Experimental models of sepsis and septic shock: an overview

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ABSTRACT - Sepsis remains a major cause of morbidity and mortality in surgical patients and trauma victims, mainly due to sepsis-induced multiple organ dysfunction. In contrast to preclinical studies, most clinical trials of promising new treatment strategies for sepsis have failed to demonstrate efficacy. Although many reasons could account for this discrepancy, the misinterpretation of preclinical data obtained from experimental studies, and especially the use of animal models that do not adequately mimic human sepsis may have been contributing factors. In this review, benefits and limitations of various animal models of sepsis are discussed to clarify the extent to which findings are relevant to human sepsis, particularly with respect to the subsequent design and execution of clinical trials. Such models include intravascular infusion of endotoxin or live bacteria, bacterial peritonitis, cecal ligation and perforation, soft tissue infection, pneumonia or meningitis models, using different animal species including rats, mice, rabbits, dogs, pigs, sheep and nonhuman primates. Despite several limitations, animal models remain essential for the development of all new therapies for sepsis and septic shock, because they provide fundamental information about the pharmacokinetics, toxicity, and mechanism of drug action that cannot be duplicated by other methods. New therapeutic agents should be evaluated in infection models, even after the initiation of the septic process. Furthermore, debility conditions need to be reproduced to avoid the exclusive use of healthy animals, which often do not represent the human septic patient.


Introduction

Sepsis is a major cause of morbidity and mortality in surgical patients and trauma victims, in spite of all technical improvements and advances in supportive treatments. Sepsis affects approximately 700,000 people annually and accounts for about 210,000 deaths per year in the United States, contributing to an annual healthcare expenditure of $16.7 billion. Sepsis is a clinical syndrome that results from a complex interaction between host and infectious agents, and it is characterized by systemic activation of multiple inflammatory pathways, including cytokine network and coagulation. Hemodynamic changes, widespread microcirculatory disturbances and cellular alterations, leading to an uncoupling between blood flow and metabolic requirements, are implicated in the development of multiple organ dysfunction, responsible for most of deaths.

Unfortunately, while epidemiologic data show that the incidence is rising at rates between 1.5% and 8% per year, there is little evidence of substantial improvement in survival since the 1970s. This upsurge has been attributed to a host factors, including the increased use of cytotoxic and immunosuppressive therapies, the aging of the population, a heightened frequency of infection from antimicrobial-resistant pathogens, a high prevalence of chronic debility disease and the increased use of invasive devices for diagnostic, treatment and monitoring. In the last three decades, considerable effort and expenses have been spent in animal and clinical studies addressing the pathophysiology and treatment of this syndrome. However, in contrast to preclinical studies, most clinical trials of promising new treatment strategies for sepsis have failed to demonstrate efficacy. Although many reasons could account for this discrepancy, the misinterpretation of preclinical data obtained from experimental studies, and especially the use of animal models that do not adequately mimic human sepsis may have been contributing factors.

In this review, benefits and limitations of various animal models of sepsis are discussed to clarify the extent to which findings are relevant to human sepsis, particularly with respect to the subsequent design and execution of clinical trials.

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Animal models

Several animal models replicate the signs and laboratory findings seen in human sepsis. Such models include intravascular infusion of endotoxin\textsuperscript{3-5},\textsuperscript{8,10-17} or live bacteria\textsuperscript{2,10,18-20}, bacterial peritonitis\textsuperscript{8,25,31}, cecal ligation and perforation\textsuperscript{32-34}, soft tissue infection\textsuperscript{35}, pneumonia model\textsuperscript{16,37}, and meningitis model\textsuperscript{38}. Different animal species have been used including rats, mice, rabbits, dogs, pigs, sheep and nonhuman primates\textsuperscript{39}. Even though those models replicate many of the features of sepsis, it is important to critically evaluate the extent to which they mimic the septic picture\textsuperscript{2}. With this objective, we present some of the important features of the sepsis models that have been currently used.

Endotoxicosis models

Endotoxin is commonly used in animal models of sepsis, although there is controversy over their relevance to our understanding of human sepsis. When administered to human subjects, endotoxin may mimic many of the features of sepsis\textsuperscript{39}. In critically ill patients, increased concentrations of serum endotoxin have been associated with the development of sepsis, disease severity, and mortality\textsuperscript{4,6,39}. Detectable levels of LPS are identified in up to 75 per cent of patients with sepsis in intensive care setting\textsuperscript{10}. Serum endotoxin levels often remain undetectable in more indolent forms of uncomplicated sepsis with the recorded levels being of no prognostic significance\textsuperscript{4}. Very high levels are occasionally found in meningococccemia and at the start of bactericidal antibiotic therapy\textsuperscript{10}. The plausibility of the hypothesis that endotoxin plays a significant role in the pathogenesis of sepsis is supported by many studies that show that antibiotic administration may lead to an sudden release of massive amounts of endotoxin from dead bacteria and an acute hemodynamics worsening\textsuperscript{6,8,10,12,21,24}.

Endotoxin or lipopolysaccharide (LPS), the principal component of the gram-negative bacterial cell wall, stimulates the release of inflammatory mediators from various cell types, responsible for initiating the process of sepsis\textsuperscript{6,39}. LPS is a stable, relatively pure compound that can be stored in lyophilized form. An accurate dose can be measured and may be administered as a bolus or infusion\textsuperscript{39}. This has formed the basis for the simplest sepsis model and many endotoxicosis models have been described\textsuperscript{40}.

There are considerable differences between species in sensitivity to endotoxin. Rodents, cats and dogs are relatively endotoxin resistant, whereas humans and other animals (rabbits, sheep and nonhuman primates) show an enhanced response\textsuperscript{39,40,42}. In insensitive animals, presensitization with killed organism or D-galactosamine reduces the dose of LPS needed to produce an inflammatory response\textsuperscript{40}. Despite lower doses being more physiological, most endotoxicosis studies have continued to use high doses in non-sensitized animals. The duration and route of administration have varied between studies.

A large intravenous dose of LPS in rats produces rapid cardiovascular collapse and early death\textsuperscript{11}, whereas lower doses produce a hyperdynamic response with an early increase in cardiac output\textsuperscript{12}. Similarly, rabbits challenged with high-doses of LPS (5mg/Kg), develop a hypodynamic circulatory pattern with low cardiac output and high systemic vascular resistance, and when challenged with much smaller dose (1-3ug/Kg), they manifest a hyperdynamic state\textsuperscript{39}. In sheep, low dose of LPS (0.75ug/Kg) results in a biphasic response characterized by an early reduction and late increase in cardiac output\textsuperscript{13}, whereas prolonged extremely low-dose of LPS (9, 12 or 24mg/Kg-hr for 24 hr) produces a delayed hyperdynamic state characterized by an increased cardiac output and vasodilatation\textsuperscript{14}. As is the case in human sepsis, higher dosage of endotoxin in sheep has been associated with a more profound myocardial depression\textsuperscript{9}. Endotoxin administration in dogs (2mg/Kg) provokes a severe hypodynamic state with a sharp decrease in arterial pressure, cardiac output, hepatic blood flow, and an increase in systemic vascular resistance and blood lactate levels\textsuperscript{15}. Most nonhuman primates endotoxicosis models have used massive intravenous doses that resulted in rapid circulatory collapse and early death, while a low dose more closely resembled human sepsis with coagulopathy and progressive multiorgan dysfunction\textsuperscript{16}.

In spite of evidences that endotoxin may play an important role in the pathogenesis of sepsis, several authors have expressed concerns that the infusion of endotoxin is not a suitable model to study sepsis\textsuperscript{4,39}. These concerns are based on a number of observations: a) it is likely that the use of endotoxin, in high doses in animals that are resistant to endotoxin, has toxic effects that are not seen when low doses are administered to endotoxin sensitive species, such as man; b) endotoxin, although released by gram-negative sepsis, is not released in gram-positive bacteria, and yet mortality for these infections is similar\textsuperscript{4}, and c) administrations of corticosteroids\textsuperscript{17} and anti-TNFα\textsuperscript{41} have been shown to be effective in animal models of endotoxemia, but then failed to show efficacy when used in clinical trials\textsuperscript{4,6,7,44}. Moreover, it has been demonstrated that killed $E$. coli are much more lethal than endotoxin. As the endotoxin is only one component of gram-negative bacteria, it is theorized that the other cell wall components also contribute to systemic inflammatory response\textsuperscript{44}.

Thus, caution is needed in assessing the clinical efficacy of novel therapeutic agents in animal models of endotoxemia\textsuperscript{4}. Currently, there is general agreement among researchers that LPS injection may serve as a model for endotox shock but not for sepsis\textsuperscript{4,39}.

Intravascular infusion of live bacteria

Sepsis is a syndrome that usually evolves in a spectrum from systemic inflammatory response to septic shock. With increasing disease severity, the frequency rate of positive blood cultures increases (sepsis [17%], severe sepsis [25%], septic shock [69%]), therefore several studies suggest that bacteremia may play an important role in determining outcome in sepsis\textsuperscript{39}. Several studies have investigated the response to intravenous administration of live bacteria\textsuperscript{44}. Many different aerobic bacterial species have been investigated: $Escherichia$ coli is the most common. As with endotoxicosis models, the dose of organism and duration of infusion have varied considerably between models.

In small mammals, low doses of $E$. coli, administered over several hours, have
been associated with minimal early physiological changes, while higher doses have often produced a biphasic response with an early rise and late fall in cardiac output\textsuperscript{40}.

In large mammals studies, higher microbiological doses have invariably been used. In baboons, the intravenous LD\textsubscript{50} dose of \textit{E. coli}, as a bolus, induced an exaggerated TNF\textalpha{} response with cardiovascular collapse and early death. In this model, pretreatment with a TNF\textalpha{} inhibitor improved hemodynamic performance and survival, confirming the role of TNF\textalpha{} in cardiovascular collapse with subsequent organ damage and death, observed in sepsis\textsuperscript{21}. Recently, Taylor described a severe disseminated intravascular coagulation induced by both sublethal and lethal dose of live \textit{E. coli} and endotoxin in baboons\textsuperscript{10}. This threatening condition results from a complex inflammatory and hemostatic response that involve the microvascular endothelium and its regulatory anticoagulant networks, and contributes to development of a multiorgan dysfunction\textsuperscript{10}.

Interestingly, in a porcine model using both gram-positive and gram-negative bacteria at the same dose, the hemodynamic and pulmonary changes depended on the bacterial species used. While \textit{Staphylococcus aureus} induced minimal changes, both \textit{E. coli} and \textit{P. aeruginosa} resulted in shock and acute respiratory failure\textsuperscript{22}.

In an ovine model, a nonlethal dose of \textit{E. coli} promoted a hyperdynamic cardiovascular response with hypotension, increased output, tachycardia, fever, oliguria, tachypnea and hyperlactatemia\textsuperscript{3}. In a porcine model, \textit{P. aeruginosa} infused over a week resulted in biphasic changes to the cardiac output, with late systemic hypotension and pulmonary hypertension\textsuperscript{23}.

In dogs, both sublethal and lethal intravenous dose of live \textit{E. coli} promoted early profound cardiovascular deterioration with hypotension, very low cardiac output, splanchic hypoperfusion and severe metabolic changes\textsuperscript{19,20}. Animals challenged by a lethal dose of \textit{E. coli} (1.2 x 10\textsuperscript{10} cfu/Kg) presented only partial and transient improvements in systemic and regional blood flows during fluid resuscitation, but the progressive cardiovascular collapse was unavoidable (Figure 1).

![FIGURE 1 - Experimental model of septic shock induced by lethal intravenous dose of live E. coli in dogs. Changes in mean arterial pressure, cardiac index, portal vein blood flow index and PCO\textsubscript{2}-gap (pCO\textsubscript{2} gastric mucosal-arterial gradient) during the experimental protocol (mean ± SEM). BI: bacterial infusion (1.2 x 10\textsuperscript{10}cfu/Kg over 30 min), FR: fluid resuscitation period, CT: controls, no fluid (n=7) and RL: lactate Ringer’s solution 32ml/Kg over 30 min (n=7). Modified from Garrido AG\textsuperscript{29}.](image-url)
aggressively resuscitated at the time and studied 24 hr later. The animals were infused intraarterially with viable canine model wherein animals were chronically instrumented unastetized bacteremia. Shaw and Wolfe described a collapse secondary to overwhelming infusion of live bacteria are acute prepara-
tion, specially in the absence of fluid resuscitation18,32,41.

Not all models using an intravascular infusion of live bacteria are acute preparations characterized by cardiovascular collapse secondary to overwhelming bacteremia. Shaw and Wolfe described a chronically instrumented unastetized canine model wherein animals were infused intraarterially with viable E. coli and studied 24 hr later. The animals were aggressively resuscitated at the time sepsis was induced, the dose of bacteria being invariably lethal in the absence of adequate restoration of intravascular volume. However, with the fluid resuscitation, 85% of the animals survived the protocol and, at the time of study, were hyperdynamic and hypermetabolic24. Additionally, many of the typical hormonal perturbations in septic humans were observed. Although not widely utilized, this model mimics many of the features of clinical sepsis and avoids the confounding effects of anesthesia and surgical preparations.

Since most patients are not challenged with a massive bacterial load at any time, but rather harbor a septic focus that is intermittently and persistently, showering the body with bacteria, several authors have questioned the relevance of models utilizing a bolus infusion of viable bacteria39.

Additionally, concerns over the use of an appropriate strain of an infective organism extend also to studies of endotoxemia where the most commonly used strain of endotoxin is uncommonly seen in human bacteremia. There are a number of features of either the host or host-bacterium interactions that are species-specific4. For example, Salmonella typhi does not cause systemic infection in laboratory rodents but is responsible for typhoid fever in humans. In mice, a related bacterium, Salmonella typhimurium, causes a systemic infection and it is commonly used as a model of human typhoid infection, despite its low virulence in humans4. Individual bacteria may also cause a broad spectrum of disease process depending on the expression of virulence genes. These findings suggest that it may be difficult to make conclusions regarding the efficacy of a therapy based on a study looking at infection with a single bacteria strain, particularly if it is a pathogen not commonly seen in critically ill patients4.

Despite these criticisms, numerous laboratories continue to utilize intravascular infusions of viable bacteria to induce sepsis in animals, and many of these models remain very useful, provided that certain inherent limitations are recognized39.

Peritonitis models
Peritonitis may be induced in animals by several techniques. Bowel can be perforated allowing contamination with gastrointestinal contents, or inoculum of fecal material or pure bacterial cultures can be instilled into the peritoneal cavity40. In early models, segments of intact bowel were isolated and the development of peritonitis was expected40. The disadvantage of this method was that the onset of peritonitis was uncontrolled and depended on the timing of gastrointestinal perforation. To overcome this limitation, the simple and reproducible cecal ligation and puncture model (CLP) was developed and has been used widely in sepsis research33. The cecum is ligated distal to the ileocecal valve and perforated using two needle punctures. Needle size can be used to manipulate CLP to give a lethal and nonlethal sepsis3. The model was originally described in rats, but has been extended successfully to other species32,34.

The principal advantage of CLP models is their simplicity. Since sepsis is induced by a straightforward surgical procedure, there is no need to grow and quantify bacteria or in other ways prepare the inoculum. Furthermore, these are models of sepsis due to peritoneal contamination with mixed flora in the presence of devitalized tissue and thus bear an obvious resemblance to clinical problems.
like perforated appendicitis and diverticulitis. This technique, without fluid resuscitation, promotes rapid onset of shock, while after fluid resuscitation the mortality rate may be reduced with pathophysiological responses resembling those noted in human sepsis.

However, in CPL model, it is difficult to control the magnitude of the septic challenge. In studies using small animals (mice and rats), the problem of variability is easily overcome by increasing sample size; whereas, variability remains a problem in larger species. The gastrointestinal contents of animals, particularly herbivores, vary between species. Initial attempts at inducing peritonitis by intraperitoneal implantation of feces were often disappointing, with animals appearing tolerant to their own fecal flora. To overcome those limitations, human feces were used, or barium sulphate, bile salts, or autologous haemoglobin added to the fecal material. Polymicrobial peritonitis induced in rats, by a standard inoculum of pooled fecal material derived from several animals, produced a hyperdynamic sepsis response, with a high mortality rate in unresuscitated animals. The advantages of this model include good reproducibility and the possibility to study dose-response relationship both systematically and in remote organ systems, where the intensity and the kinetics of systemic and pulmonary inflammatory responses to polymicrobial peritonitis in mice substantially depend on the inoculum size.

Both cecal ligation and puncture and fecal inoculation models deliver a variable microbiological dose, so pure bacterial culture peritonitis models have been developed. In 1980, Ahrenholz and Simmons showed that 24-hr mortality was 100% when viable E. coli suspended in saline were injected intraperitoneally in rats. However, when the same number of bacteria was implanted intraperitoneally in a bovine clot, early mortality was prevented, but the rats developed abscess and 10-day mortality was 90%. Thus, fibrin delays the systemic absorption of the entrapped bacteria and promotes the development of chronic intraperitoneal abscess, a more local septic focus. Based on this original work, a canine model of sepsis, produced by the intraperitoneal implantation of a fibrin clot containing viable E. coli, was developed.

This highly reproducible model, has been described in small and large mammals and displays many features of human sepsis including insidious onset, hyperdynamic cardiovascular state, reversible left ventricular dilatation with impaired systolic performance, and a significant mortality rate. Unlike other peritonitis models using fecal implantation or cecal ligation/perforation, the fibrin clot model allows the investigator to have complete control over the dose of bacteria and the type of organism implanted. Some have used single-organism cultures, whereas others have used mixed cultures that more accurately mimic the gastrointestinal flora. However, a single-organism fails to reproduce the synergy between aerobic and anaerobic organisms seen in human peritonitis, where the aerobic gram-negative organism appear responsible for many of the acute physiological features of sepsis while anaerobes appear to contribute to the development of intraperitoneal abscesses.

**Limitations of all sepsis models**

Animal models have been chosen largely on the basis of traditional practices, familiarity of individual investigator, economic considerations, availability, and ethical acceptability. Rodents should generally be the animals of choice at the beginning of preclinical investigations. Yet rodents are quite endotoxin resistant, have limited vascular access and blood volume, and have cardiovascular physiologies that differ substantially from humans. Endotoxemia and bacteremia represent models without an infectious focus. They reproduce many characteristics of sepsis and are highly controlled and standardized. However, they reflect a primarily systemic challenge and create neither an infectious focus nor the protracted immune reaction that characterizes sepsis. In this respect, any model with an infectious focus is decisively closer to clinical reality.

In most animal models of infection, gram-negative bacteria have been used. This does not reflect the diversity of infectious agents, sites of infection, and progress of the infection encountered clinically. For example, in recent clinical trials, gram-positive organisms and fungi have exceeded gram-negative organisms as a cause of sepsis, but they are infrequent in animal studies. Additionally, there is an increasing concern about possible important differences in host inflammatory responses to sepsis due to gram-positive versus gram-negative bacteria. Experimental evidences suggest that the efficacy of mediator-specific anti-inflammatory agents in sepsis may be altered by the bacteria type of underlying infection with significant differences between gram-negative and gram-positive strains.

Recently, the use of antibody to TNFα improved the host defense and survival rates with both lethal E. coli and S. aureus pneumonia, but the protection was greater with E. coli, where TNFα concentrations were higher than with S. aureus. In some experimental models, anticytokine therapy has been effective in the setting of systemic intravascular challenge with live bacteria or endotoxin, but is of little benefit, or potentially harmful, in models of localized infection. Intravenous challenge, due to the relatively large inoculum required, probably constitutes a model of endotoxin intoxication rather than evolving infection. Intraperitoneal challenges typically require 100- to 1000-fold fewer bacteria. Thus, the models used most extensively do not precisely replicate many important clinical parameters and do not duplicate the dynamic interactions among investigational drugs, microbial pathogens, and host defenses that occur in patients with sepsis.

While promising agents are studied later in larger animals, including primates, and attempts are made to mimic various aspects of human septic shock, the experimental conditions encountered in human sepsis trials are more complicated than simulated even in large-animal models. Animals are carefully selected to have no intercurrent illnesses, and to be similar genetic background, age, weight, gender, and nutritional status. These animals are then challenged with a single, well-defined, precipitating event. In contrast, patients with sepsis are heterogeneous with respect to age, preexisting conditions, sources of infection, types of infecting microorganism, and many of them have experienced trauma or major surgery. Adequacy of care, including promptness and quality of resuscitation, appropriateness of antibiotics, and timing...
quality of surgical intervention also determine survival in human settings. The natural history of severe sepsis in laboratory animals is generally distinct from human sepsis, with animals more often having a rapid onset of hypodynamic circulatory collapse and a more rapid resolution or decline to mortality. In clinical sepsis, the mortality is most commonly due to the development of multiple organ failure, days to weeks after initial presentation. For this reason, animal models that lead to significant mortality within the first 6 to 12 hours may not describe an outcome that is relevant to human.

Agents under investigation are too often administered to animals before or immediately after the septic challenge, conditions that can rarely be achieved in clinical trials. Finally, nonblinded and/or nonrandomized studies are common in animal research, and introduce an easily avoidable source of bias. In addition, the desire to demonstrate a therapeutic affect may create experimental conditions that are not clinically realistic.

Conclusions

A major concern is that animal models have demonstrated protective effects that have not been reproduced in subsequent clinical trials. Many of the problems with the use of animal data in sepsis drug development stem, not from the animal models, per se, but from how those results have been adapted to clinical trial design. Animal models provide insights about specific components of the septic process but cannot truly mimic the full clinical complexity and intrinsic heterogeneity of patients with sepsis. Despite these limitations, animal models will remain essential in the development of all new therapies for sepsis and septic shock, because they provide fundamental information about the pharmacokinetics, toxicity, and mechanism of drug action that cannot be duplicated by other methods.

Nonetheless, there is much to be improved in animal experiments. Examples are the need for long-term studies with intensive care unit-like conditions to simulate the often delays onset of organ dysfunction in the clinical setting, using sepsis or organ dysfunction criteria to start treatment instead of a fixed time schedule. New therapeutics agents should be studied in infection models even after initiation of the septic process. Furthermore, debility conditions need to be reproduced to avoid using healthy animals, which often do not represent the human septic patient.

References

27. Goldfarb RD, Marton A, Szabo E, Virag L, Salzman AL, Glock D, Akhter I, McCarthy