e-ISSN:2321-6190
p-ISSN:2347-2294

Research & Reviews: Journal of Zoological Sciences

Laboratory Animal Models to Mimic Human Sepsis: A Review

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Review Article

Received date: 21/03/2016
Accepted date: 14/05/2016
Published date: 18/05/2016

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Keywords: Sepsis, Toxaemia, Cecal ligation and puncture (CLP), Colon ascendens stent peritonitis (CASP), Systemic inflammatory response syndrome (SIRS), Compensatory anti-inflammatory response syndrome (CARS).

ABSTRACT

Inflammation is a protective measure taken by body against exogenous and endogenous pathogens. It is a transient mechanism that resolves with removal of pathogenic agent, resulting in re-gain of body homeostasis. But this transient protective mechanism transforms into destructive tissue-damaging response, if dysregulated. Such a condition is called as systemic inflammatory response syndrome which further leads to multiple organ failure and ultimately death, if untreated. In order to understand the physiology, mechanism of sepsis and to test the potential therapeutics for its treatment, a suitable animal model is required. Various laboratory animal models have been developed to mimic human sepsis in laboratory, primarily rodents. Some of these commonly used models include injectable models i.e., toxaemia models and bacterial infection models and surgical 'immune barrier disruption models' i.e., cecal ligation and puncture (CLP) and colon ascendens stent peritonitis (CASP). In the present review, these models have been reviewed with respect to their advantages and disadvantages in mimicking human sepsis.

INTRODUCTION

Sepsis

Sepsis is a serious pathological condition which is characterised by dysregulated immune response followed by immune-suppression [1,2]. An immune response which is protective in nature, maintains body homeostasis after a pathogenic attack. But this protective function of the immune system transforms into tissue-damaging response if not regulated tightly. A normal inflammatory process resolves once the infective agent is removed. However, if dysregulated immune response persists, it results in systemic inflammatory response syndrome (SIRS) or sepsis. The SIRS is followed by another phase of sepsis i.e., compensatory anti-inflammatory response syndrome (i.e., CARS) [3]. The balance between SIRS and CARS influences the host survival [4]. The criteria for defining human sepsis are summarized in **Table 1** [3].

Animal Models for Sepsis

Various animal models have been developed to mimic human sepsis and their suitability for pre-clinical tests is being checked [5-7]. An appropriate animal model aims to investigate the biology of normal and pathological process, to understand the mechanism of disease, to have pre-clinical tests of potential therapeutic agents and to test the ability of any intervention to interrupt the normal or pathological process [7]. The mice are most popularly used pre-clinical animal model for sepsis research. Being small in size, easy to rear, little or no harm to laboratory personnel and availability of inbred strains and diagnostic/immunological assay kits are some of the key features that mice are preferred over large animals including dogs, cats, horses and non-human primates [8]. In the present review, some of the commonly used animal models of sepsis have been reviewed for their advantages and disadvantages. But before that, we will discuss some of the common concepts of animal modelling to mimic a syndrome like sepsis.

e-ISSN:2321-6190
p-ISSN:2347-2294

Table 1. Criteria for defining sepsis and associated terms.

Medical Term	Clinical Symptoms	Sepsis Related Term
Hypo/ hyperthermia	Core body temperature <36°C or >38°C	SIRS
Tachycardia	Heart beat >90 beats per minute	
Tachypnea	Heart beat >20 breaths per minute or $PaCO_2$<32 mmHg	
Leukocytosis/ Leukopenia	WBC count >12000/mm^3 or <4000/mm^3 or presence of >10% immature/ band forms	
SIRS+Infection		Sepsis
Sepsis+Organ failure		Severe Sepsis
Severe sepsis+Hypotension		Septic Shock
SIRS due to sterile cause+Organ failure		Severe SIRS
Severe SIRS+Hypotension		Shock

The central goal of all animal models is to faithfully reproduce clinically relevant pathogenesis that is similar to human disease. Human sepsis is characterized by various clinical features which are used to validate relevance of animal models [5]. These characteristic features of human sepsis divide the clinical sequence of sepsis in two distinct phases: early and late septic phase. The early phase is referred as hyper-dynamic phase, characterized by low systemic vascular resistance (SVR) and increased cardiac output (CO) [9-11]. With progression of sepsis, CO declines without any change in SVR, resulting in hemo-dynamic shock. The combination of low CO and SVR is the hallmark of septic shock and defines the late/ second hypo-dynamic phase of sepsis [9]. Models that do not closely mimic the hemo-dynamic changes of human sepsis are not considered as clinically relevant [6,7,12] **(Table 2).**

Table 2. Criteria for validation of an animal model for mimicking sepsis.

Early Phase Sepsis	Late Phase Sepsis
▪ Hyper-dynamic Cardiovascular State (Elevated cardiac output, Low systemic resistance) ▪ Hyper-metabolic State (Hyper-insulinaemia, Increased gluconeogenesis)	▪ Hypo-dynamic Cardiovascular State (Low cardiac output, Low systemic resistance) ▪ Hypo-metabolic State (Hypo-insulinaemia, Hypo-glycaemia)

It is also difficult to extrapolate animal model studies results to human because of the following reason: while designing an *in vivo* experimental setup, pre-requisite taken into consideration is to regulate the underlying confounding variables. To achieve so, healthy, inbred animals of same sex, age and weight are chosen to limit the baseline variability. Also, the experimental insult is kept constant ensuring same stimulus is provided to each animal for the purpose of comparing the results. But it is a well-known clinical reality that the patients are outbred; have variable age, sex and weight; have variable health parameters and different causes of sepsis [13]. A summary of differences between clinical reality of sepsis and available animal models is represented in **Table 3** [14]. Thus in order to mimic the human sepsis for the purpose of therapeutic intervention, different models of animal sepsis should be used. In other words, successful clinical trial can be predicted by the success of pre-clinical trials using a number of distinct animal models [7].

Table 3. Difference between clinical and experimental conditions of Sepsis.

Factor	Clinical Condition	Experimental Condition
Genetic pool of sex	Heterogenous of both the sexes	Homogenous of single sex
Age	Variable (More of neonatal and elderly)	Usually young
Species	Single i.e., Human	Variable i.e., Mice, Rat, Pigs, dogs, etc.
Treatment Differences	Active treatment including resuscitation, antibiotics, organ support, etc.	No organ support, No or limited antibiotics and resuscitation
Infection	Natural	Induced
Type of microbes	Usually virulent	Usually avirulent
Degree of insult	Variable	Uniform
Time course	Natural	Imposed

On the basis of initiating agent, sepsis models can be divided into three categories: exogenous administration of toxin (such as LPS), exogenous administration of viable pathogen (such as bacteria), alteration of animal's endogenous protective barrier (inducing colonic permeability, allowing bacterial translocation). These distinct models of sepsis **(Figure 1)** are dealt in detail in next sections.

Toxaemia Model

Toxaemia models are basically injectable mode of sepsis induction where an exogenous toxin is administrated intra-peritoneally [15,16]. Immune pathology of endotoxicosis model using bolus injection is characterized by overwhelming innate immune response with inflammatory cytokines such as TNF-α representing crucial mediators [17-19]. But bolus injection of LPS commonly induces a hypo-dynamic cardiovascular state immediately and does not reproduce the hemo-dynamic changes as observed in human sepsis [6,7].

The bolus injection based sepsis model differs from human sepsis in showing a very rapid and transient increase in systemic cytokine levels, which is not the case in human sepsis. Human sepsis is characterized by prolonged elevation of systemic cytokines

that are several orders of magnitude lower than endotoxicosis models [6,7]. Also, comparison of response to endotoxin in mouse and human has demonstrated that mice are relatively more resistant to endotoxin [20]. The endotoxin dose leading to 50% mortality in mice (i.e., LD50) is about 1–25 mg/kg [21-23] whereas in humans it is 2-4 ng/kg [24,25]. The biological mechanism(s) responsible for difference in responsiveness to LPS in mice and humans is yet to be fully elucidated, but Warren and colleagues have suggested that some factor(s) which are present in murine sera but not in human sera may be responsible for the same [26]. These factors are capable of suppressing the production of pro-inflammatory cytokines. One such factor is hemopexin i.e., iron binding acute phase protein [27]. Such differences further limit the extrapolation of these experimental studies across species.

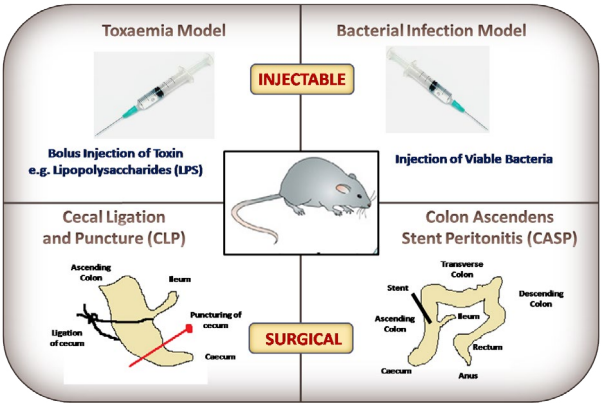

Figure 1. Commonly used murine sepsis models i.e., injectable toxaemia and bacterial infection models and surgical cecal ligation and puncture (CLP) and colon ascendens stent peritonitis (CASP) models.

Bacterial Infection Model

Bacterial infection model refers to injectable mode of introducing live bacteria inside the body against which immune response is elicited. Though this model fails to recapitulate many important clinical features of sepsis, it provides an insight into mechanisms of host response against pathogen. Inoculation of animals with pure or mixed bacterial flora has been a common tool for studying septic mechanisms [5-7]. High dose of bacteria inoculated does not colonize and replicate inside the body but rapidly lysed by complement system [28]. Thus, it leads to a potential model of intoxication with endotoxins rather than true septic model [28]. Variable responses are observed based on the bacterial load, strain type, mode of administration, host etc. Also, the resulting outcomes do not mimic the true human septic conditions.

Host-Barrier Disruption Models

The endogenous protective barrier that maintains the sterile compartment inside body, are manipulated in these model so as to result in septic conditions. The gut harbours a number of microbial species which are restricted to intestine by specialized intestinal epithelium. With the disruption of this barrier, microbial flora can ooze out to peritoneal cavity to elicit an immune response. Cecal ligation and puncture (CLP) model and colon ascendens stent peritonitis (CASP) models represent the examples of this category of septic models [4]. These models have been described below in detail, but an overview of how intestine maintains this sterile compartment and what are the innate and adaptive immune specializations it possesses are discussed first in the next subsection.

The mammalian gut harbours a dense and dynamic community of micro-organisms. These resident populations are mainly represented by bacteria, although viruses [29] and even archaea [30] are also reported. The microbial profiling studies have revealed that the individual variation in terms of gut resident bacterial species is very high though common patterns have emerged at the phylum level. Gram negative Bacteroidetes and gram positive Firmicutes are the most common intestinal bacterial phyla [30,31] followed by actinobacteria, proteobacteria [32] etc. These bacteria contribute to host's digestive efficiency, immune system development and pathogen colonization limitation. In turn, they are provided with nutrient-rich, protected habitat. Thus, a mutual symbiotic relationship is constituted among host and microbes. But this mutualism can turn to pathogenicity if the microbial penetration remains unchecked by intestinal mucosal surface. This property of intestinal mucosal barrier is disrupted in surgical models of sepsis (i.e., CLP and CASP) detailed below.

Colon Ascendens Stent Peritonitis (CASP) Model

In this model, a stent of a defined diameter is implanted into the ascending colon of the experimental model, leading to persistent leakage of fecal content into the peritoneal cavity and infection with intestinal microbial flora [33-35]. The model can be manipulated in terms of outcome by varying the diameter of stent used. CASP model is accompanied with multi-organ failure [36]. It represents an acute polymicrobial septic peritonitis model where both SIRS and CARS responses are observed. This model is still limited in use and thus, the confounding variables are not known in detail. Also, it presents a very challenging surgical procedure for placing the stent.

Cecum Ligation and Puncture (CLP) Model

The CLP model is one of the most stringent models of sepsis, and is considered by many investigators to be the crucial

pre-clinical test for any new treatment to human sepsis [5]. Compared to other models, CLP provides a better representation of the complexity of human sepsis. CLP involves a combination of three insults: tissue trauma due to laparotomy, necrosis caused by cecal ligation and infection due to microbial leakage. The latter results in peritonitis and further followed by translocation of bacteria into bloodstream which activates inflammatory response. The advantage of CLP is that the pathogens are endogenous, therefore mimicking traumatic injury leading to peritonitis in humans. CLP technique became readily acceptable and popular because it satisfies many of the essential criteria that are required in a potent septic model: simple procedure, poly-microbial in nature, localized infectious focus etc. [1]. Furthermore, sepsis shows a high degree of similarity to human sepsis progression and displays both hyper- and hypo-inflammatory response of human sepsis [5]. Being one of the best representatives of human sepsis, it has been recognized as gold standard for sepsis research [1]. Still there are a number of discrepancies in CLP model and human sepsis which must be considered for any therapeutic approach [1].

A comparative account of various rodent sepsis models is summarized in **Table 4.** These models are compared on the basis of type of sepsis induction (i.e., injectable or surgical), factors that may cause variability in results and advantages and disadvantage in using respective model.

Table 4. Comparison of various commonly used animal models of sepsis.

SEPSIS MODEL	TYPE	VARIABILITY FACTORS	ADVANTAGES	DISADVANTAGES
Endotoxemia/ Toxicosis Model	Injectable	• Type of toxin used • Dose • Route of administration • Fluid Resuscitation • Host species and strain	• Simple and reproducible • Induced response is acute • Highly controlled and standardized model	· LPS mediated signaling is strictly TLR-4 dependent · Does not mimic human sepsis in terms of cytokine profile · Variability in dose, toxin and route of administration
Bacterial Infection Model	Injectable	• Bacterial load • Route of administration • Time of infusion • Bacterial and host strain • Antibiotic/fluid resuscitation	• Presence of bacterium allows insights into mechanisms of host response to pathogens	• Growth and quantification of bacteria is required before administration • Single bacterium model does not reflect true human sepsis • Variability in bacterial load, route of administration and bacterial strain • High dose causes endotoxic not septic shock
Colon Ascendens Stent Peritonitis (CASP)	Surgical	• Stent lumen diameter • Load of stool transferred into peritoneum • Sex, age and strain	• Polymicrobial • Presence of infection focus	• Surgically more difficult • Length of colon is not defined • Less characterized hemodynamic phase
Cecal Ligation and Puncture (CLP)	Surgical	• Needle size and number of puncture • Amount of cecum ligated/ necrosis induced • Uncontrolled bacterial load • Antibiotic/ fluid resuscitation • Sex, age and strain	• Simple procedure • Presence of infection focus • Polymicrobial • Mimics human sepsis most • Prolonged and lower elevation of cytokines as in human	• Abcess Formation • Variability in severity due to differences in experimental procedure

Non-human Primate Models of Sepsis

Considering the gap in rodent septic models and human sepsis, non-human primate models are tested for mimicking human sepsis. Intravenously injected live *E. coli* and LPS in baboons have been used as model system for sepsis [37,38]. But owing to the limitations of ethical committee and lack of intensive care facilities, these models are not propagated.

CONCLUSIONS

A number of animal models have been developed to faithfully mimic human sepsis but unfortunately the interventions that have been shown protective in animal sepsis fail in human clinical trials. This failure does not indicate that the animal models are irrelevant. It might be probably because either we do not understand the complex nature of sepsis completely yet or we have not been able to mimic this complexity in a single satisfactory pre-clinical model. Though these animal models do not truly and completely mimic the clinical complexity and intrinsic heterogeneity of human sepsis in all the respects but still they provide an insight about specific components of this syndrome. Thus, despite all the limitations these models have, we need to use them for pharmacokinetic studies as no other substitute is available yet. The future prospects lies with development of new rodent models, improvising the existing ones and to look for the feasibility of more successful non-human primate model considering the existing differences between clinical reality and experimental models.

e-ISSN:2321-6190
p-ISSN:2347-2294

ACKNOWLEDGEMENTS

The guidance and intellectual support from Prof. Madan Mohan Chaturvedi, Professor, Department of Zoology, University of Delhi is highly acknowledged. I also acknowledge the Council of Scientific and Industrial Research (CSIR), Delhi for financial assistance.

REFERENCES

1. Dejager L, et al. Cecal ligation and puncture: the gold standard model for polymicrobial sepsis. Trends Microbiol. 2011;198-208.
2. Ward PA and Bosmann M. A historical perspective on sepsis. Am J Pathol. 2012;181:2-7.
3. Bone RC, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Committee, American College of Chest Physicians/Society of Critical Care Medicine. Chest 1992;101:1644-1655.
4. Buras JA, et al. Animal models of sepsis: setting the stage. Nature Reviews Drug Discovery 2005;4:854-865.
5. Wichterman KA, et al. Sepsis and septic shock: a review of laboratory models and a proposal. J Surg Res 1980;29:189-201.
6. Fink MP and Heard SO. Laboratory models of sepsis and septic shock. J Surg Res 1990;49:186-196
7. Deitch EA. Animal models of sepsis and shock: a review and lessons learned. Shock 1998;9:1-11.
8. Fink MP. Animal models of sepsis. Virulence 2014;5:143-153.
9. Abraham E, et al. Sequential cardiorespiratory patterns in septic shock. Crit Care Med. 1983;11:799-803.
10. Parker MM and Parrillo JE. Septic shock, Hemodynamics and pathogenesis. JAMA 1983;250:3324-3327.
11. Parker MM, et al. Serial cardiovascular variables in survivors and nonsurvivors of human septic shock: heart rate as an early predictor of prognosis. Crit Care Med. 1987;15:923-929.
12. Deitch EA. Rodent models of intra-abdominal infection. Shock 2005;24:19-23.
13. Dyson A, Singer M. Animal models of sepsis: why does preclinical efficacy fail to translate to the clinical setting? Crit Care Med 2009;37:S30-7.
14. Chen P, et al. Differences between murine and human sepsis. Surg Clin North Am 2014;94:1135-1149.
15. Johnston TD, et al. Septic hemodynamics produced by infusion of endotoxin. Curr Surg 1989;46:101-103.
16. Traber DL, et al. Cardiopulmonary responses to continuous administration of endotoxin. Am J Physiol 1988;254:H833-839.
17. Beutler B and Poltorak A. Sepsis and evolution of the innate immune response. Crit Care Med 2001; 29: S2-6.
18. Cohen J. The immunopathogenesis of sepsis. Nature 2002;420:885-891.
19. Riedemann NC, et al. The enigma of sepsis. J Clin Invest 2003;112:460-467.
20. Copeland S, et al. Acute inflammatory response to endotoxin in mice and humans. Clin Diagn Lab Immunol 2005;12:60-67.
21. Glode LM, et al. Significant contribution of spleen cells in mediating the lethal effects of endotoxin *in vivo*. Infect Immun 1976;14:626–630.
22. McCuskey RS, et al. Species differences in Kupffer cells and endotoxin sensitivity. Infect Immun 1984;45:278–280.
23. Reynolds K, et al. Pretreatment with troglitazone decreases lethality during endotoxemia in mice. J Endotoxin Res. 2002; 8: 307–314.
24. Barber AE, et al. Influence of hypercortisolemia on soluble tumor necrosis factor receptor II and interleukin-1 receptor antagonist responses to endotoxin in human beings. Surgery 1995;118:406–410.
25. Suffredini AF, et al. Effects of recombinant dimeric TNF receptor on human inflammatory responses following intravenous endotoxin administration. J Immunol. 1995;155:5038–5045.
26. Warren HS, et al. Resilience to bacterial infection: difference between species could be due to proteins in serum. J Infect Dis. 2010;201:223–232.
27. Liang X, et al. Hemopexin down-regulates LPS-induced proinflammatory cytokines from macrophages. J Leukoc Biol. 2009;86:229–235.
28. Cross AS, et al. Choice of bacteria in animal models of sepsis. Infect Immun. 1993;61:2741-2747.
29. Zhang T, et al. RNA viral community in human feces: prevalence of plant pathogenic viruses. PLoS Biol. 2006;4:e3.

30. Eckburg PB, et al. Diversity of the human intestinal microbial flora. Science. 2005;308:1635-1638.

31. Ley RE, et al. Worlds within worlds: evolution of the vertebrate gut microbiota. Nat Rev Microbiol. 2008;6:776-788.

32. Backhed F, et al. Host-bacterial mutualism in the human intestine. Science 2005; 307: 1915-1920.

33. Lustig MK, et al. Colon ascendens stent peritonitis--a model of sepsis adopted to the rat: physiological, microcirculatory and laboratory changes. Shock 2007;28:59-64.

34. Maier S, et al. Cecal ligation and puncture versus colon ascendens stent peritonitis: two distinct animal models for polymicrobial sepsis. Shock 2004;21:505-511.

35. Zantl N, et al. Essential role of gamma interferon in survival of colon ascendens stent peritonitis, a novel murine model of abdominal sepsis. Infect Immun. 1998;66:2300-2309.

36. Feterowski C, et al. CC chemokine receptor 2 regulates leukocyte recruitment and IL-10 production during acute polymicrobial sepsis. Eur J Immunol. 2004;34:3664-3673.

37. Bengtsson A, et al. Anti-TNF treatment of baboons with sepsis reduces TNF-alpha, IL-6 and IL-8, but not the degree of complement activation. Scand J Immunol. 1998;48:509-514.

38. Minnema MC, et al. Recombinant human antithrombin III improves survival and attenuates inflammatory responses in baboons lethally challenged with *Escherichia coli.* Blood 2000:95:1117-1112.