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Research paper

Comparison of sepsis rats induced by caecal ligation puncture or *Staphylococcus aureus* using a LC-QTOF-MS metabolomics approach

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ABSTRACT

Sepsis is a whole-body inflammatory response to infection with high mortality and is treated in intensive care units (ICUs). In the present study, to identify metabolic biomarkers that can differentiate sepsis models induced by caecal ligation puncture (CLP) or *Staphylococcus aureus* (*S. aureus*), small molecular metabolites in the serum were measured by liquid chromatography quadruple time-of-flight mass spectrometry (LC-QTOF-MS) and analysed using the multivariate statistical analysis (MVA) of partial least square-discrimination analysis (PLS-DA) method. The results demonstrated that the body showed obvious metabolic disorders in the sepsis groups compared with the control group. A total of 8 potential biomarkers were identified in the CLP group, and 10 potential biomarkers were identified in the *S. aureus* group. These potential biomarkers primarily reflected an energy metabolism disorder, inflammatory response, oxidative stress and tissue damage, which occur during sepsis, and these markers might potentially be used to differentiate CLP from *Staphylococcus aureus* sepsis.

1. Introduction

Sepsis describes the disruption of inflammatory homeostasis triggered by an infection. Sepsis and sepsis-associated multi-organ failure might rapidly deteriorate fast severe sepsis, eventually resulting in an irrecoverable state or death if efficient treatments are not immediately administered (Buras et al., 2005). Despite intensive basic research and clinical trials, sepsis largely contributes to the morbidity and mortality of patients in ICUs, and the incidence of severe sepsis in the United States is estimated at 750, 000 cases with 210, 000 deaths per year (Angus et al., 2001). Therefore, it is obvious that efforts to develop novel therapies to treat sepsis will be of great value.

Animal models play an indispensable role in understanding the host response to an infection or anti-inflammatory reactions during sepsis. Sepsis models can be divided into three categories: exogenous administration of a toxin (such as lipopolysaccharide (LPS), endotoxins or zymosan), exogenous administration of a viable pathogen (such as bacteria), or destruction of the animal's endogenous protective barrier (such as colonic permeability) (Buras et al., 2005; Fink, 2013). However, when considering the use of animal models in studies on the development of sepsis, different models present advantages and drawbacks. The toxaemia model is less attractive because this model has short-term effects on the inflammatory cascade and shows a lack of an active nidus of infection (Buras et al., 2005; Remick and Ward, 2005). Although bacterial infection models cannot replicate some features of human sepsis, these models provide important clues about the mechanisms of host reactions to a pathogen and, more importantly, facilitate studies of a particular type of bacterial infection (such as Gram-positive versus Gram-negative bacteria). In the last decade, Gram-positive bacterial infections of *Staphylococcus aureus* (*S. aureus*) have been reported to cause >50% of sepsis cases (Hiramatsu et al., 1997; Opal and Cohen, 1999). When considering destroying the endogenous protective barrier, caecal ligation and puncture (CLP) are typically used, and this strategy has been regarded as the gold standard for experimental sepsis. For caecal ligation and puncture (CLP), the cecum is opened to facilitate the release of faecal material into the abdominal cavity to generate a systemic inflammatory response that is induced by poly-bacterial infection.

Metabolomics is a representation of the actual physiological status of a biological system in response to external stimulation and

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perturbation (Nicholson et al., 1999). Jeremy Nicholson (Imperial College in London) initially pioneered this approach, which has currently been developed into an irreplaceable tool in fields such as biomarker discovery of diseases (Kenny et al., 2010), drug toxicity and efficacy evaluations (Nicholls et al., 2000), lipids (lipidomics) studies (German et al., 2007). Combined with the high-resolution platform of liquid chromatography and mass spectrometry (LC-MS), metabolomics possesses exceptional advantages, as this method can be used to detect and quantify up to several hundred metabolites in one sample in a relative short time without any preconception or selection bias (Wilson et al., 2005; Zelena et al., 2009). To our knowledge, there is no investigation to explore the similarities or differences of sepsis models induced by endogenous protective barrier destruction versus bacterial infection based on a metabolomics approach (Izquierdo-García et al., 2011; Lin et al., 2009; Su et al., 2014; Xu et al., 2008). Consequently, in the present study, we proposed a LC-QTOF-MS-based metabolomics approach to differentiate sepsis induced by CLP or *S. aureus*. The aim of the present study was to enhance the current understanding of sepsis and provide more valuable clues for future sepsis studies and treatment development.

2. Materials and methods

2.1. Chemicals

Acetonitrile (LC-MS grade) and methanol (LC-MS grade) were purchased from JT Baker (NJ, USA). Formic acid (spectroscopic grade) was purchased from Sigma/Aldrich (MO, USA). Distilled water was obtained using a milli-Q20 system Millipore (MA, USA). *S. aureus* (ATCC 29213) was obtained from American Type Culture Collection.

2.2. Animals

Male specific-pathogen-free Sprague Dawley (SD) rats (250–300 g), purchased from Shanghai Experimental Animal Centre of the Chinese Academy of Sciences (Shanghai, China), were maintained under a 12 h light/dark cycle and constant temperature (20 ± 0.5 °C) and humidity (60%–70%). Animals were provided free access to a normal rat diet and tap water. The rats were fasted overnight with water ad libitum on the night before the experiment. The experiments were conducted in accordance with the protocols reviewed and approved by the

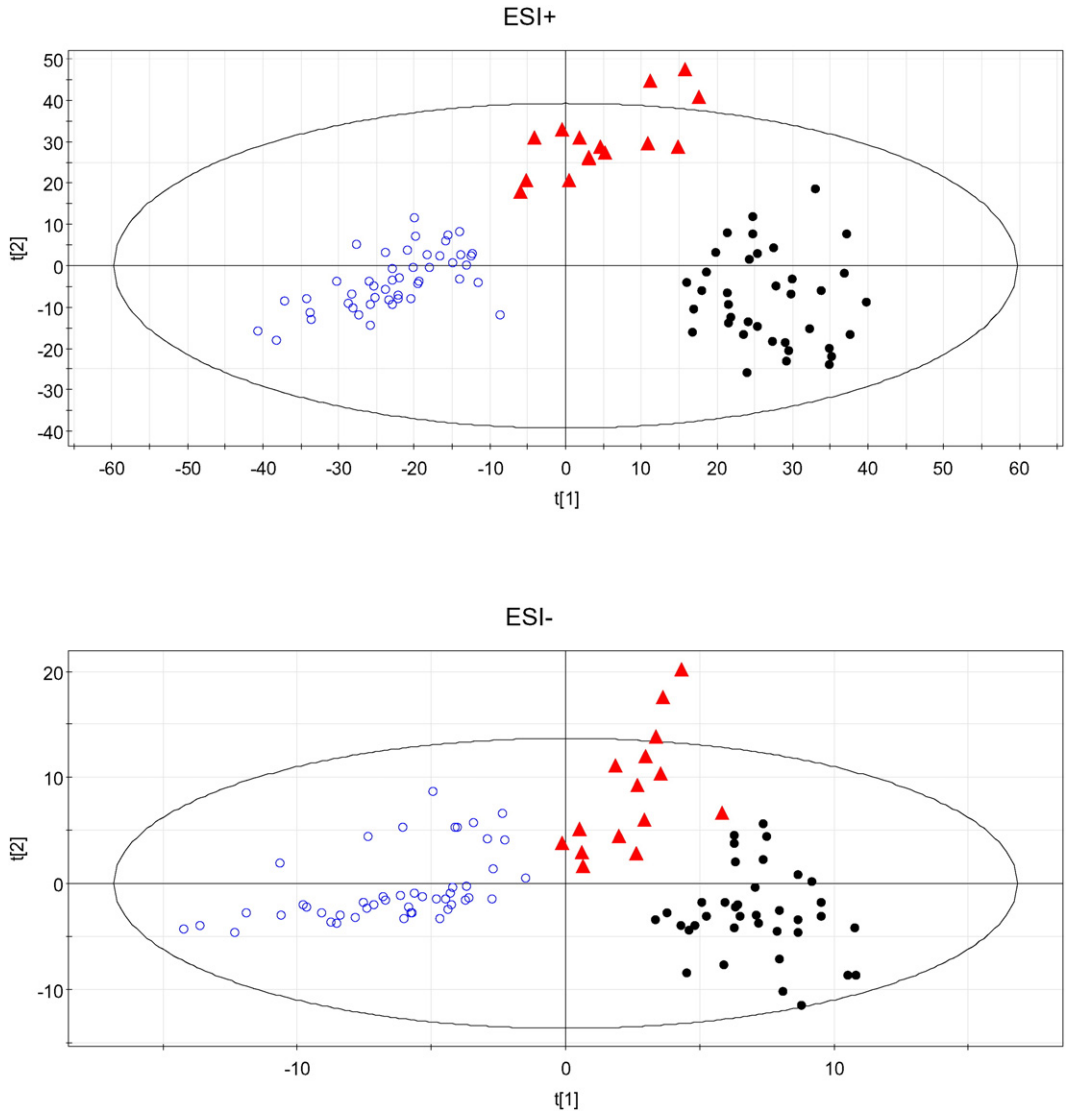

Fig. 1. PLS-DA score plots of rat serum from the control (red triangle), CLP (blue circle) and *S. aureus* (black dot) groups in ESI+ and ESI− modes.