonic solutions have been shown to improve contractility in animal and human studies of sepsis [14,54]. These improvements in myocardial performance were unrelated to changes in coronary flow or myocardial oxygen consumption [14].

### Neural effect: the role of lung innervation

Two forms of experimental evidence indicate that a pulmonary reflex mechanism may participate in the resuscitative effects of hypertonic saline.

First, it has been suggested that passage of hypertonic saline through the pulmonary circulation is necessary for resuscitation. In dogs, prepulmonary (right atrial, pulmonary arterial) administration resulted in resuscitation, but postpulmonary (left atrial, aortic) administration did not provide effective resuscitation [55,56]. However, data from sheep [57] suggested that the site of administration has no influence on the resuscitative effects of hypertonic saline. The differences between the reports may be explained by the different species investigated.

The second form of evidence for lung innervation is provided by studies indicating that vagal blockade attenuates the hemodynamic response to hypertonic saline administration in hypovolemic dogs [50,55,58]. In contrast to this finding, nearly identical improvements in mean arterial pressure, cardiac output, heart rate, and blood chemistry parameters in response to administration of hypertonic saline into innervated and denervated pulmonary circulations were reported [59,60]. However, Younes and coworkers [50] conducted studies 7 days after surgery in a model of total lung denervation. In that model, HSS administration produced a sustained hemodynamic improvement in the innervated group as compared with the denervated group. The different preparations may explain those contrasting results [50,59].

### Endothelial effects

In the initial phase of hypovolemia and shock, the hypoxia and activation of polymorphonuclear cells in the endothelium of postcapillary venules produce endothelial cell edema. This leads to capillary lumen narrowing, which can cause complete obstruction of local blood flow and reduction in oxygen transport [45,61]. During small volume resuscitation, the intracellular fluid is primarily mobilized from microvascular endothelial cells and erythrocytes. This effect is more pronounced in capillaries in which edema is greater. It produces a reduction in hydraulic resistance and an improvement in tissue perfusion. It has been demonstrated that a reduction in

endothelial volume of 20% after infusion of HSS/dextran, as well as an increase in sinusoidal perfusion, may occur, resulting in significant improvements in hepatic energetic status and excretory function [34,45,61].

### Vasoactive mediators

Studies have revealed increased cardiac output and restoration of peripheral blood flow mediated by vasodilating substances released after HSS infusion, especially prostacycline, together with an increase in the 6-cheto-prostaglandin $F_{1\alpha}$ : thromboxane $B_2$ ratio [62]. The decrease in total peripheral resistance is the main factor responsible for the hypotension that occurs immediately after infusion of HSS [19].

The neuroendocrine response to 7.5% HSS/dextran after hemorrhagic shock was quantified in pigs [63]. The result of hemodilution in association with plasma volume expansion was decreased plasma levels of adrenocorticotropic hormone, cortisol, and aldosterone. Also, reductions in plasma concentrations of norepinephrine (noradrenaline), epinephrine (adrenaline), lysine, vasopressin, and renin were greater with hemodilution combined with plasma volume expansion than with hemodilution alone, indicating that alterations in hormone release have a role to play in cardiovascular response in this model of resuscitation.

### Immunomodulatory effects

Hemorrhage and sepsis often initiate a systemic inflammatory response that is accompanied by organ dysfunction, most commonly acute lung injury [41]. Neutrophil sequestration in the lung is a necessary prerequisite for development of lung injury in most models of hemorrhagic and septic shock [41]. Ischemia has been shown to lead to accumulation of neutrophils and other leukocytes in the microvascular bed of many organs [64–66]. HSS has been shown to reduce lung injury after hemorrhagic shock [41,47]. Those studies showed that HSS produced the following improvements in the lung: reduction in neutrophil accumulation, less neutrophils recovered on bonchealveolar lavage, reduced albumin leak and a lower degree of histopathologic injury. The mechanism of neutrophil sequestration or adhesion depends on the particular inflammatory condition. The CD11b integrin is a vital component of neutrophil–endothelial interactions, and in this respect Rizoli and colleagues [47] showed that HSS prevented lipopolysaccharide-stimulated expression and activation of CD11b. Corroborating those data, HSS has been shown to decrease neutrophil L-selectin expression and to eliminate neutrophil priming by mesenteric lymph production [40,49]; this suggests that HSS reduces lung injury by preventing neutrophil adhesion to endothelium. Also, Oreopoulos and colleagues [24] showed inhibition of ischemia/reperfusion-induced hepatic expression of intercellular adhesion molecule-1 mRNA with HSS as compared with normal saline.

Studies into the action of HSS on cellular mechanisms [21–29] have yielded data that indicate that HSS regulates

the expression and release of elastase, cytokines, free radicals, and adhesion molecules. T cells incubated at NaCl levels of up to 180 mmol/l exhibited 100% enhancement of proliferation (these concentrations of salt are similar to the plasma sodium levels that are introduced by the traditional dose of 4 ml/kg of a 7.5% NaCl solution) [26]. Several circulating factors with T-cell suppressive activity have been identified in trauma patients, suggesting that these factors cause a down-regulation of T-cell function after trauma [26]. Prostaglandin $E_2$ is a T-cell suppressor that interferes with calcineurin-dependent signaling pathways, and thereby inhibits interleukin-2 production and T-cell proliferation. Human peripheral blood mononuclear cells were suppressed when incubated with prostaglandin $E_2$ [26]. T-cell proliferation was significantly enhanced when the cells were exposed to HSS. Cell-mediated immune function and splenocyte proliferation is significantly suppressed after hemorrhage, and HSS resuscitation clearly restored splenocyte function and cell-mediated immune function.

*Hypertonic saline modulates cellular signaling pathways*

Most knowledge in this area stems from work with *Saccharomyces cerevisiae* [67–69]. Hyperosmotic conditions trigger activation of a mitogen-activated protein kinase (MAPK) termed high-osmolarity glycerol response (HOG)1 in yeast cells. Han and colleagues [70] identified a mammalian equivalent of HOG1 in monocytic cells. This protein, namely MAPK p38, shares approximately 60% amino acid sequence with HOG1. MAPK p38 is tyrosine phosphorylated and activated under hypertonic conditions, suggesting the existence of an osmolarity sensing system in mammalian cells. A human T-cell line (Jurkat cells) was used to investigate whether HSS triggers signaling events through protein phosphorylation [71]. HSS exposure permitted tyrosine phosphorylation of cellular protein in a dose-dependent manner.

Sepsis, trauma, and hemorrhage activate neutrophils and can trigger excessive release of cytotoxic mediators, damaging host tissues and resulting in major post-traumatic complications [25]. Clinically relevant hypertonicity suppressed degranulation and superoxide formation in response to *N*-formyl-methionyl-leucyl-phenylalanine (fMLP), and blocked the activation of the MAPKs ERK 1/2 and p38. HSS did not suppress neutrophil oxidative burst in response to phorbol myristate acetate. This indicates that HSS suppresses neutrophil function by intercepting signal pathways upstream of or apart from protein kinase C. Neutrophils incubated in hypertonic saline showed a reduction in platelet-activating factor mediated MAPK p38 signal transduction. Clinically relevant levels of hypertonic saline attenuated platelet-activating factor mediated $\beta_2$ integrin expression, superoxide radical production, and elastase release [28]. Recent evidence [72] suggests that cytoskeletal reorganization is critical for receptor-mediated signal transduction. Cytoskeletal disruption prevented attenuation of receptor-mediated MAPK p38 activation by hypertonic saline. Therefore, hypertonic saline alters cell shape, and this is followed by cytoskeletal reorganization with a resultant immunomodulatory effect [72,73].

Data have been reported [74] that indicate that HSS augments interleukin-10 induction by lipopolysaccharide at the gene level and reduces tumor necrosis factor levels, independent of nuclear factor-κB signaling. These actions may explain the lesser degree of injury following HSS administration. However, because HSS reduces but does not completely abrogate proinflammatory pathways, there is an adequate balance between proinflammatory and anti-inflammatory cytokines, thus maintaining the ability to fight bacteria efficiently.

## Conclusion

The potential beneficial effect of small volume resuscitation with HSS, which has been extensively studied in hypovolemic shock, appears to be reproducible in various models of experimental septic shock. The anti-inflammatory effects of hypertonic saline on neutrophils, oxidative burst, and cytokine release are mediated through the signaling molecule MAPK p38. These effects may reduce the excessive proinflammatory action found in sepsis, reducing the degree of damage to multiple organs. Hemodynamic effects have been widely demonstrated, and recent data showed that early goal-directed therapy is very important in reducing mortality. The vicious circle of ischemia, inflammation, fluid extravasation, and ischemia that occurs in sepsis may perpetuate damage to organs. A therapy that simultaneously blocks both of the damaging components of sepsis, namely ischemia and inflammation, will probably have an enormous impact on our ability to manage this condition. HSS is emerging as a possible preventive therapeutic in sepsis.

## Competing interests

None declared.

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