

Animal Models for Sepsis Research

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INTRODUCTION

The typical reaction to an infectious agent is a much localized inflammatory response, counteracted by an anti-inflammatory reaction. The septic syndrome develops when the infection results in a systemic activation of inflammatory pathways with no counteraction from anti-inflammatory mechanisms. The signs of human sepsis are well-defined: body temperature higher than 38°C or lower than 36°C, heart rate higher than 90/min, hyperventilation evidenced by respiratory rate higher than 20/min or PaCO₂ lower than 32 mmHg and white blood cell count higher than 12,000 cells/μl or lower than 4,000/μl. Although these same signs may be the result of a specific illness (e.g. acute pancreatitis) or trauma, if the patient does not present measurable bacteria levels in the blood, the syndrome is called “Systemic Inflammatory Response Syndrome” (**SIRS**), but when caused by an infectious agent the syndrome is called “septic syndrome” or “sepsis” [1].

Sepsis leads to a progressive systemic damage resulting in a sequential organ failure. This is caused by an imbalance between the metabolic requirements of each particular organ and its blood supply [2]. Because the progressive damage implies different levels of disease and different levels of medical care (clinical specification and definitions are reviewed elsewhere [3]), it is crucial to specify the degree of the damage. Thus, sepsis in addition to hypotension, hypo perfusion or at least one organ dysfunction is called “severe sepsis”. Likewise, sepsis accompanied with refractory arterial hypotension, is denominated “septic shock”.

Considering that sepsis is an uncontrolled inflammatory response, it should be expected that the most appropriated treatment would be anti-inflammatory therapies. But, they are not as successful as anticipated [4]. Moreover, although many advances in diagnostic tools, patient care and drugs development, sepsis remains as an important cause of morbidity and mortality [4–6]. Regrettably, as the management of septic patients remains essentially supportive, the organ failure leads to death in 28-47% of the cases and rises up to 40 to 70% in patients with septic shock. In addition, the incidence of sepsis is about 750,000 cases per year, only in USA. Altogether, sepsis not only is a severe illness, but also a very expensive health-care related item [4,7,8].

ANIMAL MODELS OF SEPSIS

Healthy people are exposed to many different ways of infection, but the infectious agent must cross the physical or the immunological barrier of the host. Some of the hazards are obvious -like an exposed fracture- and some are subtle such as insect bites or minor skin scratches. Moreover, even a well-defined illness as appendicitis with signs and symptoms clear enough to identify the condition, may develop into peritonitis, which sometimes may lead to sepsis.

People under medical care, especially those in intensive care units, are exposed to concealed infection threats. For example; mucosa ruptures in the respiratory tract due intubation protocols or in the genitourinary tract by urinary catheters. Likewise, contaminated venous cannulas can directly inoculate pathogens to the blood stream.

Because of the different origin, localization and pathogens of the initial infection, it is difficult to perform controlled studies in patients. Furthermore, it is very difficult to establish a time line of the condition unless the sepsis begins when the patient is already under medical care and/or in a medical facility. Although encouraged, research using animal models as preclinical studies cannot translate to clinical studies [9,10]. Too often, the positive outcome in a particular animal model fails in clinical studies [11–16]. Two of the reasons for these disappointing results are: a) the experimental approach to the problem; specifically, the animal model used may not correlate with the sepsis development in humans, and b) the direct progression of the therapeutic agent from an over-controlled pre-clinical study to clinical trials, with poor to none validation in different models.

Starting from 1960s and 70s, various animal models were developed to study sepsis and its progression [17–19]. Because of the nature of each model, not all of them resemble the features of septic condition in humans [20]; nonetheless, all animal models have been useful for the study of different components of the sepsis syndrome.

Choosing a species must account for many factors, some are practical and –nowadays- some are socio-cultural. Practical factors are easy to identify and resolve. Small mammals like mice, rats, guinea pigs and rabbits are easy and inexpensive to obtain, maintain and reproduce, hence much appropriated for protocols related to survival or protocols that need large sample numbers. Mice can also be breed or mutated to obtain strains with desirable features [21,22]. While rabbits are large enough to endure protocols requiring several samples (e.g. blood samples), rats and guinea pigs are suitable for preparations that evaluate function and/or metabolism of isolated perfused organs. Regarding animal size, cats and dogs are appropriate for surgery, blood sampling and implementation (i.e. surgical implantation of electronic devices to remote monitoring physiological variables, such as temperature, blood pressure, etc.). Both species are proper to assess cardiac output, ventilatory variables and even pulmonary artery pressure, all three parameters comparatively difficult to obtain in smaller animals, such as rats or mice. It is noteworthy that dogs and cats have been adopted as pets worldwide. A very active and strong “animal rights” movement has pushed so hard, that legislation regarding the use of animals for experimentation changed in several countries. Nowadays, it is almost impossible the use of a single cat or dog, and literally impossible to justify the sample number needed to obtain statistical significance. Pigs and sheep also have an appropriate size for all the above-mentioned experiments. Their advantage is the social impact of experiments using those species is far less than those using dogs or cats. Their large size allows the dispose of large volumes of sampling material (e.g. blood, tissues, organs), very useful to detect substances in small concentrations, such as hormones. Also, sheep are docile, a desirable feature for chronic studies. In addition, anatomy and physiology of pigs cardiovascular, renal and digestive systems are remarkable similar to humans. Of course, pigs and sheep require facilities much larger than those needed to maintain small animals. Finally, non-human primates are the most similar to humans and have a suitable size for almost all kind of experiments. On the other hand, work with primates is challenging and they are by far the most expensive and very noticeable in terms of animal rights. Hence, experiments with non-human primates must require a very small sample size. Therefore, non-human primates are usually reserved for preclinical studies.

Toxemia

Endotoxin is a part of the wall (the outer membrane) of bacteria and lipopolysaccharide (LPS) is its main bioactive component. Thus, both terms are interchangeable although the latter is a purified fraction of the first. The relation between endotoxin and human sepsis [23,24] was suggested only a decade after the discovery that gram-negative bacteria produce endotoxin [25].

The suggestion -accepted by many investigators- was the beginning of the conundrum with animal models of sepsis, because it leads to two and a half decades of experimental research models of sepsis injecting endotoxin to a variety of laboratory animals. Despite the many species used, none of these models satisfactory resembles human sepsis [26–28]. Although, as expected, responses of non-humans primates (like baboons or monkeys) are the closest to humans [17,29].

One of the main issues with endotoxemia models is the blood-level of endotoxin. Although endotoxin is detectable in the blood of septic patients, its level is very low and it is far from the microgram order equivalent to a lethal dose. In addition, the dose needed to produce endotoxin shock depends on the animal species, some -like rabbits and sheep [30,31]- respond to a low human-related dose [32], but others need doses up to 1,000-fold larger, like dogs [33]. In addition, responses to low or high doses are variable even in the same species, e.g., when challenged with a low dose of LPS, rats will develop a hyperdynamic cardiovascular response, but larger doses lead to cardiovascular collapse -hypodynamic phase- and death.

Anyhow, the administration of LPS causes a systemic response that partially resembles the pathophysiological responses of human sepsis, but the differences are huge. Dogs are very good candidates to illustrate this point. Endotoxemic dogs develop a hypodynamic state characterized by a rapid reduction of both blood pressure and cardiac output, but with an augmented peripheral resistance [18], whereas human patients develop both hypotension and diminished cardiac output, but also a significant fall of the peripheral resistance. Another remarkable difference is that human patients develop hyperglycemia and hyperinsulinemia [34], while dogs present a severe hypoglycemia caused by endotoxin-induced sepsis [35]. Interestingly, septic rats also present hypoglycemia, exacerbated in chemo-baro denervated animals [2].

Other issue with endotoxemia models is the endotoxin. There is no doubt that LPS must be related with the pathogenesis of sepsis. In fact, detectable plasma levels of LPS predict in a precise way the development of sepsis in human patients [36]. Nonetheless, LPS is just only one component of gram-negative bacteria wall. Intravascular injection of dead *Escherichia coli* is more lethal than comparable dose of endotoxin. Thus, other components of the bacteria wall may also play a role in the induction of the systemic response. Besides, infections leading to sepsis and septic shock are not restricted to Gram-negative bacteria and may be due to Gram-positive bacteria or fungi.

All above disadvantages considered, LPS does have advantages as well. It is a stable and pure compound, easy to storage and measure for an accurate administration. Thus, LPS-induced sepsis is very reproducible.

Bacteremia

A different model of sepsis is the intravenous administration of bacteria, most frequently *E. coli*. The early model, using a single bolus of bacteria, was rapidly criticized [37] because it

does not reproduce the constant load of bacteria in a patient with a septic focus. Nonetheless, the model is useful for studies assessing the effect of pharmacological agents when lethal doses of bacteria are used. In these studies, the main variable is the survival rate, although other physiological or pathological parameters were studied. Considering the protocol does not include medical care -beside the drug assessed- the results are not comparable with human patients, and the effectiveness of the drug does not translate into clinical trials [14]. However, using primates and large doses of bacteria, the model reproduces many of the characteristics of severe sepsis and septic shock in humans, including low systemic vascular resistance, arterial hypoxemia and reduced glomerular filtration rate [17]. This resemblance allowed the study of the effect of intravenous administration of fluid upon systemic and pulmonary hemodynamics and oxygen metabolism, aiming to a protocol that recovers the sepsis-induced hypotension and its consequences [38]. The model also provides the opportunity to study the effect of the intravascular infusion of different microorganisms. A variation of the model using anaesthetized swine continuously infused with bacteria concluded that *E. coli* and *Pseudomonas aeruginosa*, both have important cardiorespiratory effects, while *Staphylococcus aureus* has minimal or none effect. Because of its fast and dramatic impact on the pulmonary function, the model has been used for the study of acute respiratory distress syndrome, but the clinical relevance of these studies is dubious, since the protocols do not consider the restoration of intravascular volume [39,40]. Finally, since the anesthesia may induce some unwanted effects, a model of intraarterial infusion of *E. coli* in unanaesthetized dogs showed that all animals die if not resuscitated replenishing intravascular volume, but 85% survive if resuscitated. Interestingly, recovered dogs were not only hyperdynamic and hypermetabolic, but they also manifest both the hyperinsulinemia and hyperglucagonemia characteristics of human patients [41].

Another sepsis approach is the creation of a septic focus in the animal tissue. Injections of LPS or bacteria does not resemble the time course of an infection. Rarely an injury conveys a bolus of pathogens; instead, after the injury, an infection focus will develop in time and the host immune system would have time to respond to the infection. The first model used calcium chloride to produce necrosis in the muscles of dogs' thighs, which spontaneously developed as infection focus [42]. A technique derived from the previous one also used dogs, but instead of necrosis, investigators surgically implanted small pieces of cloth soaked in feces (frequently the animal own feces) into the animal soft tissue of the thigh, to create a local infection [43]. After 24 hrs, the animal presented the typical hyperdynamic circulatory response, but none of the dogs die, suggesting that the model is more suitable for the study of localized infection.

A similar model used subcutaneous injections of live bacteria, reproducing both, the hypermetabolic and the hyperdynamic circulatory response. In this model, the size of the inoculum represents the degree of the challenge; thus, the investigator can control the level of infection and the mortality of each experimental series [44].

Cecal ligation and Puncture

The model that most closely resembles septic state in humans derived from peritonitis models. Peritonitis can be induced in many ways. The idea is very simple: to contaminate the intraperitoneal space with fecal material (or with cultures of specific bacteria). Thus, the surgical intraperitoneal implantation of clots of fibrin with bacteria, pellets with specific number of specific bacteria or –even- osmotic minipumps with a specific dose of bacteria, all three procedures developed septic state. These models can be adjusted to study chronic sepsis and are useful to study the effects of pharmacological agents against specific strands of bacteria, but none of these models accounts for the typical pool of bacteria in the fecal material [45–49].

A different approach was the cecal ligation, producing a necrotic cecum and, consequently, an intraperitoneal focus of infection [50]. Early models used dogs and pigs, reproducing many features of septic patients including fever and respiratory alkalosis [51]. As mentioned above, although very similar to humans, the use of these species does not permit the use of large number of animals. Cecal ligation in rats does not successfully produce sepsis, but adding a puncture the model mimics a ruptured appendicitis [37,52]. Cecal ligation and puncture (**CLP**) create a perforated bowel that leaks fecal material to the peritoneal cavity, establishing a focus of infection with the normal bacterial flora and the inflammatory process of the necrotic tissue. The fecal leakage can be controlled with the number of perforations and the size of the needle used to perforate the cecum. The severity of CLP model, assessed by mortality, is as adjustable that septic state can be as severe that the animals die within hours, or they may survive up to 28 days [37]. CLP model presents many advantages; it is reproducible, the technique is relatively easy and, more importantly, its similarity to human sepsis progression in signs, symptoms and host responses [37]. All considered, the main advantage of CLP is its clinical validation. Although CLP is now considered as the gold standard, it has disadvantages. The host can isolate the injury and contain the infection forming an abscess [53]. This is especially important if any type of treatment (drug and/or protocol) is studied. Animal survival may be biased if the therapy promotes abscess formation, instead of recovering from the septic state. Even though CLP technique is rather easy, small differences in the percentage of cecum ligated may represent an important change in the survival rate [54]. In addition, the amount of fecal leakage cannot be controlled between experiments, resulting in a source of unaccounted variability among different laboratories.

Colon Ascendens Stent Peritonitis

One last model of sepsis requires the puncture of the colon ascendens and uses a small device -a stent- to maintain the rupture permeable. The result is a stable leakage of intestinal content to the peritoneal space, and -consequently- peritonitis Colon Ascendens Stent Peritonitis (**CASP**). This model has almost all the advantages of CLP, including the adjustment because the diameter of the stent may regulate the flow of fecal material [55]. When compared with CLP, CASP has some advantages, such as the bowel perforation remains unaltered for days, while in CLP the

small intestine may cover the puncture. In addition, the stent can be removed, simulating the typical surgical intervention in human patients. An important disadvantage of the CASP is that the intervention is technically more complicated than the CLP intervention. This may be the reason why CASP has not expanded its use as CLP and, therefore, CASP model has fewer results to compare with all other models.

CONCLUSION

The main reason to study and develop different animal model of sepsis is for assay therapies and/or protocols to treat sepsis in humans. For that reason, clinical research must account for every possible caveat of the model in use. Likewise, to study a specific part of the sepsis syndrome, the model selected must be adequate. It is the researcher responsibility to evaluate not only animal species, but also if the model is suitable for the study. When the features of each model are considered prior to the actual experiment, the study will be benefited instead of limited.

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