International Immunopharmacology 32 (2016) 66–75

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Consistency and pathophysiological characterization of a rat polymicrobial sepsis model via the improved cecal ligation and puncture surgery

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ARTICLE INFO

Article history:
Received 3 November 2015
Received in revised form 24 December 2015
Accepted 29 December 2015
Available online 21 January 2016

Keywords:
Sepsis
Animal model
Cecal ligation and puncture
Inconsistency
Syringe needles
Three-edged needles

ABSTRACT

Sepsis is the leading cause of death for critical ill patients and an essential focus in immunopharmacological research. The cecal ligation and puncture (CLP) model is regarded as a golden standard model for sepsis study. However, this animal model is easily affected by variability problems and dramatically affects pharmacological evaluation of anti-sepsis therapies, which requires standardized procedures and stable outcomes. Herein, the traditional syringe needle based puncture method was used as the major unstable factor for CLP models. Syringe needles created varied mortality in parallel experimental groups of CLP rats; they were inconsistent for severity control as mortality in CLP rats was not correlated with change in punctures, ligation lengths, or needle sizes. Moreover, the use of drainage tubes or strips, which was supposed to strengthen drainage stability, also failed to improve consistency of traditional syringe needles. To solve the consistency problem, an improved design of CLP surgery by puncture with newly-developed three-edged needles was described herein. In contrast to traditional syringe needles, these three-edged needles ensured more stable outcomes in repetitive groups. Furthermore, increased severity was found to be consistent with the enlarged needle size, as shown by the elevated mortality, increased proinflammatory cytokines, abnormal coagulation, worsen acidosis and more severe acute lung injury. In conclusion, application of the newly-developed three-edged needles provides a simple and feasible method to improve stability when conducting CLP surgery, which is significant for pharmacological studies on sepsis.

1. Introduction

Sepsis is a life threatening clinical syndrome induced by infection and characterized by uncontrolled inflammatory reaction [1,2]. Currently, sepsis affects more than 19 million people worldwide each year and is ranked as the leading cause of death in critically ill population [3,4]. Despite advancement in modern medicine, sepsis treatment has been poorly progressed. Consequently, the development of novel anti-sepsis drugs still represents a major focus in the immunopharmacological field.

The progress of sepsis is complicated, involved with both pathogen invasion and host immunological dysfunction [5]. Therefore, standardized and clinically resembling animal models are indispensable to provide insights into the pathogenesis for sepsis and to testify the pharmacological characteristics of potential drug therapies [6]. The most accepted sepsis model currently is produced via cecal ligation and puncture (CLP), a surgery characterized by cecum ligation and perforation which induced polymicrobial peritonitis and ultimately leads to sepsis [7]. CLP typically presents similar pathophysiological progress and comparable cytokine kinetics and magnitude with clinical sepsis. It is thereby regarded as the golden standard for experimental sepsis models [7–10]. It has been revealed that the severity of sepsis in rodent CLP model is generally affected by animal background and surgical factors such as the length of ligation, sizes and numbers of puncture [11,12]. Controlled procedures of rodent CLP surgery was also described previously attempting to provide standardized surgical procedures and facilitate consistency [13]. However, the variability problem persists even when identical surgery is given to identical groups of animals, demanding the necessity to further explore the underlying factors for the inconsistency problem in CLP model [14,15].

Infection and sepsis in CLP model are triggered by translocation of enteral content from the perforated cecum into the abdominal cavity. Therefore, the consistency of cross cecum drainage induced by puncture principally decides the stability of severity. The standardized procedures of CLP surgery by introducing syringe needles for cecum puncture were firstly established by Wichterman, which was assumed to create more predictable outcomes [16]. Thereafter, syringe needles have

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http://dx.doi.org/10.1016/j.intimp.2015.12.041
1567-5769/

been used almost exclusively as the puncture tool in performing the CLP surgery, with the needle sizes ranging from 13 to 25 G to achieve sepsis with different severity [12,17–19]. However, the mortality still varied when identical needles were used in inter-lab studies or even just in the same study [13,16,20].

In our lab, inconsistent mortality of CLP rats punctured with a 16 G needle was also observed [14]. Meanwhile, a novel kind of puncture needles was discovered to create consistent outcomes in rat CLP model and applied for anti-sepsis drug evaluation. Also, a brief explanation for the reason of good consistency was provided for the interested researchers [14,21]. In the present study, a more comprehensive evaluation of the three-edged needle used for CLP surgery was introduced, and its advantages over routinely applied CLP surgery were presented in detail, too.

2. Materials and methods

2.1. Animals

Male S-D rats (200–220 g, 6–8 weeks, obtained from the Experimental Animal Centre of Third Military Medical University) were kept in independent ventilated cages (IVC) with free access to autoclaved food and water. All the animal experiments were approved by institutional Animal Ethic Committee and conducted in accordance with national and institutional guidelines for animal care and use of experimental purpose.

2.2. Puncture needles used for CLP surgery

Two types of needles were used. One type of needle was a syringe needle defined by their outer diameters described as gauge (G). The diameters of 14, 16 and 18 G needles were 2.108, 1.651 and 1.270 mm, respectively. Another type needle was a three-edged needle with three sharp blades, and their sizes were defined for the edge widths (3, 4 and 5 mm).

2.3. Procedures of CLP surgery with traditional syringe needles or newly-developed three-edged needles

S-D rats were subject to the CLP surgery as previously described [14]. In details, rats were fasted for 12–16 h before the CLP procedure and then anesthetized by isoflurane (Keyuan Pharmaceutical, Shandong, China) inhalation. After a loss of consciousness within approximate 1–2 min, rats were placed in a supine position with a nosecone cover on their face to maintain the sedate status (Fig. S1). The abdominal region was opened via a 1–1.5 cm longitudinal midline incision. The cecum was exposed and the mesentery was carefully sheared from the cecum. The free cecum was gently extruded to ensure a uniform distribution of inside content in each rat. Then the cecum was ligated in a vertical position with the 4–0 silk thread. In syringe groups, a perforating puncture was made vertically on the central position of the ligated cecum with a syringe needle (BD, Franklin Lakes, NJ) alone or in combination with drainage strips or tubes. While in three-edged needle groups, a perforating puncture was made with a three-edged needle (Figs. S2, S3). Rats were then resuscitated via subcutaneous injection of 50 ml/kg pre-warmed normal saline (NS) solution (Tuopai Pharmaceutical Co. Ltd., Sichuan, China) and kept in a thermostatic room (22 ± 2 °C) with a 12-hour interval of day and night alternation and ad libitum access to water and food.

2.4. Post surgery treatment

For survival analysis, the mortality rate in each group was observed till 7 days after the surgery. For pathophysiological comparison, blood and histological samples of rats were collected at indicated time points. Blood drawn via abdominal aorta was used for cytokines detection, blood culture and various laboratory tests. Blood drawn via heart puncture was collected for blood bacterial culture and LPS detection. The lung tissue samples were extracted and prepared for wet to dry ratio measurement, cytokines expression detection, signaling molecules detection and histological examination.

2.5. Laboratory blood tests

Blood samples were taken for laboratory tests including arterial blood gas, blood cell count and coagulation parameters. Arterial blood pH and lactate (Lac) was detected instantly after sampling via an ABL700 blood gas analyzer (Radiometer, Brønshøj, Denmark). Blood cellular examination for platelet (PLT) was analyzed by a BC-5800 auto hematology analyzer (Mindray, Mahwah, NJ). Blood prothrombin time (PT) and activated partial thromboplastin time (APTT) were measured by a CA-7000 coagulation analyzer (Sysmex, Chuo-ku, Kobe, Japan).

2.6. Broncho-alveolar lavage fluid (BALF) sampling and cell counts

Rats were anesthetized under isoflurane inhalation. BALF samples were obtained as described previously [22]. Briefly, the trachea was exposed and cannulated with a small tube. The entire airway was lavaged by gently aspirated and pooled with 1 ml of sterile PBS. The BALF samples were collected and neutrophil counts in the lavage were determined by detection using a hemocytometer. The remaining samples were used for staining or bacterial culture.

2.7. Blood bacterial culture

The whole blood samples, lung homogenates and BALF were diluted in NS, smeared on blood agar plates and incubated at 37 °C overnight for blood bacterial culture.

2.8. Plasma lipopolysaccharide (LPS) detection

Blood samples were obtained via heart punctures and centrifuged for the isolation of plasma. Subsequently, 100 μl of each plasma sample was added to 100 μl of the quantitative LAL reagents (A&C Biological Ltd., Zhanjiang, Guangdong, China) and reacted at 37 °C for 60 min in an ATI 320-06 kinetic tube reader (L ab Kinetics Ltd., Bruton, UK).

2.9. Western blot analysis

Equal amounts of protein lysates extracted from lung tissue by RIPA lysis buffer kit (Thermo Pierce, Rockford, IL) were separated by SDS PAGE and transferred onto PVDF membranes (Millipore, MA). Blots on the membranes were blocked in 5% BSA and probed with the anti-p-p38, p38, p-ERK, ERK, Tubulin (1:1000 dilutions, Cell Signaling, Danvers, MA) and neutrophil elastase (NE), GAPDH (1:1000 dilutions, abcam, Cambridge, UK). The blots were then incubated with secondary IgG antibodies (1:2000 dilutions, Cell Signaling, Danvers, MA) and developed with SuperSignal West Femto Maximum Sensitivity Substrate kit (Thermo Pierce, Rockford, IL) for chemiluminescence assay under a ChemiDoc XRS gel imaging system (Bio-Rad, Hercules, CA).

2.10. ELISA

For serum procalcitonin (PCT), interleulin 1β (IL-1β), IL-17, complement 5a (C5a) and monocyte chemotactic protein 1(MCP-1) detection, blood samples were centrifuged and serum was obtained and added to the 96 well microplates for ELISA detection (Uscn Life Inc., Wuhan, Hubei, China). For lung IL-6, C5a and neutrophil elastase detection, equal amounts of protein lysates were extracted from lung tissue homogenate and added to plates for ELISA detection (Uscn Life Inc. Wuhan, Hubei, China).