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Possible biomarkers of early mortality in peritonitis-induced sepsis rats

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ABSTRACT

Background: Sepsis induced by cecal ligation and puncture (CLP) is accompanied by circulatory failure, multiple organ dysfunction syndrome, metabolic acidosis, and electrolyte imbalance in rats. However, it remains uncertain which parameters can be used to predict the mortality of septic rats. Thus, the aim of this study was to examine which possible biomarkers were associated with mortality in the CLP-induced sepsis model.

Materials and methods: After the carotid artery and vein were cannulated, rats were subsequently subjected to CLP or sham operation. The changes of hemodynamics, biochemical variables, blood gas, and electrolytes were monitored during the 18-h observation.

Results: The CLP surgery caused circulatory failure, multiple organ dysfunction syndrome, metabolic acidosis, electrolyte imbalance, and death. Compared with survivors, nonsurvivors showed significant difference in (1) blood glucose; (2) lactate dehydrogenase, alanine aminotransferase, aspartate aminotransferase, creatinine, and blood urea nitrogen in serum; and (3) base excess, HCO_3^-, $PaCO_2$, potassium, and calcium in whole blood at 9 h after CLP. No significant difference in blood pressure, heart rate, pressor response to noradrenaline, rectal temperature, total protein, albumin, PaO_2, and sodium was observed between nonsurvivors and survivors. However, after multifactor dimensionality reduction analysis, the union of HCO_3^- and blood glucose had the biggest testing balanced accuracy.

Conclusions: These results indicate that HCO_3^- plus blood glucose serves as the best biomarker of early death in rats with CLP-induced sepsis. Thus, these parameters could guide experimental procedures for making the right interventions when utilizing CLP as a sepsis model in rats.

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1. Introduction

Sepsis is regarded as a pathologic state after infection that is associated with a systemic inflammatory response. Patients with sepsis initially have a proinflammatory state and later shift toward an immunosuppression period. Indeed, in severe sepsis, an extreme and combined proinflammatory and anti-inflammatory state potentially leads to multiple organ dysfunction syndrome (MODS) and death [1]. Despite considerable knowledge of the pathophysiology of systemic inflammatory response syndrome and the improvement of intensive care techniques, mortality from severe septic complications in trauma and surgical patients remains very high.

Different animal models have been developed to study the pathophysiology and treatment of sepsis. Polymicrobial sepsis induced by cecal ligation and puncture (CLP) is a commonly used model because it is similar to the progression and characteristics of human sepsis [2,3]. Having been developed over more than 30 y, the CLP model is considered to be a realistic model for studying the mechanisms of sepsis [4,5]. As in human sepsis, CLP surgery results in an early hyperdynamic phase, which is followed by a hypodynamic phase in animals [6,7]. Over the past year, we have focused on the treatment of sepsis, attempting to develop new therapeutic drugs to improve the survival of animals in which sepsis was induced by CLP [8–12]. Our previous studies demonstrated that the CLP surgery caused significant changes in hemodynamic and serum data at 9 h and 18 h after CLP. Indeed, CLP animals developed circulatory failure, MODS, metabolic acidosis, and electrolyte imbalance, as seen in patients with severe sepsis. After therapeutic interventions, the alterations of these parameters were attenuated along with the improvement of survival rate [8–12].

We observed that if some of these parameters are dramatically changed in CLP-induced sepsis, rats will survive less than 18 h. It seems that these parameters could predict the early death of animals in this model. Only few examples show a high correlation between serum interleukin 6 (IL-6) levels and mortality in CLP-induced sepsis [13,14]. Mice with higher levels of IL-6 have a significantly increased mortality and would lack benefits after treating with antibiotics. However, the relationships between mortality and hemodynamic or serum parameters in the CLP model have not yet been studied. Therefore, we examined and compared the changes of hemodynamic and serum parameters in survivors (at 18 h) and nonsurvivors (died between 9 h and 18 h) after CLP-induced sepsis, endeavoring to determine which parameters could serve as possible biomarkers correlating with early death of animals in the CLP model.

2. Materials and methods

2.1. Animal experiments

Male Wistar rats (10–12 wk old, 280–350 g) were purchased from BioLASCO Taiwan Co (Taipei, ROC, Taiwan) and were guaranteed free of particular pathogens. All animal experiments were approved by the local Institutional Review Board according to the recommendations from the Declaration of Helsinki, and were performed in adherence to the National Institutes of Health guidelines for the treatment of animals and ethical animal research. Rats were bred and maintained under a 12-h light/dark cycle at room temperature (21°C ± 2°C) with free access to food and water. To increase reliability of the survival and to examine the reproducibility in the CLP-induced sepsis model, as well as to follow the regulation of 3R in using animals, we included some of our previous data in this study.

2.2. Surgical procedures and experimental protocols

Rats were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg). The left carotid artery was cannulated and exteriorized to the back of the neck and connected to a pressure transducer (P23ID; Statham, Oxnard, CA) to measure mean arterial blood pressure (MAP) and heart rate (HR), which were displayed on a MacLab/4e poly-graph recorder (AD Instruments Pty Ltd, Castle Hill, Australia). The right jugular vein was cannulated and exteriorized to the back of the neck for the administration of drugs. Intraperitoneal sepsis was induced by CLP as described by Wichterman *et al.* [2] after the cannulated animals were allowed to recover to the normal condition overnight. The rats were again anesthetized with sodium pentobarbital (30–40 mg/kg intravenously), a small midabdominal incision was performed, and the cecum was exposed. The cecum was then isolated and ligated with a 3-0 silk ligature just distal to the ileocecal valve, punctured twice at opposite ends with an 18-gauge needle, and returned into the abdominal cavity. Sham-operated (SOP) rats underwent the same surgical procedure except that the cecum was neither ligated nor punctured. All animals were resuscitated with normal saline (10 mL/kg subcutaneously) immediately after the surgery.

Our previous studies showed that the mortality rate of animals that received CLP surgery was approximately 62% at 18 h after CLP [8–12]. Because of this high mortality of CLP animals at 18 h after this surgery, we chose 18 h as a cut-point time. Animals were divided into three groups: (1) SOP ($n = 44$); (2) survivors (at 18 h) of CLP ($n = 49$); and (3) nonsurvivors (died between 9 h and 18 h) of CLP ($n = 34$). During the experimental period, we examined changes in hemodynamics (ie, MAP, HR, and pressor responses to 1 μg/kg noradrenaline [NA]), rectal temperature, blood glucose, hepatic function index (ie, alanine aminotransferase [ALT], aspartate aminotransferase [AST], albumin [ALB], and total protein [TP]), renal function index (ie, creatinine [CRE], blood urea nitrogen [BUN]), cell injury index (ie, lactate dehydrogenase [LDH]), blood gas (ie, HCO_3^-, base excess [BE], $PaCO_2$, and PaO_2), and blood electrolytes (ie, potassium, sodium, and calcium). To normalize the baseline value of pressor responses to NA in all groups, we calculated the value of pressor responses to NA (by area under curve) at the resting state (ie, time 0) of each group as 100%. Blood samples (1 mL at each time point) were obtained at baseline (ie, time 0) and specified times (ie, at 9 h and 18 h) throughout all procedures. Each volume of blood removed was immediately replaced by the injection of an equal volume of sterile saline.