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Original article

Low dose of lipopolysaccharide pretreatment can alleviate the inflammatory response in wound infection mouse model

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ABSTRACT

Purpose: To assess the effects of lipopolysaccharide (LPS) pretreatment on wound infection mouse model and evaluate the biological safety of the optimal pretreatment dose *in vivo*.

Methods: Mice were pretreated with LPS of different doses at 48 and 24 h before femoral medial longitudinal incision was made and infected with different bacteria.

Results: It is showed that 0.5 mg/kg/time of LPS pretreatment can significantly alleviate the inflammation in mouse model infected with methicillin-resistances *Staphylococcus aureus*, methicillin-sensitive *S. aureus*, *Pseudomonas aeruginosa*, or *Escherichia coli* compared with doses of 0.25 mg/kg/time, 1 mg/kg/time, and 1.5 mg/kg/time.

Conclusions: LPS pretreatment can alleviate the inflammation in mouse model and the optimal dose is 0.5 mg/kg/time, and meanwhile it does not damage organs' function.

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Introduction

Lipopolysaccharide (LPS) is one of the most potent stimulators to trigger immune response.¹ In recent years, there have been many researches on the biological effects of LPS.^{2–4} High dose of LPS is toxic to the body. Only when the bacteria dissolved or bacterial cells destroyed by artificial methods, will LPS be released. LPS is called endotoxin and the main toxic substance is lipid A. The effect of LPS is by affecting the body's immune system.^{5–7} LPS is a toll-like receptor 4 (TLR4) agonist and can strengthen the body's ability to fight infection.^{7–9} TLR is the surveillance and recognition receptor of a variety of disease-associated molecular patterns (PAMP). TLR4 can identify not only exogenous pathogens but also endogenous substances and its degradation products.

Surgical infection is a common postoperative complication.¹⁰ How to prevent and treat wound infection is always a thorny problem.¹¹ In our study, we used BALB/c mice wound infection model to explore the effects of different doses of LPS pretreatment and assess the bio-safety of the optimal pretreatment dose.

Materials and methods

Bacteria

Methicillin-resistances *Staphylococcus aureus* (MRSA, ATCC33591), methicillin-sensitive *S. aureus* (MSSA, ATCC25923), *Pseudomonas aeruginosa* (PA, ATCC27853), and *Escherichia coli* (EC, ATCC25922) used in this study were all from American Type Culture Collection.

Animals

Adult female BALB/c mice (20 g) and adult female Wistar rats (200 g) were purchased from the Charles River Laboratories, Beijing, China. All the animals were housed at an individual plastic cage in a sterilized room with the temperature of 23.6 °C and humidity of 35% and a 12 h light/dark cycles (light on at 7:00 am). Each animal was used only once and fed sterile water and chow. All the animal experiments were in ABSL-2 laboratory and have been adopted by the Ethical Committee of Capital Medical University in China.

Reagents

Phenol extracted LPS of *E. coli* 055: B5 was from Sigma in USA and the product number is L2880. ELISA kits for detection of IL-1 β , IL-6, IL-10, and TNF- α were purchased from Dakewe Bioengineering Company in China.

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Preparation of bacterial suspension

Bacteria were grown in trypticase soy broth (Beijing, China) and cultivated in an incubator at 37 °C and containing 5% CO₂; 0.2 ml of bacterial culture solution was diluted at the ratio of 1:10 with sterile saline and the OD value was measured at 600 nm of the diluted solution each hour. The growth of bacteria was in exponential phase when the OD value was increased fast.^{12,13} Then the bacteria were segmented (4 °C, 6000 r/min for 15 min), washed, and suspended in sterile saline; 0.2 ml of suspension was diluted at the ratio of 1:10⁴ with sterile saline; 0.2 ml of the diluted suspension was added into 0.4 ml of 0.4% trypan blue solution, and mixed well to stain 2 min; 2 µl of the stained solution was flowed into the cell counter. According to the counts consequences, bacterial suspension was set at a density of about 1.8×10^9 CFU/ml.

Wound infection model

BALB/c mice were anesthetized by intraperitoneal injection of pentobarbital sodium (40 mg/kg). A longitudinal incision was made on the medial region of the right thigh. The length of the incision was 5 mm and the depth was 3 mm. The deep fascia was not cut. The bacterial suspension was gradually dripped into the incision and a sterile bacterial inoculation needle was utilized to embrocate the solution onto the incision surface.¹⁴ Notice that the bacterial suspension was not overflowed the incision.

LPS pretreatment

The temperature was measured by the digital thermometer (Shenzhen Life Technologies Corporation, China). Mice were adjusted to rectal probe to minimize the stress response 2–5 days before the experiments. Every time, the mice were gently handled. The probe was inserted 2 cm into the rectum. Each measurement was taken six times, and started at 9:00 am. At 48 and 24 h before the wound infection model was established, the mice were treated with LPS via intraperitoneal injection between 10:20 and 10:40 am. The control group animals were intraperitoneally injected with 0.2 ml of sterile saline. The mice's temperatures were recorded for 7 days. Blood sample was collected to measure the white blood cells, granulocyte, and lymphocytes at 72 h and 7 days after wound infection. Serum IL-1β, IL-6, IL-10, and TNF-α were measured at the 7th day after wound infection. At the end of the experiment, all the mice were sacrificed and the wound tissue and spleen were taken. The tissues were observed under microscopy to assess the inflammatory responses.

Biological safety assessment

Rats in the experiment group were intraperitoneally injected with LPS and those in the control group injected with sterile saline. The temperature was measured with a digital thermometer after LPS injection at every 20 min. Serum sample was harvested to detect cytokine at every 2 h and blood biochemistry at every 6 h after LPS injection. All the rats were sacrificed at last and the liver was taken for biopsy 24 h after LPS injection.

Statistical analysis

All data are expressed as means ± SD and the results were analysed by ANOVA for repeated measurement or one-way ANOVA with SPSS17.0 software. Values of $p < 0.05$ were considered significant.

Results

The wound was observed 4 days after the bacteria suspension embrocated onto the incision surface. BALB/c mice were evenly distributed into 7 groups with each group having 12 animals. The MRSA group was neither given LPS nor sterile saline pretreatment. The control group was neither subjected to wound infection nor administrated LPS pretreatment. The experiment group was given LPS pretreatment with the dose of 0.25 mg/kg/time, 0.5 mg/kg/time, 1 mg/kg/time, or 1.5 mg/kg/time. The temperature was obviously changed in all the groups after infection. The magnitude of temperature elevation was the lowest in animals receiving 0.5 mg/kg/time of LPS pretreatment (Fig. 1). There was no significant difference in basic temperatures between groups ($p = 0.964$). White blood cells, granulocytes, and lymphocytes were obviously changed between the 3rd day and the 7th day after wound infection in all the groups besides control group. On the 3rd day, there was a least increase in white blood cells in mice with 0.5 mg/kg/time of LPS pretreatment; while for granulocytes and lymphocytes, the least increase was found in mice with 0.25 mg/kg/time and 0.5 mg/kg/time of LPS pretreatment ($p = 0.166$ and 0.194 respectively). On the 7th day, the white blood cells and lymphocytes showed least increase in mice with 0.5 mg/kg/time of LPS pretreatment. Granulocytes returned to normal in mice with 0.25 mg/kg/time, 0.5 mg/kg/time, and 1 mg/kg/time of LPS pretreatment ($p = 0.522$, 0.809 , and 0.297 respectively).

The wound tissue and spleen of the mice were observed under microscopy and mice with 0.5 mg/kg/time of LPS pretreatment had the lightest wound inflammation and the lowest lymphoid nodules (Fig. 2). The levels of IL-1β, IL-6, and TNF-α were least elevated but IL-10 was highest in mice with 0.5 mg/kg/time of LPS pretreatment. The inflammatory response was lightest in mice with 0.5 mg/kg/time of LPS pretreatment. The optimal pretreatment dose was 0.5 mg/kg/time.

BALB/c mice were evenly divided into 6 groups with each group having 12 animals. The wounds of mice were infected by MSSA, PA, or EC. Half of the mice infected with each bacterial strain were pretreated with 0.5 mg/kg/time of LPS and the others were pretreated with sterile saline. LPS pretreatment could reduce the temperature of the mice and alleviate the increase of white blood cells, granulocytes, lymphocytes, and serum cytokines (Fig. 3). Mice with LPS pretreatment had the milder wound inflammation as compared with mice receiving sterile saline pretreatment (Fig. 4).

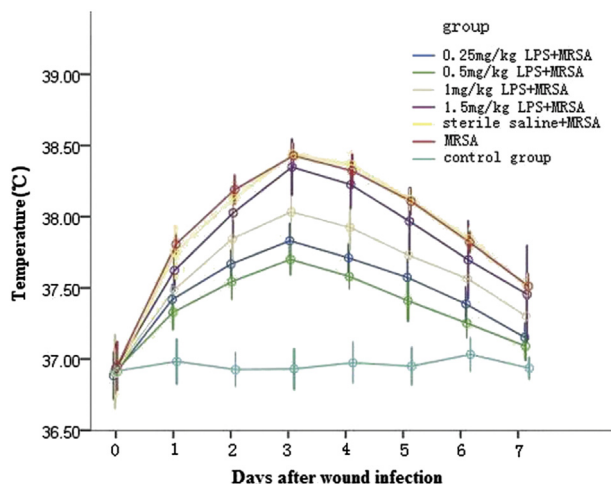


Fig. 1. Temperature alterations of mice infected by MRSA with different doses of LPS pretreatment.

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