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Mimicry of human sepsis in a rat model—Prospects and limitations

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ABSTRACT

Background: Sepsis and systemic inflammatory response syndrome (SIRS) continue to represent critical conditions with persistently high mortality and continue to need experimental and clinical research. We developed a rat model of gram-positive and gram-negative SIRS/sepsis with *in vivo* visualization of the pulmonary microcirculation to evaluate the optimal dosage and application path for SIRS/sepsis-inducing agents.

Methods: Male Sprague-Dawley rats ($n = 8$ per group) were assigned to control, lipopolysaccharide (LPS), alphatoxin, or living *Staphylococcus aureus* (strain 68/50) groups. SIRS/sepsis was induced by intraperitoneal injection of the differing agents. The onset of SIRS was determined through human sepsis parameters and fluorescence video microscopy-based measurement of platelet and leukocyte velocity within the pulmonary vascular system (injection of 5×10^6 calcein AM-labeled nonactivated platelets; leukocytes labeled *in vivo* by rhodamine).

Results: The optimal dosage to induce SIRS was 30 mg/250 g body weight for LPS (bolus injection) and 60 μg/250 g body weight for alphatoxin (2 h continuous perfusion). Sepsis was not achieved by injection of living *S. aureus*. The onset of SIRS was seen after 2–5 h for LPS and after 2–4 h for alphatoxin after intraperitoneal administration with a significantly increased heart rate, breathing rate, and body temperature ($P < 0.05$) and significantly decreased cell velocity ($P < 0.05$).

Conclusion: Our study represents an effective approach for a gram-negative (LPS) and gram-positive (alphatoxin) SIRS model to mimic human sepsis. Human sepsis-based criteria were used to define SIRS in our rats to achieve an optimal analogy for the human system. In our model, higher dosages were needed for SIRS induction than have been previously reported. The resulting, considerable heterogeneity of current SIRS-inducing models suggests that additional studies in this field are required to define standard procedures.

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1. Introduction

Sepsis, with its host of associated complications, remains a major clinical challenge with persistently high mortality of 30%–70% [1] There is continued need for experimental and clinical research in this field, and several animal models of sepsis and systemic inflammatory response syndrome (SIRS) have been previously developed [2–10], making use of different agents and application techniques. These models can be divided into three categories by the mechanism of sepsis initiation: (1) exogenous administration of an agent (i.e., toxin, microbial component) into the bloodstream or peritoneal cavity (e.g., lipopolysaccharide [LPS] or alphatoxin); (2) exogenous administration of a living pathogen (e.g., bacteria); or (3) alteration of the animal's endogenous protective barrier (e.g., mechanical or chemical damage of the colon for fecal contamination). The variety of advantages and disadvantages of the existing sepsis models have been discussed in many studies and reviews [3,11–14] Animal models that do not closely mimic the typical changes occurring with human sepsis are often regarded as less clinically relevant [3,9] As discussed very appropriately by Buras *et al.* [11], 2 major impairments exist with most sepsis models: the interval to disease development and the deficiency of supportive therapeutic interventions. Animal models are usually designed to develop reproducible and efficient sepsis compared with human sepsis. In most animal models, the onset and progression of sepsis leads to multiorgan failure within hours. However, in humans, septic conditions and complications usually occur over a longer period. In addition, septic patients are immediately treated, but sepsis-related therapies are generally not used in animal models. The timing of sepsis initiation is obviously very important, because the onset of sepsis leads to several pathophysiologic and immunologic changes that cannot be influenced and could alter the whole experimental setting. Also, the term "sepsis" has been used incorrectly in many toxin- or microbial component-based studies, because sepsis is defined by the evidence of bacterial infection. Therefore, several studies have used SIRS animal models and not sepsis models.

Many sepsis/SIRS models have demonstrated typical SIRS symptoms, such as hyperthermia, tachycardia, and tachypnea; however, other parameters, such as the levels of pro- and anti-inflammatory cytokines, differ between humans and animals in sepsis/SIRS. Therefore, the methods of measuring sepsis/SIRS are important. Several studies have measured plasma endotoxin or cytokine levels [2,5] to demonstrate SIRS; however, the well-defined criteria for sepsis in humans [15] were not taken into account in most studies. In addition, the different models used significantly different dosages and application techniques [2,4–8,10] for SIRS initiation, and most studies used one defined sepsis/SIRS model with gram-negative or gram-positive toxins (or bacteria), although each of these agents is responsible for ~45% of human sepsis cases.

The aim of the present study was to evaluate adequate dosages of SIRS-inducing agents and suitable methods for their application to find the optimal conditions for additional animal SIRS/sepsis studies. Therefore, we analyzed the typical sepsis criteria for humans [15] in our rat model and measured the reaction of platelets and leukocytes as SIRS/sepsis-sensitive cells in the lung. The lung, in turn, represents an organ prone to SIRS- and sepsis-related changes from gram-negative and -positive SIRS/sepsis in rat models.

Thus, a typical gram-negative agent, such as LPS, and gram-positive agent/bacteria, such as alphatoxin and living *Staphylococcus aureus* bacteria, were chosen for our SIRS model. LPS is one of the most used agents for SIRS induction in animals and has several mechanisms of action. For example, the ability of LPS to activate the complement system corresponds well with its ability to induce platelet responses and rapid shock in animal models [16] It has been proposed that the structure of the O-antigen region of LPS is important for the platelet response to endotoxemia by activation of the lectin pathway of the complement system. Furthermore, administration of LPS into mice or other animal models induces a rapid accumulation of platelets in the lung [17] When degradation of the accumulated platelets occurs, anaphylactic shock can follow rapidly, caused by the release of mediators. The severity of the hemodynamic reaction appears to correlate with the quantity of platelets accumulated in the lung [16] As a prototype of pore-forming exotoxins, alphatoxin represents the major cytotoxin of *S. aureus* [18,19] and is known to cause SIRS with severe cardiovascular damage. Although a model with living bacteria results in more uncertainties, it represents a sepsis model that seems to be closest to human sepsis. Therefore, we chose to induce gram-positive sepsis using living *S. aureus* bacteria to optimally mimic the human sepsis.

2. Materials and methods

2.1. *Sepsis definition*

To reflect the human system as closely as possible, we used the sepsis definition developed by the American College of Chest Physicians/Society of Critical Care Medicine Consensus Panel [15] as the sepsis criteria for our model. From that consensus, sepsis has been defined by the presence of $\geq$2 of the following symptoms, along with confirmation of bacterial infection: temperature >38°C or <36°C; heart rate >90 beats/min; respiratory rate >20 breaths/min or an arterial partial pressure of carbon dioxide <32 mm Hg; and/or white blood cell count >12,000 cells/μL or <4,000 cells/μL, or >10% band forms.

If these criteria were fulfilled, but an infectious focus was not detected, the state of systemic inflammation should be termed "systemic inflammatory response syndrome" or SIRS.

2.1.1. *Macrohemodynamics*

Because the physiologic heart and breathing rates of Sprague-Dawley rats differ significantly from those of humans, we defined a $\geq$30% increase of heart and breathing rates (*versus* the initial rates) as the beginning of SIRS.

2.1.2. *Microhemodynamics*

To confirm the septic conditions, we evaluated the cell reactions of leukocytes and platelets using intravital microscopy, as described previously [20] In the present study, LPS-associated SIRS resulted in a velocity decrease of 20% in