REVIEW

J Crit Intensive Care 2023;14:96–100
https://doi.org/10.37678/dcybd.2023.3488

Experimental Animal Models of Sepsis and Septic Shock

Esin OZCELEBI[1], **Alper Bektas ISKIT**[1]

[1]Hacettepe University Faculty of Medicine, Department of Medical Pharmacology, Ankara, Turkey

Cite this article as: Ozcelebi E, Iskit AB. Experimental Animal Models of Sepsis and Septic Shock. J Crit Intensive Care 2023;14:96–100

Corresponding Author: Alper Bektas Iskit
E mail: alperi@hacettepe.edu.tr

Received: Oct 02, 2023
Accepted: Oct 11, 2023
Available online: Oct 17, 2023

ABSTRACT

A typical inflammatory reaction to several infections leads to sepsis. It continues to provide a picture of a complicated heterogeneous disease that frequently results in the emergence of many organ dysfunctions and kills millions of individuals globally. Numerous attempts have been made to simulate sepsis because it is challenging to perform extensive studies on sepsis in humans. To explain the pathogenetic causes of the disease, its clinical and paraclinical aspects, as well as chances for its therapeutic management, the goal of this review is to provide the most popular experimental sepsis models ever created. This review is based on a thorough analysis of the literature. The types of laboratory animals, infections used, and ways in which they are introduced into the organism vary between the cited models. Models can be classified as "non-surgical" (with parenteral administration of endotoxins or pathogenic bacteria) or "surgical" (referring to preceding operative intervention intended to induce peritonitis, such as ligation and subsequent puncture of the cecum, insertion of a stent in the wall of the ascending colon, implantation of bacterial cultures, or pathogens included in the composition of different carriers). The pros and cons of the models under examination are taken into account, as well as how closely they resemble clinical sepsis in all of its manifestations, grading each step in the septic process. The reality is that none of the models that have been developed can accurately capture the intricate, polymorphic, and dynamic nature of sepsis. However, any of them can offer trustworthy details on specific steps in the septic process.

Keywords: sepsis, septic shock, experimental, animal, lipopolysaccharide, cecal ligation and puncture

The goal of animal-based research is to better understand the pathophysiology of various diseases, unravel complicated physiological processes, and test therapeutic approaches and medications for their potential before progressing to clinical trials. Findings from animal studies are increasingly being used to advance both human and veterinary care. Similar to other medical specialties, the use of animal models in the study of sepsis, shock, and trauma has been recognized, and *in vivo* results have led to numerous significant advancements in those domains. Animal-based research does, however, occasionally contain errors, use subpar investigative techniques, and lacks standards in some instances (1). Such flaws result in incorrect data, which may then be translated into inaccurate conclusions that mislead decisions on the transition from animals to humans (such as whether to begin phase 1 clinical trials or not).

To enable interventions that are more closely correlated with the clinical intensive care situation and are therefore considered valuable for proof-of-concept studies before human investigations, large mammals like sheep and pigs have been utilized in sepsis research. These studies include the invasive measurement of numerous physiological parameters, which can be technically difficult in smaller species, and high-volume fluid resuscitation to simulate the hyperdynamic condition of the cardiovascular system (2). The most popular species in preclinical research is rodents. This is because of the simplicity of genetic alteration, huge litter sizes, rapid generation times, and very simple housing and maintenance requirements (3).

It is impossible to avoid conducting rodent experiments due to the well-studied nature of the species, the similar gene expression patterns that are seen during inflammation in rodents and humans, the similarity between the physiological, genetic, and biochemical characteristics of rodents and humans, the low cost, simplicity, and

Available online at
http://www.jcritintensivecare.org/

ethical complexity of rodent experiments. In addition, there is a sizable panel of genetically altered rodents with altered immune system components that offer more chances to look into the molecular pathways behind sepsis pathogenesis. Because the disease development in humans is more accurately reflected in such models, it is crucial to use humanized rodent strains more widely to experimentally simulate sepsis. However, it should be remembered that humanization largely affects immune system components (4).

Experimental septic shock models

Septic shock is a significant clinical entity with a death rate of 30 to 90%, despite the numerous therapy regimens using cutting-edge medications, antibiotics, or immunomodulatory medicines. Preclinical investigations using animal models of septic shock provide useful information for clinical studies, although the suitability of these experimental models is still up for debate (5). There is no common experimental model because diverse models can be applied to different animal species. One animal model cannot adequately represent all clinical facets of this complex illness. The absence of research with Gram (+) bacteria as opposed to Gram (-) bacteria and variations in endotoxin response between laboratory animals and human clinical investigations are the other causes of difference. Combining current techniques can greatly advance analysis (6).

1. Live pathogen administration through intravenous or intraperitoneal route

To mimic septic shock, bacteria, particularly an *E. coli* strain, might be infused or injected once. Another technique for mimicking septic shock is intraperitoneal inoculation, which involves inserting gelatin capsules containing germs or fecal material (inoculum) after an abdominal midline incision. Animals in the control group have sterile gelatin capsules inserted into their abdomens.

After intravenous delivery of 10^8-10^9-10^{10} Colony Forming Unit (CFU) per kilogram, the hyperdynamic phase of septic shock is reached in roughly two hours. A clinically acceptable model will be one that incorporates fluid resuscitation or vasoactive support for the improvement of the hemodynamic condition in bigger experimental animal species. A rapidly lethal model, intraperitoneal injection of bacteria (*E. coli*, *Klebsiella*, or *B. fragilis*) had a 100% mortality rate within 24 hours. Intraperitoneally implanted osmotic minipumps are used to achieve regulated bacterial discharge and delay early mortality. A controlled bacterial release model investigation has been run for 18 to 20 days.

Models involving delivery of live pathogen are not clinically correlated since animals are exposed to large bacterial loads over time. In some investigations, excrement or agarose are added to balance out the bacterial load (7).

It should be emphasized that sepsis is typically caused by a polymicrobial infection, whereas just one bacterial strain is frequently used in these models. Lab workers and researchers should take contamination danger into account.

2. Intravenous or intraperitoneal lipopolysaccharide administration

One of the intricate pathogen-associated molecular patterns released by an organism is lipopolysaccharide (LPS). Lipopolysaccharide is a glycolipid compound that is extracted from the cell wall of Gram (-) bacteria for use in septic shock research. Commercially, LPS is offered as lyophilized powder. The manufacturer carries out the chemical process of lyophilization, which entails phenol/trichloroacetic acid/phenol-chloroform-ether extraction. Lipopolysaccharide is produced by a variety of Gram (-) bacterial species, including *E. coli*, *S. typhimurium*, *K. pneumoniae*, *P. aeruginosa*, and others, and it is often used in experimental models of septic shock. Different strains of *E. coli* have different serotypes. In septic models induced with LPS administration metabolic, immunological, physiological, toxicological, and pharmacological data are provided even if endotoxin and LPS administration (8–12). Although LPS and endotoxin are sometimes referred to as the same thing, they differ from one another in terms of molecular structures. Lipopolysaccharide is a chain of glycolipids that has been purified, and Lipid A is the hazardous component. Endotoxin also includes cell wall proteins, lipids, lipoproteins, and polysaccharides in addition to the lipopolysaccharide chain.

Lipopolysaccharide that has been dissolved is injected intraperitoneally or intravenously as a single dosage or as part of an infusion. The LPS dose can be adjusted from 1 to 80 mg/kg according to the study's goal. For investigations on septic shock survival, higher dosages are desirable (5). Another important consideration for choosing a dose is the bacterial strain from which LPS is produced. The clinical profile change depending on the bacterial strain or LPS dosage.

The best laboratory animal to use for this model is still up for debate. The following is a list of factors influencing the selection:

a) Cost,
b) Accessibility,
c) The researchers' prior experiences,
d) Compliance with the study's purpose and premise.

For LPS studies, mice, rats, and guinea pigs perform better in scientific settings than dogs, pigs, and sheep because they are more readily available, less expensive, and require less upkeep before and after experiments. Small species are chosen in particular for investigations on survival. It is simpler and more effective to design an experimental study using smaller species to assess isolated organ functions using an *in vitro* or perfusion method (3). The same is said for histological investigations. On the other hand, it is difficult to offer supportive care, such as fluid resuscitation, intubation, or mechanical ventilation, to balance the hemodynamics in smaller laboratory animals (2).

Septic shock models with LPS delivery have drawbacks

1. The outcomes of investigations using LPS that used various extraction techniques or serotypes are inconsistent with one another. Since the purity of the various products is different, it is important to take into account the bacterial strain, LPS serotype, and extraction technique while designing an experimental investigation.

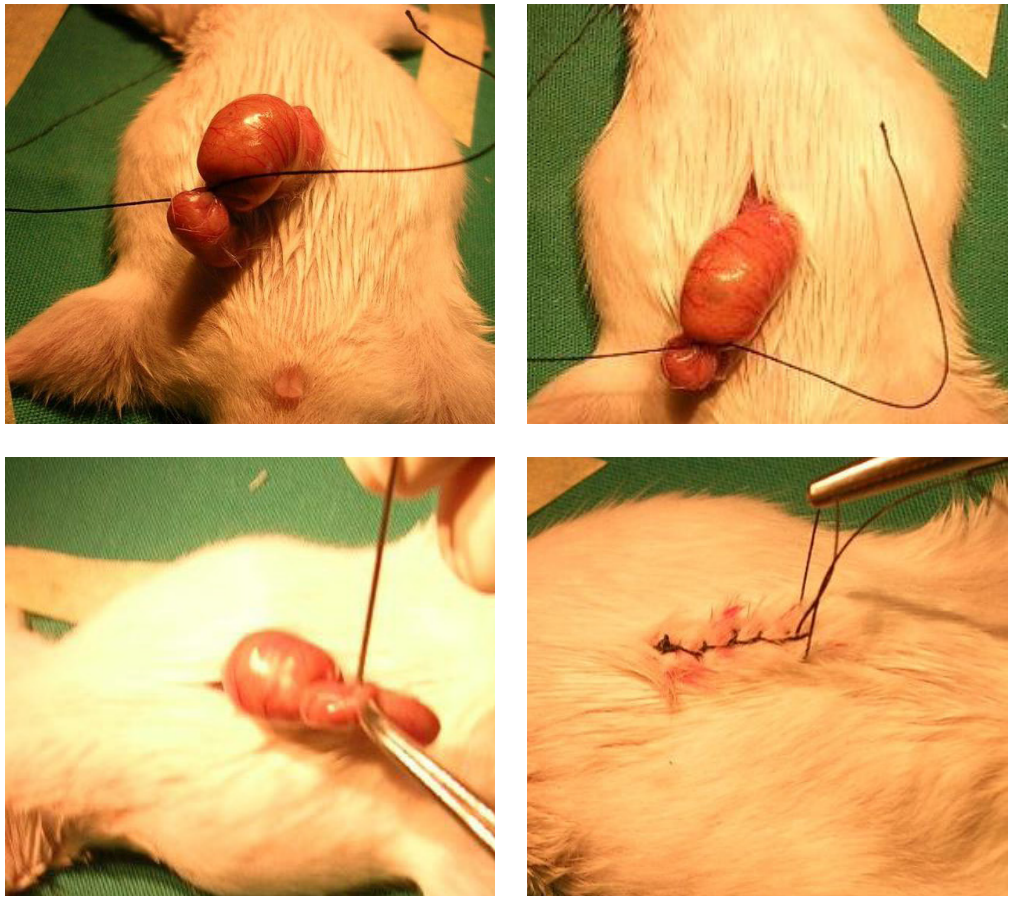

Figure 1. Representation of cecal ligation and puncture method on rat

2. Plasma endotoxin levels, clinical or microbiological results, or prognosis do not appear to be correlated with one another. In this model, higher endotoxin levels attained with a single injection or infusion are not clinically associated with septic patients. Due to a significantly higher LD_{50} for LPS in rats, LPS doses differ from those causing equivalent effects in humans.
3. Human septic shock, which possesses microbial diversity, cannot be replaced by models with the endotoxin of a single microbe. For its microbiological variety (polymicrobial), the "Cecal ligation and puncture" approach is favoured in several research (13).
4. There is an early phase of sepsis termed as the hyperdynamic phase of sepsis which is characterized by a compensatory increase in cardiac output and lower systemic vascular resistance. Low systemic vascular resistance, which is an indication of prognosis, persists as cardiac output declines over time. High dosages of LPS in animal models reduce cardiac output and increase or do not effect on vascular resistance. Low dosages of LPS trigger the hyperdynamic phase, which the model uses to depict the early, compensatory stage of human sepsis.

Septic shock models with LPS delivery have the following benefits

1. The intravenous or intraperitoneal injection of a single dose makes the model simple to perform.
2. Due to the stability and purity of its lyophilized state, LPS is easier to store than live pathogens and has a lower risk of contamination.

The multistage clinical course of sepsis is greatly simplified by these controlled reproducible models, which instead replicate some characteristics of endotoxemia or the acute phase of Gram-negative sepsis, such as the absence of an infection focus, the development of a hypodynamic stage without an earlier hyperdynamic stage, lactic acidosis, short-term and abundant production of proinflammatory cytokines, increased expression of DAMPs (such as HMBG-1), and strong activation.

3. Cecal ligation and puncture (CLP)

In the therapeutically most appropriate contemporary models, the gut integrity is damaged, simulating polymicrobial peritonitis by allowing microbiota components to enter the peritoneal cavity. Other sepsis-related characteristics, such as the activation of both proinflammatory and antiinflammatory immune responses, are also present in the model. The rapidly increasing proinflammatory phase of sepsis is followed by a compensatory antiinflammatory phase, which frequently results in immunosuppression. From another perspective an early hyperdynamic phase, characterized by a higher cardiac output and a higher systemic vascular resistance, and a late hypodynamic phase, characterized by numerous organ failure, hypothermia, metabolic changes, the formation of DAMP, and a comparable kinetics of the cytokine response, observed in this model (14,15).

Cecal ligation and puncture is based on the closure of a section of the cecum with a standard thread without obstructing the intestinal tract, followed by a single or double puncture (cecal

colotomy) with a standard-sized syringe needle. Following surgery, the abdominal cavity is sealed off, and the animals are given subcutaneous fluid for resuscitation based on their weight (16). Despite being largely used on rats, this technique has also been used on mice and lambs.

Using this method, there are three insults: 1. Polymicrobial sepsis from fecal spills following needle punctures; 2. Surgical damage to tissues; and 3. Ischemic tissues of the ligated cecum. The ligated and ischemic cecal tissue adds to the observed immunological dysfunction in addition to facilitating bacterial spread.

Cecal ligation and puncture models have drawbacks

It's possible to see variations and discrepancies in the findings of each study. Here are some potential explanations for this circumstance:

1. Different anatomical levels or lengths of ligation in the cecum,
2. Digestive passageway obstruction,
3. The quantity of cecal colotomies performed and the needle's gauge (often between 18 and 25 gauge)
4. Increased intestinal motility following puncture,
5. The results are conflicting in starved and fed animals like other shock models (17),
6. The requirement of fluid resuscitation. Death rates will rise if fluid resuscitation is not perfomerd, since the hyperdynamic phase won't start.

Because the method is difficult to standardize due to the variation in surgery parameters, such as the type of anesthesia, the laparotomy technique, the length of the cecal ligation, the needle size, and the number of punctures, as well as the dependence on the mouse genetic strain, gender, age, microbiota composition, and rearing conditions, it should be noted that the model is only reproducible when the experimental animal sample size is large enough.

Benefits of the cecal ligation and puncture technique

1. The method is affordable and simple to use. Lipopolysaccharide or microorganisms do not need to be administered,
2. Constant source of bacterial release is ensured,
3. Unlike other models, this one is polymicrobial, and different microorganisms are in charge of producing the clinically appropriate septic shock profile in septic patients,
4. Cecal ligation and puncture mirror a clinically comparable pattern that results in septic shock (perforated appendicitis, diverticulitis, and colonic perforation).

By altering the length of the cecal region to be ligated, the needle size (18–25G), and the number of punctures (to a lesser extent), as well as by implementing infusion therapy, giving antibiotics, or simulating an appendectomy by removing the necrotic cecal region through a subsequent operation, sepsis dynamics can be controlled.

4. Syndrome of systemic inflammatory response and multiple organ failure caused by zymosan

The *Saccharomyces cerevisiae* fungus's cell wall is where zymosan is found. It has diverse inorganic structures, 15% protein, 7% fat, and 73% polysaccharide. In experimental animals, administration of zymosan results in the production of several inflammatory mediators and a protracted inflammatory response. Zymosan causes a more prolonged, three-phase inflammatory response than LPS or CLP administration. It is an appropriate method for investigations that call for ongoing follow-up. Acute peritonitis is seen at a dose of 0.8–1.0 mg/g (body weight) and progresses through three phases: acute peritonitis, relative recovery, and broad systemic inflammation that results in multiple organ failure or death. It is an experimental paradigm that allows researchers to look at the extensive, chronic inflammatory response without an infection (18,19).

Conclusions

In a variety of disease areas and research fields, including sepsis, there is now heated discussion on the predictive validity and translational utility of many animal models utilized in medical research. It is crucial to recognize that every experimental model has flaws and that no animal model can ever completely mimic every aspect of human disease. However, understanding disease mechanisms and the therapeutic efficacy of novel pharmacological/genetic interventions is of critical importance, and it is for this reason that animal studies are currently conducted.

Animal models are useful for studying the pathophysiology of septic shock and collecting data prior to clinical trials in order to develop new treatment protocols and techniques, even though it may be difficult to imitate the complete clinical profile of septic shock using these models.

AUTHOR CONTRIBUTIONS:

Concept: EO, ABI; **Design:** EO, ABI; **Supervision:** EO, ABI; **Fundings:** EO, ABI; **Materials:** EO, ABI; **Data Collection and/or Processing:** EO, ABI; **Analysis and/or Interpretation**: EO, ABI; **Literature Search:** EO, ABI; **Writing Manuscript**: EO, ABI; **Critical Review:** EO, ABI.

Peer-review: Externally peer-reviewed.

Conflict of Interest: Authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

References

1. Nandi M, Jackson SK, Macrae D, et al. Rethinking animal models of sepsis - working towards improved clinical translation whilst integrating the 3Rs. Clin Sci (Lond). 2020 Jul 17;134(13):1715–34. [CrossRef]
2. Parker SJ, Watkins PE. Experimental models of gram-negative sepsis. Br J Surg. 2001 Jan;88(1):22–30. [CrossRef]
3. Korneev KV. Mouse models of sepsis and septic shock. Mol Biol (Mosk). 2019 Sep-Oct;53(5):799–814. [CrossRef]
4. Remick D, Szabó A, Juffermans N, et al. Basic research in shock and sepsis. Shock. 2023 Mar 1;59(3S Suppl 1):2–5. [CrossRef]
5. Iskit AB, Guc MO. The timing of endothelin and nitric oxide inhibition affects survival in a mice model of septic shock. Eur J Pharmacol. 2001 Mar 2;414(2-3):281–7. [CrossRef]
6. Iskit AB, Senel I, Sokmensuer C, et al. Endothelin receptor antagonist bosentan improves survival in a murine caecal ligation and puncture model of septic shock. Eur J Pharmacol. 2004 Dec 3;506(1):83–8. [CrossRef]
7. Poli-de-Figueiredo LF, Garrido AG, Nakagawa N, et al. Experimental models of sepsis and their clinical relevance. Shock. 2008 Oct;30 Suppl 1:53–9. [CrossRef]
8. Ozer EK, Goktas MT, Kilinc I, et al. Celecoxib administration reduced mortality, mesenteric hypoperfusion, aortic dysfunction and multiple organ injury in septic rats. Biomed Pharmacother. 2017 Feb;86:583–9. [CrossRef]
9. Kavuklu B, Iskit AB, Guc MO, et al. Aminoguanidine attenuates endotoxin-induced mesenteric vascular hyporeactivity. Br J Surg. 2000 Apr;87(4):448–53. [CrossRef]
10. Baykal A, Kavuklu B, Iskit AB, et al. Experimental study of the effect of nitric oxide inhibition on mesenteric blood flow and interleukin-10 levels with a lipopolysaccharide challenge. World J Surg. 2000 Sep;24(9):1116–20. [CrossRef]
11. Ozer EK, Goktas MT, Toker A, et al. Thymoquinone protects against the sepsis induced mortality, mesenteric hypoperfusion, aortic dysfunction and multiple organ damage in rats. Pharmacol Rep. 2017 Aug;69(4):683–90. [CrossRef]
12. Özer EK, Barışkaner H, Kurtgöz S, et al. Alterations in hepatic gene expressions of CYP2C11, CYP2C6V, and CYP2D3 enzymes in endotoxemic rats. Yoğun Bakım Derg. 2017; 8: 50–3. [CrossRef]
13. Ertac-Serdar S, Atilla P, Iskit AB. Effects of PARP Inhibitör 3-Aminobenzamide on impaired mesenteric blood flow and organ injury in CLP-induced septic shock model. J Crit Intensive Care. 2022;13:32–42. [CrossRef]
14. Yang S, Chung CS, Ayala A, et al. Differential alterations in cardiovascular responses during the progression of polymicrobial sepsis in the mouse. Shock. 2002 Jan;17(1):55–60. [CrossRef]
15. Ulu N, Sokmensuer C, Iskit AB. The effects of inducible nitric oxide synthase blockers on survival and organ injury in a murine caecal ligation and puncture model of septic shock. J Crit Intensive Care. 2022;13:73–8. [CrossRef]
16. Baker CC, Chaudry IH, Gaines HO, et al. Evaluation of factors affecting mortality rate after sepsis in a murine cecal ligation and puncture model. Surgery. 1983 Aug;94(2):331–5. PMID: 6879447.
17. Agalar F, Iskit AB, Agalar C, et al. The effects of G-CSF treatment and starvation on bacterial translocation in hemorrhagic shock. J Surg Res. 1998 Aug;78(2):143–7. [CrossRef]
18. Volman TJ, Hendriks T, Goris RJ. Zymosan-induced generalized inflammation: experimental studies into mechanisms leading to multiple organ dysfunction syndrome. Shock. 2005 Apr;23(4):291–7. [CrossRef]
19. Tanriverdi P, Yuksel BC, Rasa K, et al. The effects of selective nitric oxide synthase blocker on survival, mesenteric blood flow and multiple organ failure induced by zymosan. J Surg Res. 2005 Mar;124(1):67–73. doi: 10.1016/j.jss.2004.09.004. PMID: 15734481. [CrossRef]