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Effects of *Pseudomonas aeruginosa* mannose-sensitive hemagglutinin (PA-MSHA) pretreatment on septic rats ☆

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ABSTRACT

To evaluate the effects of *Pseudomonas aeruginosa* mannose-sensitive hemagglutinin (PA-MSHA) injection on the survival rate of rats post cecal ligation and puncture (CLP), Sprague–Dawley (SD) rats were subcutaneously injected with 0.125 ml, 0.25 ml or 0.5 ml PA-MSHA for 8 days or 16 days before CLP. The survival rate and physiological appearance of rats in each group were monitored daily post CLP. The expression of Toll-like receptor 4 (TLR4) and cytokines related to inflammation was evaluated. We found that the 0.5 ml-8d (0.5 ml PA-MSHA injected for 8 days) group had the highest 7-day survival rate (91.7%), which was significantly improved compared with the CLP-only group (33.3%). Furthermore, our results showed that PA-MSHA effectively increased serum pro-inflammatory mediators (TNF-α, IL-1β and IL-6) at the early stage (8 days) but increased anti-inflammatory mediators (IL-4 and IL-10) at the late stage (16 days). PA-MSHA significantly up-regulated the mRNA expression of TLR4 at 8 and 16 days. After PA-MSHA pretreatment, CLP had no marked effect on the levels of most inflammatory factors. To explore potential protective mechanisms of PA-MSHA against CLP, we examined the effect of PA-MSHA on murine macrophage-like RAW264.7 cells and found that PA-MSHA induced endotoxin tolerance. In conclusion, this study suggested that precisely controlling the dosage and time of PA-MSHA administration can effectively increase the rat survival rate post CLP, which may be mediated through regulating inflammatory mediators and inducing endotoxin tolerance.

1. Introduction

Sepsis is a potentially deadly medical condition. It is a severe response to infection, trauma or pathogenic microorganism invasion that causes overwhelming inflammatory host reactions (called systemic inflammatory response syndrome or SIRS) with subsequent inadequate organ perfusion or organ dysfunction. Despite continuing progress in the development of antibiotics and other supportive care therapies, sepsis remains a leading cause of high morbidity and mortality in the intensive care unit [1]. Although sepsis is often unpredictable, there are patient populations that have a higher risk of becoming septic, such as the patients with implanted central venous catheter [2] or splenectomy [3]. This makes the preventative treatment of sepsis a possible therapy.

Sepsis is caused by an imprecise control of immunological monitoring, and it causes a complex immunological cascade reaction. In the initial phase of sepsis, the manifestations are frequently "nonspecific, systemic activation of the innate immune system and human proinflammatory cascade", known as SIRS [4]. The later phase is associated with immunodeficiency characterized by monocytic deactivation defined as "compensatory anti-inflammatory response syndrome (CARS)" [4]. Therefore, monitoring the immune status in septic patients, regulating the inflammatory balance, and targeting interventions have become the key in studying sepsis and sepsis prevention [5].

Pseudomonas aeruginosa mannose-sensitive hemagglutinin (PA-MSHA), developed through biological engineering technology based on *P. aeruginosa* mannose-sensitive hemagglutination pilus vaccine strains, has been successfully used as a protective vaccine. PA-MSHA has been studied as a therapy to fight against cancer [6,7] and can be used as a gram-negative pathogen-associated molecular analog to activate the gram-negative pathogen pattern recognition receptor TLR4, followed by activation of the immune system [8]. However, it has not drawn great attention in previous studies that PA-MSHA is used as an immunomodulator to prevent and treat infection and sepsis induced by gram-negative bacteria.

In the present study, we used the Sprague–Dawley (SD) rat cecal ligation and puncture (CLP) model to investigate the effect of PA-MSHA injection in preventing sepsis by monitoring the survival rate and physical appearance of rats, measuring TLR4 expression in blood and tissue samples, and analyzing pro- and anti-inflammatory factor production. Here, we aimed to evaluate whether PA-MSHA injection could enhance non-specific immune ability, thereby reducing the morbidity and mortality of gram-negative-induced sepsis.

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2. Materials and methods

2.1. SD rat sepsis model

Healthy male SD rats weighing 200–220 g were purchased from the Chinese Shanghai Institutes for Biological Science animal center. CLP was performed to mimic sepsis in the rats. All experiments were approved by the Animal Research Ethics Committee, Fudan University, China. Each rat was individually anesthetized through the intraperitoneal injection of 10% chloral hydrate at 350 mg/kg of body weight. Abdominal hair was shaved and cleaned with Anerdian solution and then covered with a sterile towel. The skin was cut along the midline, and the cecum was pulled out. A sterile thread was used to measure the length of the cecum. A ligature was placed in the middle of the cecum and fastened after the extrusion of cecal contents into the descending part. A sterile stainless steel needle of 3 mm diameter was used to punch holes along the ligation of the distal bowel three times, and the cecal ligation was gently squeezed to extrude feces. The section was closed and sutured with 4-O sterile thread. The surgery time was controlled at approximately 20 min, and the cecal exposure time was limited to 7 min. At the end of the operation, each rat was subcutaneously injected with saline at 20 ml/kg of body weight until it was fully conscious, and it had free access to water and food.

2.2. Physiological state and mortality

During the first 2 days, the survival rate and physiological state were assessed every 12 h, followed by every 24 h in the following 5 days.

2.3. Blood and tissue collection

The rats were individually anesthetized through the intra-peritoneal injection of 10% chloral hydrate at 350 mg/kg of body weight. Blood was collected from the abdominal aorta [9–11], transferred into a tube and centrifuged at 2000 rpm for 20 min. The supernatant was collected and stored at 4 °C until analysis. Rats were scarified after blood collection, and lung tissue (approximately 200 mg) was harvested and stored at −80 °C.

2.4. Experimental design (Fig. 1)

SD rats weighing 200–220 g were randomly divided into 0.25 ml-8d, 0.5 ml-8d, 0.125 ml-16d, 0.25 ml-16d, or 0.5 ml-16d experimental groups or a CLP-only group (12 rats/group). The medical treatment for the rats was the following: 0.25 ml-8d: subcutaneous injection of 0.25 ml PA-MSHA every other day for 8 days; 0.5 ml-8d: subcutaneous injection of 0.5 ml PA-MSHA every other day for 8 days; 0.125 ml-16d: subcutaneous injection of 0.125 ml PA-MSHA every other day for 16 days; 0.25 ml-16d: subcutaneous injection of 0.25 ml PA-MSHA every other day for 16 days; 0.5 ml-16d: subcutaneous injection of 0.5 ml PA-MSHA every other day for 16 days; and CLP-only: no PA-MSHA treatment. Following PA-MSHA treatment, CLP was performed on each rat. Their survival rate and physiological appearance were observed for 7 days after CLP (Fig. 1A).

An additional 36 SD rats weighing 200–220 g were randomly divided into 8d-N, 16d-N, 8d, 16d, non-CLP or CLP-only groups. Rats in the 8d and 16d groups were treated as 0.5 ml-8d and 0.5 ml-16d described above. The treatment of 8d-N and 16d-N rats was the same as 8d and 16d but did not include CLP. At the end of the treatment, blood and tissue samples were collected for further investigation. In the non-CLP group, no treatment or surgery was administered to rats prior to blood and tissue collection. Rats in the CLP-only group received CLP surgery 24 h before blood and tissue harvesting without PA-MSHA (Fig. 1B).

PA-MSHA (1.8×10^9 bacteria per ml) was kindly provided by Wanter Biopharma Company (Beijing, China). PA-MSHA is a killed mutant type of PA with a mannose-sensitive hemagglutination pilus, and its production has been described previously [7]. Briefly, the wild-type PA strain was fully attenuated by culturing with antibiotics for over 200 passages, and genomic DNA was isolated from the attenuated PA strain and ligated with the gene encoding MSHA. PA-MSHA was obtained from the attenuated PA strain transformed with the recombinant DNA and used in this study after inactivation and purification.

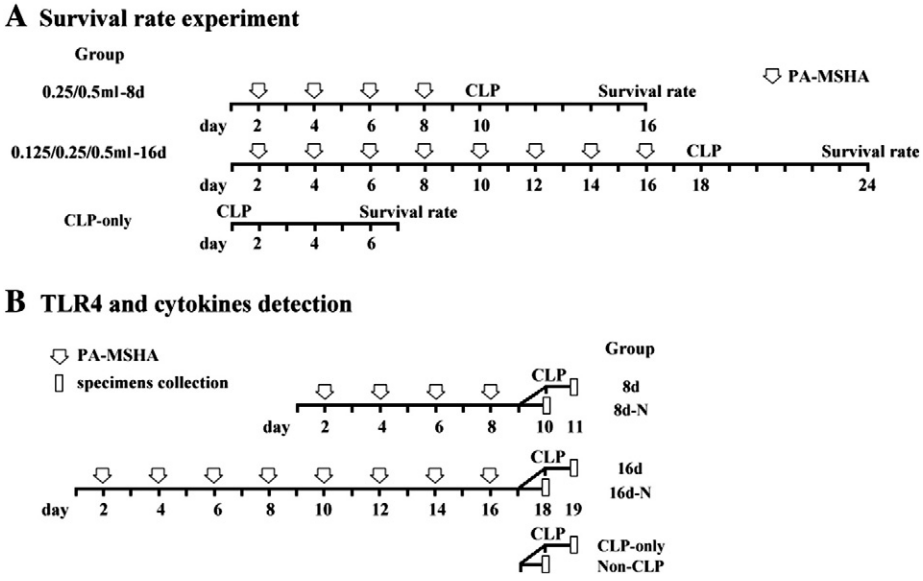

Fig. 1. Experimental design. A) Rats were divided into five experimental groups (0.25 ml-8d, 0.5 ml-8d, 0.125 ml-16d, 0.25 ml-16d, and 0.5 ml-16d) and a control group with 12 rats in each group. The 0.25 ml-8d and 0.5 ml-8d groups were given PA-MSHA every other day for 8 days, and the 0.125 ml-16d, 0.25 ml-16d and 0.5 ml-16d groups were given PA-MSHA every other day for 16 days. The control group did not receive any PA-MSHA injection. Following PA-MSHA preventive treatment, all groups underwent CLP, and the 7-day survival rate was determined. The following PA-MSHA injection volumes were used: 0.25 ml-8d, 0.25 ml/2 days; 0.5 ml-8d, 0.5 ml/2 days; 0.125 ml-16d, 0.125 ml/2 days; 0.25 ml-16d, 0.25 ml/2 days; and 0.5 ml-16d, 0.5 ml/2 days. B) Rats were divided into 0.5 ml-8d-N, 0.5 ml-16d-N, 0.5 ml-8d, 0.5 ml-16d, sham and control groups with six rats/group. The 0.5 ml-8d and 0.5 ml-16d groups were treated as described in A. The 0.5 ml-8d-N and 0.5 ml-16d-N groups were not given CLP. The control group contained normal rats. The CLP-only group was given CLP without PA-MSHA. Blood and lung tissue specimens were collected from rats at 48 h after PA-MSHA injections or 24 h after CLP.