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Abstract

Sepsis, the systemic response to infection, is the leading cause of death in intensive care units, worldwide. Mortality rates of sepsis exceed 20%, highlighting the need for new approaches of therapy for this disease. Intravenous fluid resuscitation plays a crucial role in sepsis therapy. Most preclinical studies demonstrate an association between IV fluid therapy and improved outcomes in sepsis. The purpose of this review is to summarize the preclinical studies examining outcomes of sepsis associated with different resuscitation fluid types and volume in animal models, and to review studies that have investigated underlying mechanisms by which fluid resuscitation is thought to improve outcomes in sepsis. Data obtained from preclinical studies differ due to use of varied animal species and models of sepsis. The development of appropriate animal models of sepsis and design of experiments that closely mirror the clinical progression of this disease are paramount to enhance our knowledge and understanding of sepsis pathophysiology and develop effective therapies to improve outcomes associated with sepsis.

INTRODUCTION

Severe sepsis, the documented or suspected presence of infection with manifestations of systemic response accompanied by organ dysfunction, affects about 33% of patients in intensive care units (ICUs) and is the leading cause of death in that population. Annual costs for treatment of sepsis in the United States exceed $20 billion annually, presenting a significant economic burden on health care [1]. Even with optimal treatment, mortality due to severe sepsis exceeds 20 to 40% in the case of septic shock [2], highlighting the need for development of new therapeutic strategies for management and treatment of this syndrome.

Animal models are routinely used to understand the biological cascades involved in disease processes and to help determine the viability of various treatment options for human disease. Intravenous (IV) fluid resuscitation is an important part of the optimal treatment protocol for patients with severe sepsis. Various animal models have been used to study the effect of IV fluids on clinical outcomes in sepsis. Moreover, the impact of different types of IV fluids has also been a topic of interest in sepsis research, with longstanding debates regarding the efficacy of colloid resuscitation [3-8] and recent data suggesting harm associated with the administration of large volumes of chloride-rich solutions, such as 0.9% saline [9-12].

Fluids used for volume resuscitation can be divided into two broad categories: crystalloids and colloids. An extensive review of the different types of solutions and their specific characteristics and uses for treatment in severe sepsis/septic shock was recently published and is beyond the scope of the current review [13]. The purpose of this manuscript is to examine and summarize the preclinical studies that have assessed the effect of fluid type and volume on outcomes in severe sepsis and/or septic shock animal models. Furthermore, we will review data elucidating the underlying mechanisms by which fluid resuscitation is thought to improve outcomes in sepsis.

Role of intravenous fluid resuscitation in sepsis

Fluid resuscitation serves an important role in sepsis treatment because of the hypovolemia and resultant tissue hypoperfusion that occurs during the disease. The mechanisms of hypovolemia are multifactorial, but include decreased fluid intake, increased insensible fluid loss secondary to tachypnea, and fluid extravasation or “third-spacing” secondary to loss of the barrier function of the endothelium [14,15]. Severe or prolonged
hypotension is associated with poor outcomes in patients with sepsis [16], while rapid restoration of organ perfusion appears to be associated with lower mortality [17]. Rapid or bolus intravenous fluid administration leads to short-term improvements in hemodynamics and tissue perfusion [18,19]. However, dysfunction of the vasculature leads to redistribution of this fluid into the interstitium resulting in tissue edema, an effect more pronounced with crystalloids than colloids. Nevertheless, given the vascular disruption observed in sepsis, even colloids can redistribute to the extravascular space, and due to their oncotic effects can further exacerbate tissue edema [20,21]. It has been hypothesized that this tissue edema may exacerbate preexisting organ dysfunction, particularly in the case of acute respiratory distress syndrome, and actually lead to worsened outcomes. Therefore, it is critical to understand the mechanisms related to and effects of differing types and volumes of fluid resuscitation during sepsis.

**Effect of intravenous resuscitation fluid type on outcomes in sepsis animal models**

Different animal models of sepsis have yielded differing conclusions as to which type of solution is better: crystalloid or colloid. A summary of these data are listed in Table 1. The most widely used species to study fluid resuscitation in sepsis is a small animal model, the rat. In most of these studies, fluid resuscitation is accomplished through IV administration. These studies have provided conflicting data regarding the superiority of any particular type of fluid, but comparisons are limited by differing outcomes and methods of inducing the sepsis response, as well as volume of fluid administered among the studies. In regards to perfusion, studies in a colon ascendens stent peritonitis (CASP) rat model of sepsis determined that use of the colloid hydroxyethyl starch (HES) solutions provided no enhanced benefit on microcirculation when compared to crystalloid solutions [22]. Similarly in terms of organ failure, 5% and 25% albumin showed no superiority over Ringer’s Lactate in an endotoxic rat model of sepsis in its efficacy in reducing ventilator induced lung injury [23]. In contrast, a study of acute kidney injury (AKI) using a rat cecal-ligation and puncture (CLP) model of severe sepsis found that unbalanced crystalloid solution (0.9% NaCl) was inferior compared to a balanced crystalloid (Plasma-Lyte) on development of AKI and survival time [24]. A separate study in an endotoxic rat model reported increased survival time and less metabolic acidosis after resuscitation with the synthetic colloid solution Hextend, compared to saline or Ringer’s lactate (RL) [25].

In addition to comparing crystalloid and colloid solutions alone for fluid resuscitation in sepsis, studies assessing the effect of resuscitation fluids linked with other agents or associated with other drugs have also been conducted. A solution of albumin covalently linked to polyethylene glycol was developed as a resuscitative agent and infused into both the endotoxic and CLP rat models of septic shock. In both models, the conjugated solution decreased capillary leakage and albumin was retained

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**Abbreviations:** CASP: Colon ascendens stent peritonitis; IV: Intravenous; LPS: Lipopolysaccharide; CLP: Cecal ligation and puncture; AKI: Acute kidney injury; ALI: Acute lung injury; VEGF: Vascular endothelial growth factor; HES: Hydroxyethyl starch; RL: Ringer's Lactate; PEG: Polyethylene glycol; PTX: Pentoxifylline; L-NAME: L-N^6-Nitroarginine methyl ester
in the vessels, thus decreasing the risk of edema injury from fluid resuscitation [26]. Additionally, HES resuscitation coupled with low dose dexamethasone was found to prevent septic acute renal failure in the endotoxic rat model [27]. Resuscitation with saline in conjunction with other drugs has also been tested in the endotoxic septic shock rat model. One study determined that early resuscitation with saline and norepinephrine, simultaneously, was protective against lung injury secondary to early aggressive fluid resuscitation alone [28]; and hypertonic saline combined with pentoxifylline (PTX) was superior in overall treatment of sepsis when compared with HES, RL alone or RL-PTX [29]. In contrast, it has been determined that dopamine is not beneficial in maintaining arteriolar blood flow or functional capillary density when used in conjunction with saline in the endotoxic hamster model. In fact, both doses of dopamine tested reduced tissue perfusion yielding reduced survival times and poorer outcomes compared to resuscitation with saline alone [30].

Though rodents are the most commonly used model of experimental sepsis and they conserve many of the biologic features that are common to mammals, there still remain distinct differences in their response to disease when compared to human responses. These differences may be less pronounced in larger animal models whose physiology is closer to that in humans. Furthermore, in large animals, it is possible to perform serial sampling of body fluids, and obtain detailed assessments of hemodynamic, cardiovascular and other end organ function over multiple time points, similar to what is obtained clinically [31,32]. Therefore, data obtained in larger animal models may be more translatable to treatment of human diseases. In contrast to the mixed findings in small animal models, sepsis studies in large animal models generally favor the use of colloid solution in sepsis. A study in IV LPS-induced endotoxic dogs found that resuscitation with dextran improved cardiac function to the same extent as a nitric oxide (NO) synthase inhibitor, without the risk of excessive vasodilation and ischemia [33]. Although this study compared a colloid solution with a diluted enzyme inhibitor, Qui, et al. examined the effect of fluid resuscitation, with crystallloid (0.9% NaCl and 7.5% NaCl) or colloid (HES) solutions, on capillary permeability, vascular endothelial growth factor (VEGF) levels, and development of pulmonary edema in an endotoxic dog model. The authors concluded that HES was superior to both crystallloid solutions in decreasing capillary permeability, VEGF tissue expression, and pulmonary edema [34]. In contrast, a study performed in a sheep model of peritonitis induced sepsis concluded that although the colloids albumin and HES improved cardiac output, oxygen delivery and lowered blood lactate levels, survival was no different than with the use of the crystallloid, RL [35].

In summary, the majority of rodent studies support the use of saline as the preferred resuscitative agent in treatment of severe sepsis and septic shock, though substantial variability in the method to induce sepsis, choice of study outcomes, and multiple concomitant treatment modalities significantly limits generalizations and may account for some of the variable findings among studies. There is a shortage of fluid resuscitation studies in larger vertebrate animals whose physiology and immune systems and response to sepsis may be more relevant to that of humans, though in contrast to rodent models the data that does exist seems to favor colloid resuscitation. However, this data is older and no clear conclusions can be determined from the data acquired from the few studies that have been performed in larger animals in recent years. This highlights a major gap in the area of basic mechanistic understanding of sepsis resuscitation research. Although guidelines are established for the types of fluid used for resuscitation in severe sepsis, research in animals testing these guidelines could provide insight into the mechanisms responsible for differential effects of intravenous fluids, and preclinical studies of modifications to guideline recommendations could clarify which patients may benefit from certain types of resuscitation given the significant heterogeneity of the sepsis syndrome in human patients.

**Effect of resuscitation fluid volume on outcomes in sepsis animal models**

Not only is the type of fluid utilized for resuscitation during sepsis important, but also the volume infused. The goal of fluid resuscitation is to combat hypovolemia and restore blood pressure to maintain perfusion pressure so that adequate blood is received by the tissues [13]. In administering fluids, care must be taken to not overload the patient with fluids, as this can also be detrimental to septic patients and result in edema [36,37] as well as organ damage and dysfunction [36, 38-41].

The number of studies directly comparing fluid volumes is limited, though their results appear congruent (Table 2). A mouse CLP model was used to compare the impact of varying subcutaneous fluid resuscitation regimens on cardiovascular performance and survival. In this study, the group receiving high volume (100ml/kg at T0, T6, T12; 35 ml/kg every 6 hours T18-T72) resuscitation with normal saline (5% dextrose) had the most improved CV function and higher survival compared to the low (35ml/kg at T0) and intermediate (35ml/kg at every 6 hours T0-T72) volume groups [42]. Other studies using the endotoxic pig model have drawn similar conclusions: a) higher volumes of fluid resuscitation in early stages of septic shock were superior at maintaining cardiovascular function without causing excessive lung water (pulmonary edema) [43,44]; and b) a positive fluid balance from high volume resuscitation does not negatively impact cellular metabolism during endotoxic shock [45].

Based on this data, in comparison to fluid type, very few animal studies have focused on the impact of resuscitation volume on sepsis outcomes, and have focused primarily on the impact of aggressive or high volume fluid resuscitation during early sepsis, significantly limiting the generalization of these conclusions. In the clinical setting, patients do not always present during the early stages of sepsis and the impact of fluid volume in relation to timing is an area that requires further research, especially since clinical studies do not illustrate the same uniformly positive response to large volume resuscitation [46-50]. Studies examining the impact of high, intermediate, and low volume resuscitation regimens on cardiovascular performance, development of edema, capillary leakage, organ damage, and overall outcome at various time points during sepsis, both early and late, are needed to fill this knowledge gap. Furthermore, closer observation of the response to fluid resuscitation at various times points in the course of this disease may help to develop criteria for identifying the stage of sepsis in which a patient presents based on their response to...
treatment. The ability to determine where a patient is on the disease spectrum may be of great benefit to clinicians in deciding on a course of treatment and may help to make treatment of septic patients more individualized and targeted.

Insights into the mechanisms underlying the outcomes associated with fluid resuscitation in sepsis

Equally important to the outcomes associated with fluid resuscitation type and volume in treatment of sepsis are the underlying mechanisms mediating the responses to such treatment. A limited number of studies have begun to elucidate the mechanism that mediate the physiologic and neurologic responses to sepsis and the effects of fluid resuscitation in both rodent and larger animal models of sepsis.

Early studies in the endotoxic rat determined that circulating TNF-α levels were significantly reduced in resuscitated animals compared to non-resuscitated controls. The lowered TNFα was associated with decreased total peripheral resistance (TPR), higher cardiac index, and higher overall survival [51]. Additional studies using a rabbit model of sepsis highlighted the differences that exist in mechanical responses of different vessels in an organism to fluid resuscitation with saline: fluid resuscitation resulted in relaxation of arteriolar smooth muscle cells, and contraction of aortic smooth muscle cells [52]. We must, therefore, consider the regional and systemic responses to resuscitation treatment and tailor studies and treatments to account for such differences within the individual animal and patient.

Subsequent studies in the rat and hamster determined that NO has a pivotal role in the pathophysiology of sepsis as well as the response to resuscitation with fluids. The endothelium displays a vasodilatory response to fluid loading to accommodate the increase in flow and shear stress. Losser, et al. determined that in endotoxic rats, this endothelial response is impaired due to an alteration in the NO-dependent vasodilation [53]. Using the hamster as a model of LPS-induced sepsis, a recent study determined that increasing or restoring NO bioavailability during sepsis increased functional capillary density and arteriole dilation while decreasing leukocyte endothelial interactions and sequestration. This was associated with an overall increase in survival [54]. Importantly, other studies have determined that too much NO can result in excessive microvascular permeability leading to edema and increased morbidity in sepsis [55]. This highlights the importance of maintaining the delicate balance of NO and possibly other factors during treatment to optimize outcomes associated with fluid resuscitation.

Another factor that has been identified as having an important role in the response to fluid resuscitation is the vasopressin 1 receptor (V1 R). Vasopressin is a neurohypophyseal protein that is essential for cardiovascular homeostasis and is increased in response to shock [56]. Batista, et al. determined that in the endotoxic rat, during periods of low vasopressin secretion, resuscitation with fluids can trigger the release of vasopressin to activate the V1 R which is required for the increased blood pressure response to fluids [57]. This same group later determined that the same pathway was active and responsible for responses to fluid resuscitation in the CLP rat model of severe sepsis [58]. Studies are needed to determine if the vasopressin pathway involvement is conserved in higher vertebrate models of sepsis. Confirmation of these studies may identify factors in the vasopressin pathway that may be targeted for treatment of sepsis.

Due to the small number of studies that have examined the underlying mechanisms mediating responses to fluid resuscitation (Figure 1), there remains a significant knowledge gap in this area, as well. Studies to determine the mechanisms that contribute to both positive and negative responses to fluid resuscitation can identify new therapeutic options for more targeted therapies for this disease. Furthermore, this could result in improvement in management of sepsis and a decrease in morbidity associated with fluid overload.

**CONCLUSION**

Data obtained from both pre-clinical and clinical studies has enhanced our knowledge and resulted in the improvement of treatment for patients with sepsis. However, data obtained in pre-clinical studies does not always mirror that reported in the clinical setting. These discrepancies lie in inherent differences between human and animal immunology and physiology.

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**Table 2: Articles examining effect of resuscitation fluid volume on outcomes in sepsis animal models.**

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<td>Zanotti-Cavazzoni 2009</td>
<td>mouse</td>
<td>CLP</td>
<td>subcutaneous every 6 hours</td>
<td>Low: 35 ml/kg- T0</td>
<td>High volume &gt; Low or intermediate</td>
</tr>
<tr>
<td>Wu 2013</td>
<td>pig</td>
<td>IV LPS</td>
<td>IV</td>
<td>Low: 40 ml/kg; Intermediate: 80 ml/kg; High: 120 ml/kg</td>
<td>High volume &gt; Low or intermediate</td>
</tr>
<tr>
<td>Yu 1997</td>
<td>pig</td>
<td>IV E. coli</td>
<td>IV</td>
<td>Fluid administration until pulmonary artery occlusion pressure of 12 mmHg (high) or 8 mmHg (low)</td>
<td>Higher volume = lower volume</td>
</tr>
<tr>
<td>Koci 2011</td>
<td>pig</td>
<td>IV E. coli</td>
<td>IV</td>
<td>20 ml/kg/hr ~ 24 hrs</td>
<td>Positive fluid balance does not harm cellular aerobic metabolism</td>
</tr>
</tbody>
</table>

**Abbreviations:** CLP: Cecal ligation puncture; IV: Intravenous; LPS: Lipopolysaccharide; E. coli: Escheria coli

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**Notes:**

- The abbreviations and symbols used in the table are not clearly defined in the text. They should be clarified for better understanding.

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**References:**

- For a full list of references, please consult the original publication. The reference to Cornelius et al. (2016) is included as an example.

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differences within animal species, and differences in models used to induce sepsis. There remains a need for the development of more appropriate animal models of sepsis where induction of sepsis is more closely comparable to that of sepsis in humans. Furthermore, the course of the disease and responses to current therapy should also mimic those observed in patients. Only after these conditions are optimized can we move forward in identifying underlying mechanisms that contribute to the pathophysiology associated with sepsis progression and patient responses to treatment. Identification of these pathways could then be used as reliable therapeutic targets, both immunological and physiological, and better understanding of the natural history of this devastating disease.

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REFERENCES


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