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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.

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 ceruminous* (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-1β, 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% CO_2; 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 $1:10^4$ with sterile saline; 0.2 ml of the diluted suspension was added into 0.4 ml of 0.4% trypsin 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).

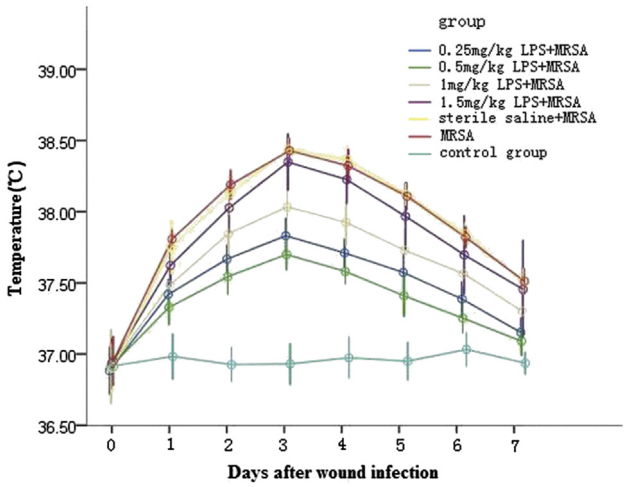

Fig. 1. Temperature alterations of mice infected by MRSA with different doses of LPS pretreatment.

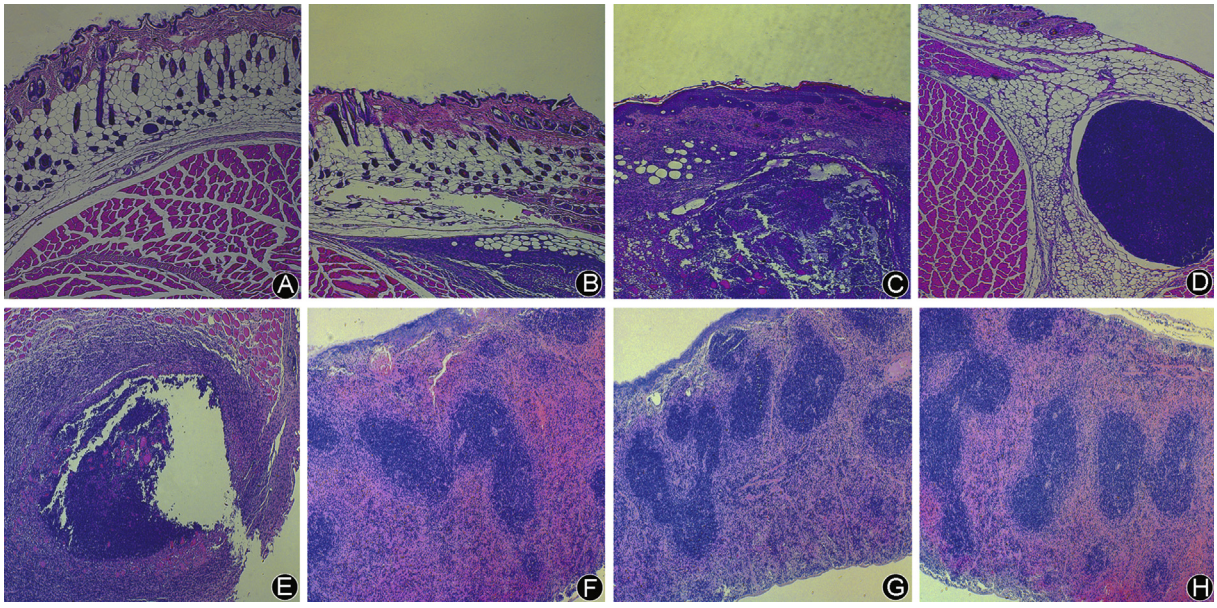

Fig. 2. A: Epithelial tissue healed in mice with 0.5 mg/kg/time of LPS pretreatment. B: Epithelial tissue did not heal and subcutaneous inflammatory response appeared in mice with 1 mg/kg/time of LPS pretreatment. C: Subcutaneous tissue damage and inflammatory cells infiltration were seen in mice with 1.5 mg/kg/time of LPS pretreatment. D: Epithelial tissue did not heal and subcutaneous abscess appeared in mice with sterile saline pretreatment. E: Epithelial tissue did not heal and tissue damage appeared in mice of MRSA group. F: Splenic lymphoid nodules were slightly increased in mice with 0.5 mg/kg/time of LPS pretreatment. G: Splenic lymphoid nodules were increased in mice with 1.5 mg/kg/time of LPS pretreatment. H: Splenic lymphoid nodules were more obviously increased in mice of MRSA group.

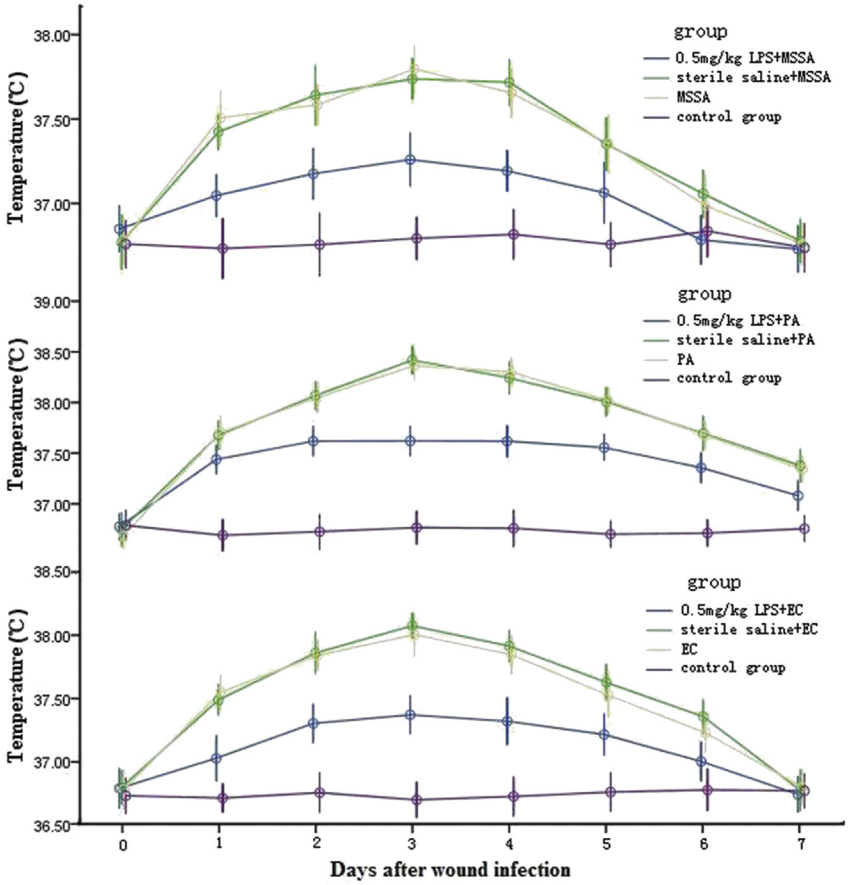

Fig. 3. The temperature alterations in mice infected by different bacteria with 0.5 mg/kg of LPS pretreatment.

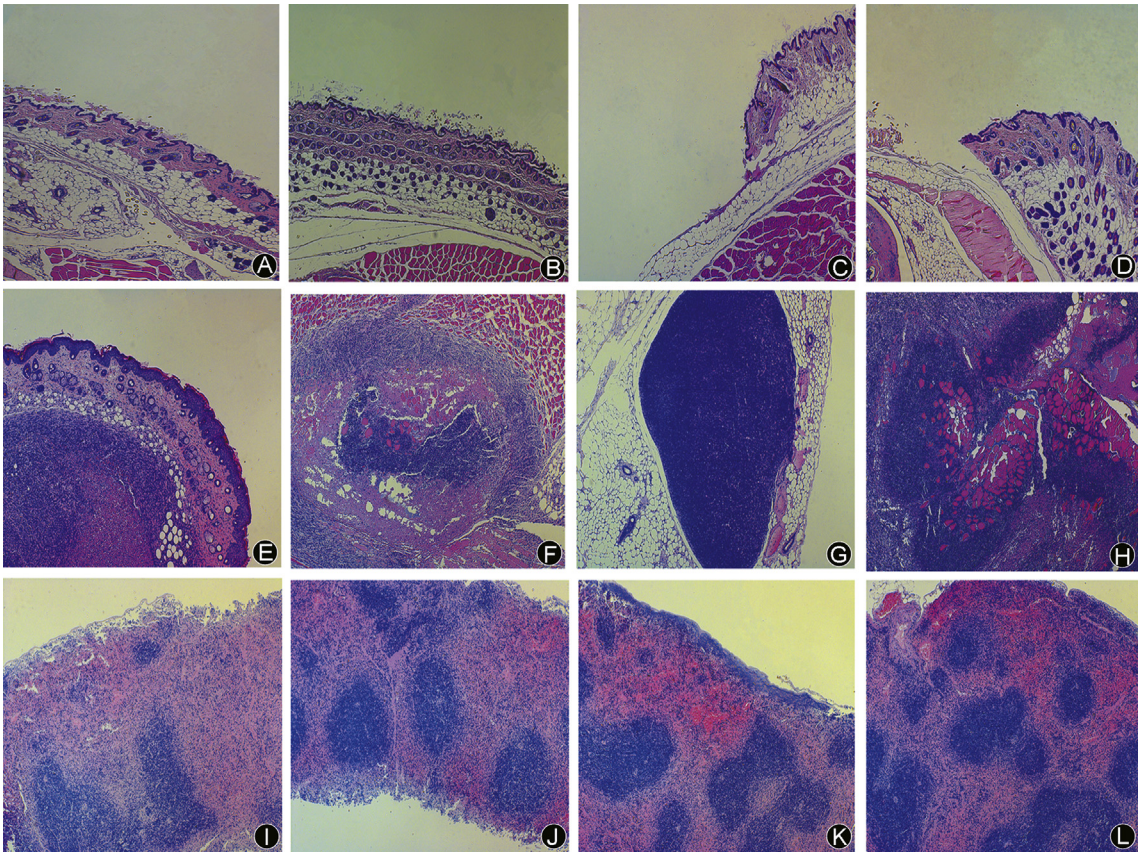

Fig. 4. The inflammation is mild in the wound tissue of mice with LPS pretreatment (A–D). The tissue damage is severe in mice without LPS pretreatment (E–H). The lymphoid nodules are less in spleen tissue of mice with LPS pretreatment (I, J) than those without LPS pretreatment (K, L).

Rats were evenly distributed into 2 groups with each group having 12 animals. The experiment group was intraperitoneally injected with LPS (0.5 mg/kg) and the control group was intraperitoneally injected sterile saline. The temperatures and serum cytokines were obviously increased and returned to normal within 24 h after LPS injection (Fig. 5); 0.5 mg/kg of LPS did not damage the structure of liver tissues. LPS could increase the levels of alanine aminotransferase (ALT) and aspartate transaminase (AST). ALT and AST returned to normal within 24 h.

Discussion

Surgical site infection (SSI) is a common complication of surgery.[15,16] It will directly influence the curative effect of an operation. Bacteria will form a biofilm on the internal fixation plate which employed in most of orthopaedic surgeries. The biofilm can resist the body immunity and antibiotics.[17,18] Once biofilm formed, the bacteria is difficult to be cleaned. In recent years, some studies have reported that LPS can enhance the body immunity to resist sepsis.[6,7] They found that LPS is a TLR4 agonist.[19,20] TLR4 will regulate and control the innate immunity and the adaptive immunity by PAMP.[21] In our study we assessed the effect of LPS pretreatment on the wound infected with different bacteria and selected an optimal pretreatment dose.

In our experiment, low dose of LPS could alleviate the inflammation by detecting the body's temperature, white blood cells, granulocyte, lymphocyte, serum cytokines, and wound tissue biopsy. It indicates that low dose of LPS could activate TLR4 and excitate lymphocytes. Once bacteria invaded, the immune system is rapidly activated. Lymphocytes rapidly proliferated and differentiated. The bacteria will be cleared out quickly.

We found that 0.5 mg/kg/time of LPS pretreatment had the best protection effect. High dose of LPS could make the mice died. Bacteria could damage the cells and made inflammatory cells to release histamine, serotonin, prostaglandins, and stimulate peptide to cause angiotelectasis, low blood pressure, and inadequacy of tissue perfusion. Thus, organs would be in a hypoxia environment. Lactic acid would accumulate to damage the organ function.

We speculate that LPS could activate not only TLR4 but also other receptors and the activation effects had a dose dependent relationship. When the dose of LPS exceeds the threshold value, it will play a harm role. However, this hypothesis needs to be further proved.

The findings suggest that low dose of LPS pretreatment can effectively reduce the inflammation of wound infection mouse model. The optimal pretreatment dose is 0.5 mg/kg/time in our study. The exact mechanism is still to be further studied.

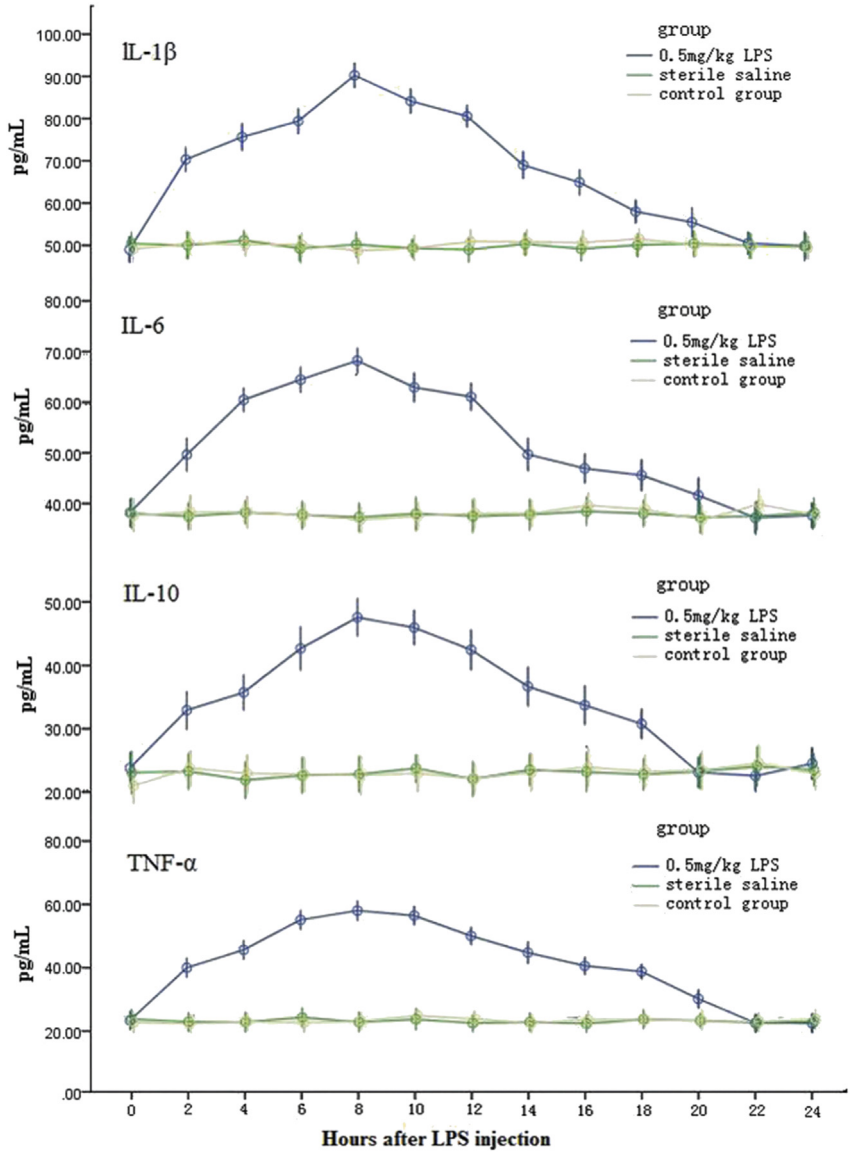

Fig. 5. Variations of the levels of IL-1β, IL-6, IL-10, and TNF-α.

References

1. Matsuura M. Structural modifications of bacterial lipopolysaccharide that facilitate gram-negative bacteria evasion of host innate immunity. *Front Immunol*. 2013;4:109. http://dx.doi.org/10.3389/fimmu.2013.00109.
2. Li X, Liu Y, Ma X. Unfractionated heparin inhibits lipopolysaccharide-induced expression of granulocyte colony-stimulating factor in human endothelial cells through Toll-like receptor 4 signaling pathway. *Zhonghua Wei Zhong Bing Ji Jiu Yi Xue*. 2015;27:81–85. http://dx.doi.org/10.3760/cma.j.issn.2095-4352.2015.02.001.
3. Zhou XY, Zhang WJ, Huang QB, et al. Role of RAGE in lipopolysaccharide-induced cytoskeletal changes in mouse pulmonary microvascular endothelial cells. *Nan Fang Yi Ke Da Xue Xue Bao*. 2015;35:6–11.
4. Rayhane N, Fitting C, Lortholary O, et al. Administration of endotoxin associated with lipopolysaccharide tolerance protects mice against fungal infection. *Infect Immun*. 2000;68:3748–3753.
5. Lin TY, Fan CW, Maa MC, et al. Lipopolysaccharide-promoted proliferation of Caco-2 cells is mediated by c-Src induction and ERK activation. *Biomedicine*. 2015;5:5. http://dx.doi.org/10.7603/s40681-015-0005-x.
6. Wang DB. Monophosphoryl lipid A is a lipopolysaccharide-derived Toll-like receptor 4 agonist which may improve Alzheimer's disease pathology. *Expert Opin Biol Ther*. 2013;13:1639–1641. http://dx.doi.org/10.1517/14712598.2013.838556.
7. Bao S, Cao Y, Zhou H, et al. Epigallocatechin gallate (EGCG) suppresses lipopolysaccharide-induced Toll-like receptor 4 (TLR4) activity via 67 kDa laminin receptor (67LR) in 3T3-L1 adipocytes. *J Agric Food Chem*. 2015;63:2811–2819. http://dx.doi.org/10.1021/jf505531w.
8. Wheeler DS, Lahni PM, Denenberg AG, et al. Induction of endotoxin tolerance enhances bacterial clearance and survival in murine polymicrobial sepsis. *Shock*. 2008;30:267–273. http://dx.doi.org/10.1097/shk.0b013e318162c190.
9. Choi YH, Kim GY, Lee HH. Anti-inflammatory effects of cordycepin in lipopolysaccharide-stimulated RAW 264.7 macrophages through Toll-like receptor 4-mediated suppression of mitogen-activated protein kinases and NF-κB signaling pathways. *Drug Des Dev Ther*. 2014;8:1941–1953. http://dx.doi.org/10.2147/DDDT.S71957.
10. Kokudo T, Uldry E, Demartines N, et al. Risk factors for incisional and organ space surgical site infections after liver resection are different. *World J Surg*. 2015;39:1185–1192. http://dx.doi.org/10.1007/s00268-014-2922-3.
11. Naik MM, Dubey SK. Lead resistant bacteria: lead resistance mechanisms, their applications in lead bioremediation and biomonitoring. *Ecotoxicol Environ Saf*. 2013;98:1–7. http://dx.doi.org/10.1016/j.ecoenv.2013.09.039.
12. Mytilinaios I, Salih M, Schofield HK, et al. Growth curve prediction from optical density data. *Int J Food Microbiol*. 2012;154:169–176. http://dx.doi.org/10.1016/j.ijfoodmicro.2011.12.035.
13. Dijkstra CE, Larkin OJ, Anthony P, et al. Diamagnetic levitation enhances growth of liquid bacterial cultures by increasing oxygen availability. *J R Soc Interface*. 2011;8:334–344. http://dx.doi.org/10.1098/rsif.2010.0294.
14. Mao XQ, Xie WY. An experimental animal model for infected wound of maxillofacial areas. *J Hainan Med Univ*. 2007;13:19–20.

15. Ovaska MT, Mäkinen TJ, Madanat R, et al. Risk factors for deep surgical site infection following operative treatment of ankle fractures. *J Bone Jt Surg Am*. 2013;95:348–353. http://dx.doi.org/10.2106/JBJS.K.01672.
16. Zhou J, Ma X. Cost-benefit analysis of craniocerebral surgical site infection control in tertiary hospitals in China. *J Infect Dev Ctries*. 2015;9:182–189. http://dx.doi.org/10.3855/jidc.4482.
17. Dhir S. Biofilm and dental implant: the microbial link. *J Indian Soc Periodontol*. 2013;17:5–11. http://dx.doi.org/10.4103/0972-124X.107466.
18. Arciola CR, Campoccia D, Ehrlich GD, et al. Biofilm-based implant infections in orthopaedics. *Adv Exp Med Biol*. 2015;830:29–46. http://dx.doi.org/10.1007/978-3-319-11038-7_2.
19. Xue P, Zheng M, Gong P, et al. Single administration of ultra-low-dose lipopolysaccharide in rat early pregnancy induces TLR4 activation in the placenta contributing to preeclampsia. *PLoS One*. 2015;10:e0124001. http://dx.doi.org/10.1371/journal.pone.0124001.
20. Jain S, Suklabaidya S, Das B, et al. TLR4 activation by lipopolysaccharide confers survival advantage to growth factor deprived prostate cancer cells. *Prostate*. 2015;75:1020–1033. http://dx.doi.org/10.1002/pros.22983.
21. Inman RD. Innate immunity of spondyloarthritis: the role of toll-like receptors. *Adv Exp Med Biol*. 2009;649:300–309.