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*Original Article*

# Role of bone marrow-derived mesenchymal stem cells in a rat model of sepsis-induced acute lung injury

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**Abstract:** Background: Acute Respiratory Distress Syndrome (ARDS) is a life-threatening condition in patients with predisposing factors, among which sepsis is a common cause. There are no effective strategies to treat this syndrome except supportive therapies. Some preclinical studies suggest a potential therapeutic effect of mesenchymal stem cells (MSCs) on ARDS. However, data specifically evaluating the impact of MSCs therapy on development of sepsis-induced ARDS are still limited. Methods: There were 25 rats in each group, including sepsis group, sepsis + antibiotic group, sepsis + antibiotic + MSCs group and sham group, twenty of which being sacrificed at 12 h, 18 h, 24 h and 48 h after surgery, respectively, five for survival analysis, and 5 normal controls. Survival rate, pulmonary physiological function, alveolar capillary barrier, lung inflammatory reaction and pathological injury were measured. Results: ① at 18 h, total nucleated cell count of bronchoalveolar lavage fluid was 12.29±7.03 in the sepsis + antibiotic + MSCs subgroup, as compared with 42.3±8.18 in the sepsis subgroup ($P$=0.007), 35.77±16.80 in the sepsis + antibiotic subgroup ($P$=0.016) and 37.80±22.96 in the normal group ($P$=0.025). ② The protein concentration of bronchoalveolar lavage fluid in the sepsis + antibiotic + MSCs subgroup was less than that in the sepsis subgroup at 24 h, (0.41±0.24 vs. 1.17±0.57 mg/ml, $P$=0.004). Conclusion: Intravenous injection of allogeneic MSCs is safe for rats with sepsis-induced acute lung injury. To be administrated in early stage of sepsis, MSCs improve alveolar inflammatory cells infiltration and protein exudation, as well as alveolar congestion and hemorrhage. However, there is a potential risk of oxygenation impairment and lung water increase with intravenous injection of MSCs.

**Keywords:** Sepsis, Acute Respiratory Distress Syndrome (ARDS), bone marrow derived mesenchymal stem cells (BMSCs)

## Introduction

Acute Respiratory Distress Syndrome (ARDS) remains a serious life-threatening condition, usually develops in patients with predisposing conditions that induce systemic inflammatory response, such as sepsis, pneumonia, major trauma, multiple transfusions, aspiration and acute severe pancreatitis [1]. The mainstay of ARDS treatment is supportive care. Some potential treatments may show benefits in improving oxygenation or accelerating alveolar fluid clearance. However, none of them has been proven to reduce mortality or improve any other fatal clinical outcomes [2-5].

Mesenchymal stem cells (MSCs) have received increasing interest for treatment of ARDS [6]. Although the pathogenesis of ARDS remains unclear, well accepted hypotheses include alveolar epithelial and vascular endothelial injury, increased permeability of the endothelial and epithelial barriers, imbalance in coagulation and fibrinolysis systems, combined with inflammatory and anti-inflammatory reactions [3, 7-10]. Most current researches demonstrate that MSCs potentially improve major abnormalities on ARDS based on a variety of mechanisms [11]. The concordance between ARDS pathophysiology and regulatory effects of MSCs therapy makes MSCs a promising option for treatment of ARDS [12]. However, application of MSCs for treatment of ARDS is still a novel field of research, with many related issues to be further clear, such as the optimal administration methods (administration route, timing, dosing frequency, etc), measurement of the effectiveness of treatment [12], safety for patients with

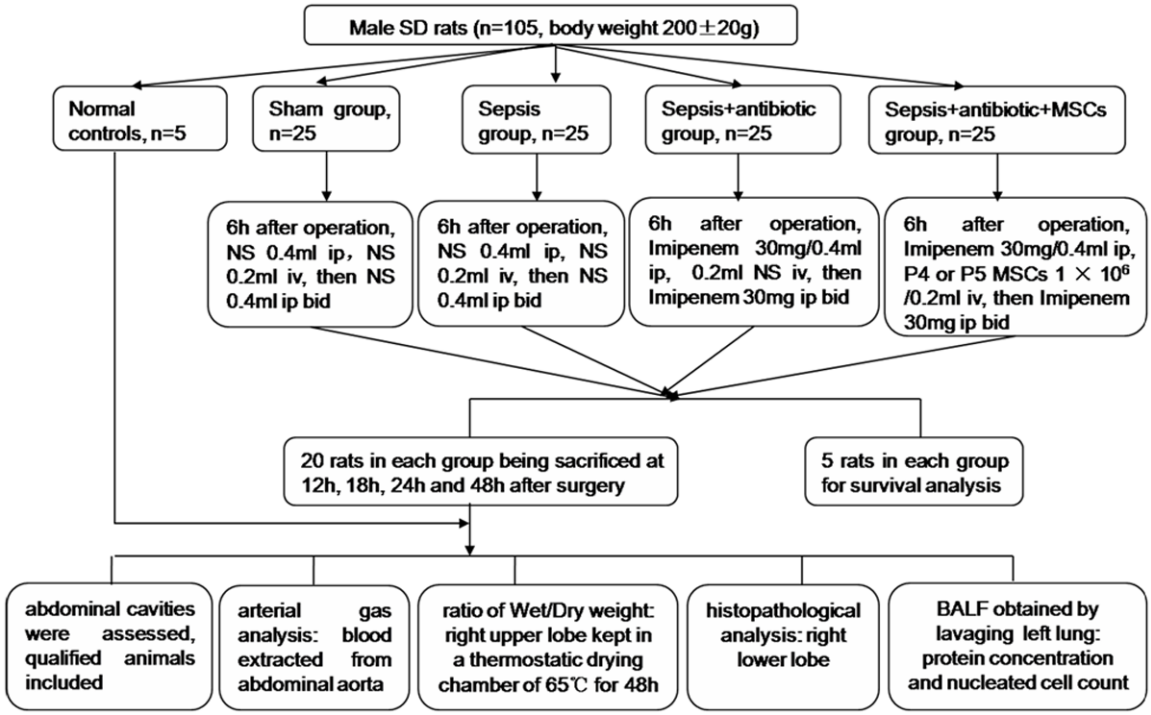


**Figure 1.** Flow diagram of study design. ip: Intraperitoneal injection, iv: Intravenous injection, NS: Normal saline, bid: Twice a day.

ARDS, etc [13]. Sepsis is one of the most common causes of ARDS [2]. Sepsis-induced ARDS has a higher overall disease severity and higher mortality than non-sepsis-induced ARDS [1]. This study was undertaken to examine the effects of BMSCs injection on sepsis-induced ARDS in a rat abdominal sepsis model.

## Materials and methods

### *Animals*

Male Sprague-Dawley (SD) rats (n=105, body weight 200±20 g) and neonatal SD rats (n=5, 2-3 wk old) in specific pathogen free (SPF) grade were obtained from animal center of Sun Yat-sen University (SCXK 2011-0029). Animals were maintained under standard laboratory conditions of 12-hour light/dark cycles, 23±3°C room temperature, 40%-70% environment humidity, and were given free access to standard rat chow and water though the whole experiment. The animals were allowed to adapt to laboratory conditions for at least 2 days. The animal experiment was approved by the Animal Care and Use Ethics Committee of Sun Yat-sen University.

### *Experimental grouping*

Male SD rats (n=105) were randomly allocated to the sepsis group (n=25), the sepsis + antibiotic group (n=25), the sepsis + antibiotic + MSCs group (n=25) , the sham group (n=25), twenty of which being sacrificed at 12 h, 18 h, 24 h and 48 h after surgery, respectively, five for survival analysis, and normal controls (n=5). Flow diagram of study design is as follows (**Figure 1**).

### *Sepsis model*

The procedure was carried out under 10% chloral hydrate anesthesia (0.4 ml/100 g body weight, i.p.). After disinfection, a 2-cm long median laparotomy was performed. The ileocecus was retracted out of abdominal cavity. Then an approximately 5-mm-long venous indwelling cannula (16 G) was inserted approximately 1 cm distal to the ileocecal valve at the antimesenteric site. The venous indwelling cannula was fixated to the wall with sutures. By careful palpation of the cecum with cotton swabs, the stent was filled with feces. The layers of the abdomen wall were sutured, followed by fluid

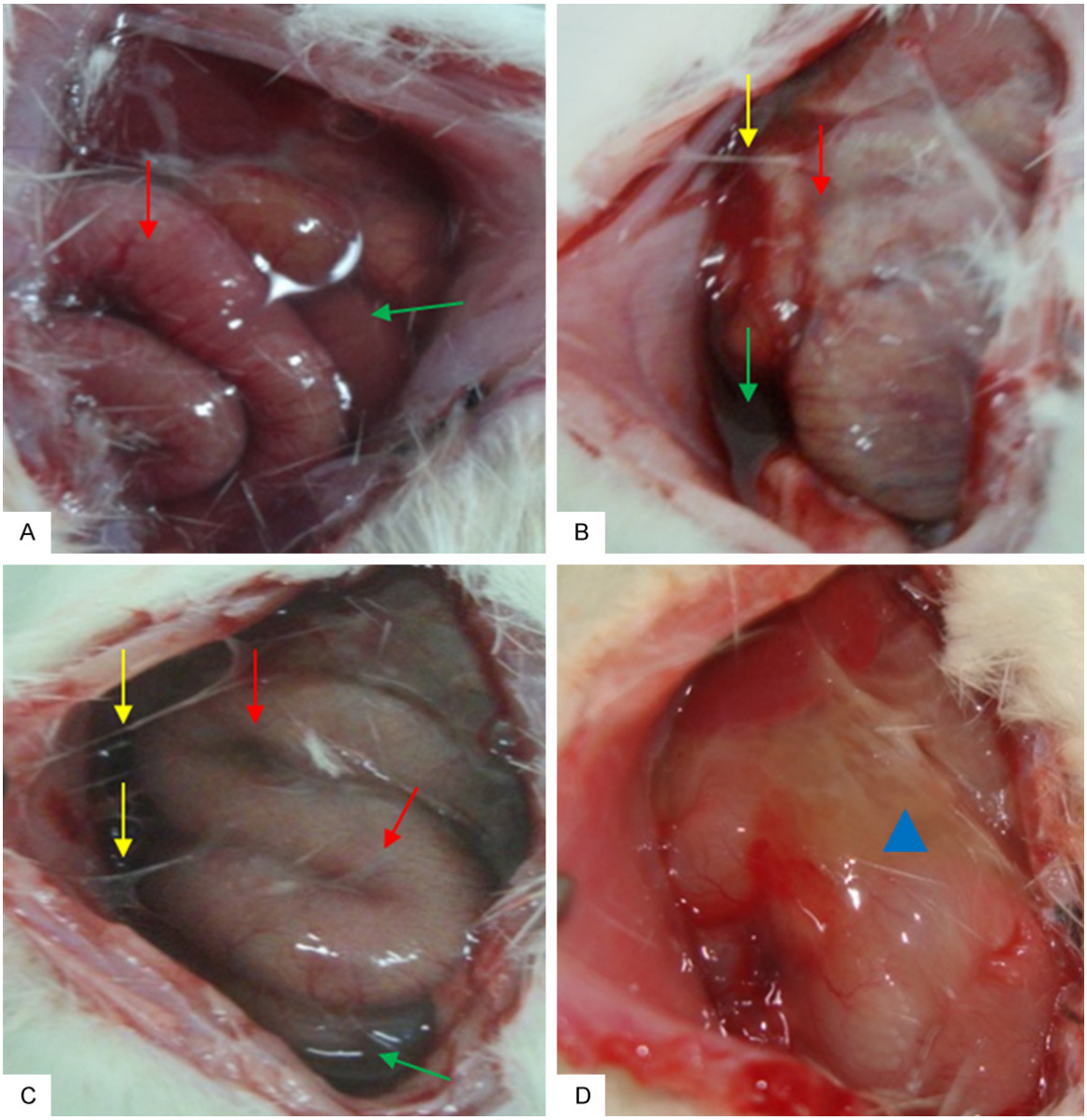


**Figure 2.** Abdominal cavity presentation in rat model of sepsis. A. At 12 h, model rats revealed obvious peritonitis. C and D. At 24 h and 48 h, the whole abdominal cavity was fixed. B. At 18 h, the severity of peritonitis was in between. Red arrows represent dilatation of bowel loops, yellow arrows represent fiber formation, green arrows represent as cites, and blue triangles represent purulent secretion.

supplement with 2 ml saline solution. Animals in sham group underwent the same surgery except that the stent was fixed outside cecum. Six hours after operation, animals in the sepsis + antibiotic + MSCs group were given one dose of Imipenem/Cilastatin (30 mg/0.4 ml) by intraperitoneal injection, and $P_4$ or $P_5$ MSCs ($1 \times 10^6$/0.2 ml) though tail vein. Then Imipenem/Cilastatin was given until sampling (i.e., 30 mg/0.4 ml i.p., twice a day). Animals in other three groups received corresponding intervention. Imipenem/cilastatin is an optimal antibiotic for enterogenous peritonitis. Drug dose is determined based on conversion coefficient method referring to "Basic Knowledge of Laboratory Animal Science and Experimental Technique Course".

*Survival analysis*

Animals in the sepsis + antibiotic + MSCs group, sepsis + antibiotic group, sepsis group

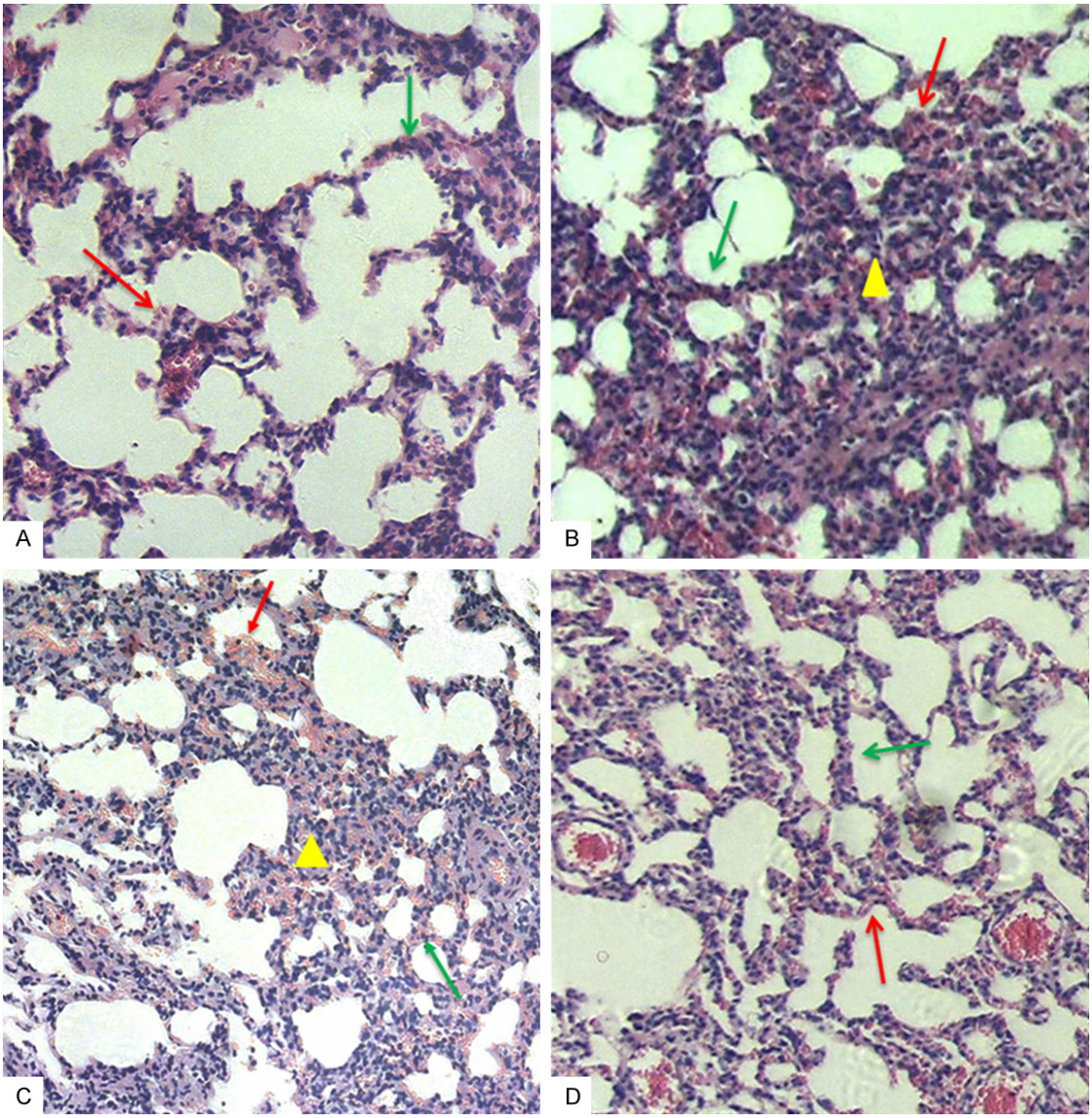


**Figure 3.** Lung histopathology for rat model of sepsis. Lung tissues were obtained at 12 h (A), 18 h (B), 24 h (C) and 48 h (D) after surgery (H&E, × 100 magnifications). Green arrows represent aggregation of inflammatory cells and thickness of alveolar wall, yellow triangles represent atelectasis, and red arrows represent alveolar congestion and hemorrhage.

and sham group (n=5 in each group) were followed up every 12 h until death.

*Sampling handling*

Survived animals were anesthetized and laparotomized at predetermined time points. Abdominal cavities were fully exposed. Severity of peritonitis was assessed and qualified animals were included. After that, exposed the abdominal aorta, extracted 1 ml of arterial blood for gas analysis, and then sacrificed the animal. Subsequently separated the trachea, performed a transverse incision between the third and fourth trachea ring, carried out endotracheal intubation with an outer diameter of 1.8 mm endotracheal tube, and fixed the tube with silk sutures, then performed thoracotomy and ligation of right lung lobes. Three aliquots (2 ml each) of sterile normal saline were instilled and aspirated 10 seconds later, repeated 3 times for each aliquot. The bronchoalveolar lavage fluid (BALF) was pooled in ice-cold tubes for testing. Separated the right upper lobe, blotted

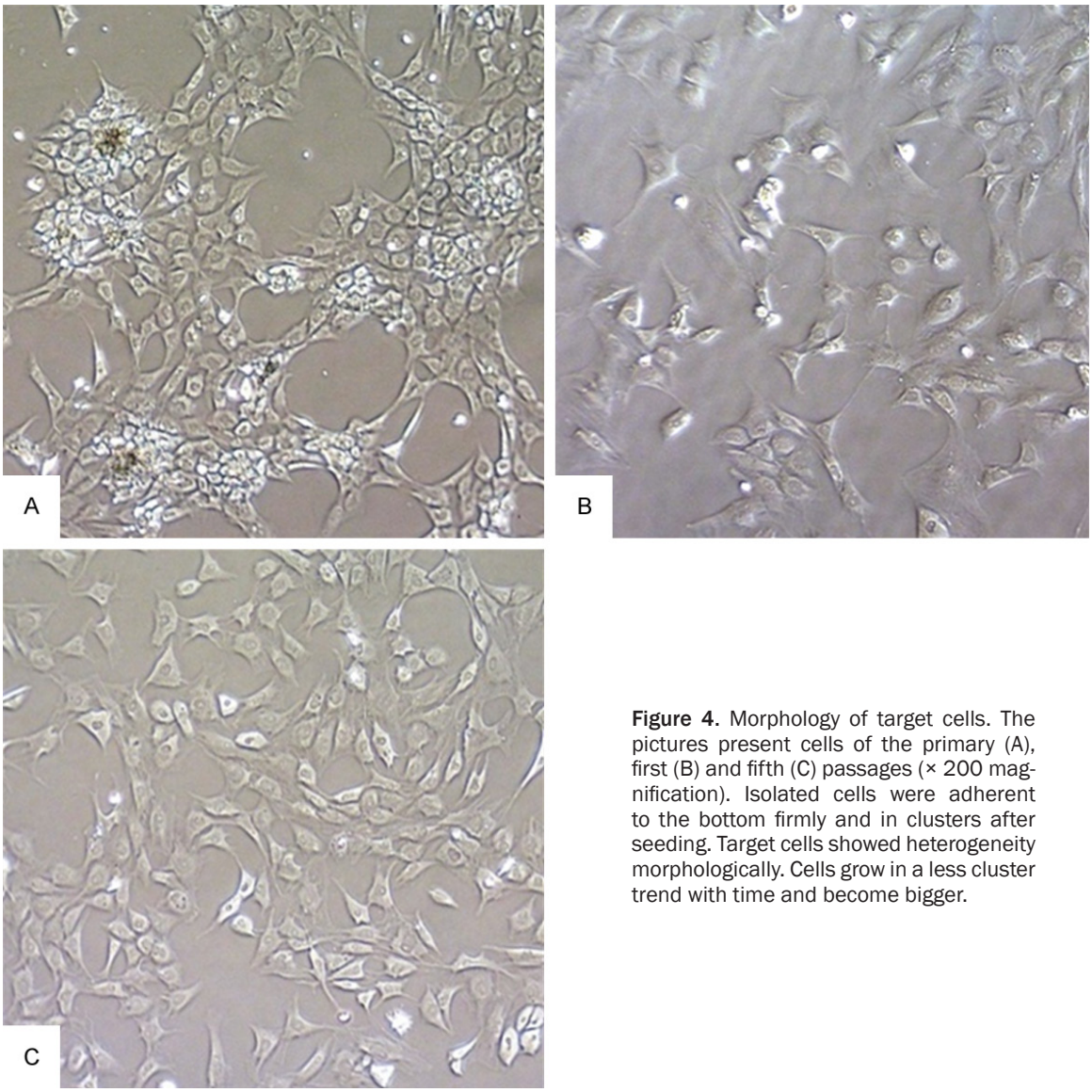


**Figure 4.** Morphology of target cells. The pictures present cells of the primary (A), first (B) and fifth (C) passages (× 200 magnification). Isolated cells were adherent to the bottom firmly and in clusters after seeding. Target cells showed heterogeneity morphologically. Cells grow in a less cluster trend with time and become bigger.

it up and weighed, then kept in a thermostatic drying chamber of 65°C for 48 hours, weighed the dried tissue again, thus calculated the ratio of Wet/Dry weight. The right lower lobe was immediately fixed in 10% formalin for histopathological analysis.

*Histopathological evaluation*

Lung injury was scored by experienced investigator blinded to the groups according to the following parameters: a) alveolar congestion and hemorrhage, b) infiltration or aggregation of inflammatory cells in airspace or interstitium, c) thickness of alveolar wall, d) hyaline membrane formation, e) atelectasis. Each parameter was scored on a 4-point scale as follows: 0 minimal damage, 1 mild damage, 2 moderate damage, 3 severe damage.

*Isolation, culture and identification of BMSCs*

MSCs derived from bone marrow of rats were isolated as Houlihan etc [14] described. SD neonatal rats (n=5, 2-3 wk old) were chosen for BMSCs isolation. The cells applied in subsequent experiments were of passage 4 or 5, identified by detecting surface markers expression by flow cytometry.

*Statistical analysis*

The SPSS software package (version 13.0) was used for the statistical tests. Continuous data

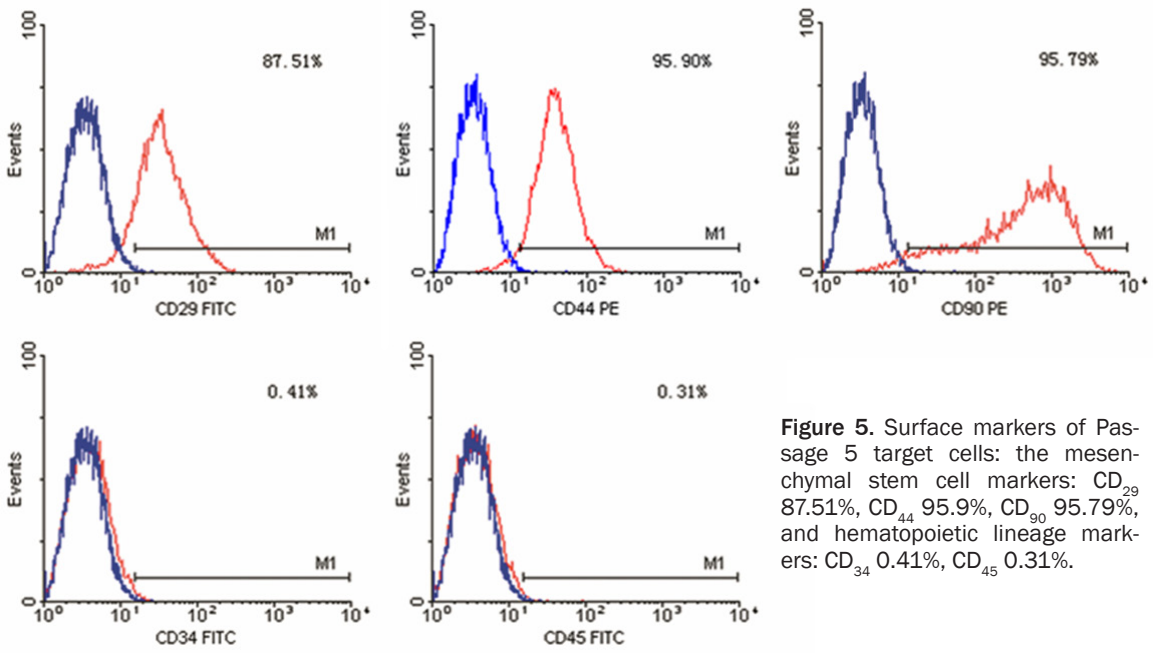


**Figure 5.** Surface markers of Passage 5 target cells: the mesenchymal stem cell markers: $CD_{29}$ 87.51%, $CD_{44}$ 95.9%, $CD_{90}$ 95.79%, and hematopoietic lineage markers: $CD_{34}$ 0.41%, $CD_{45}$ 0.31%.

were expressed as mean ± SD and categorical data as the median with interquartile ranges (IQRs). The One-Way ANOVA test and least significant difference t test (LSD-t) were used for multiple comparisons if data met corresponding conditions, otherwise Kruskal-Wallis test was done and Bonferroni method was applied for multiple comparisons. A *P* value of <0.05 was considered to be significant.

## Results

*Preparation of sepsis-induced acute lung injury model*

At 12 h after surgery, the rats revealed congestion and edema of both parietal peritoneum and intestinal walls, dilatation of bowel loops, slight adhesion, a small amount of exudates and fiber formation (**Figure 2A**). At 24 h, the rats demonstrated extensive dilatation of intestines, obvious adhesion formation, moderate amount of bloody as cites and denser fiber formation (**Figure 2C**). One model rat died between 36 h and 48 h after surgery, thus survival rate in a week was 80% (4/5).

There was a significant increase in hyaline membrane formation in the model group at 24 h compared to normal controls (*P*=0.005) and a more serious atelectasis at 48 h (*P*=0.014). All of the four model groups presented a significant increase in alveolar congestion and hemorrhage compared to the normal, (*P*<0.05) (**Figure 3**).

*Identification of target cells*

The target cells were uneven in shape (**Figure 4**). To further characterize these cells, surface markers of Passage 5 cells were examined by flow cytometry, showing the mesenchymal stem cell markers: $CD_{29}$ 87.51%, $CD_{44}$ 95.9%, $CD_{90}$ 95.79%, and the hematopoietic lineage markers: $CD_{34}$ 0.41%, $CD_{45}$ 0.31% (**Figure 5**).

*The effect of MSCs transplantation on sepsis-induced acute lung injury*

One rat in the sepsis group died between 36 h and 48 h after surgery, thus survival rate in a week was 80% (4/5). All animals in other three groups survived the observation period of 7 days.

In the sepsis group, obvious alveolar congestion and hemorrhage was detected starting from 12 h compared to normal control, (*P*=0.03, 0.048, 0.001, 0.001, respectively), significant hyaline membrane formation observed in the 24 h subgroup and more atelectasis demonstrated in the 48 h subgroup compared to normal control, (*P*=0.005, 0.014, respectively). All of the five lung injury parameters did not differ

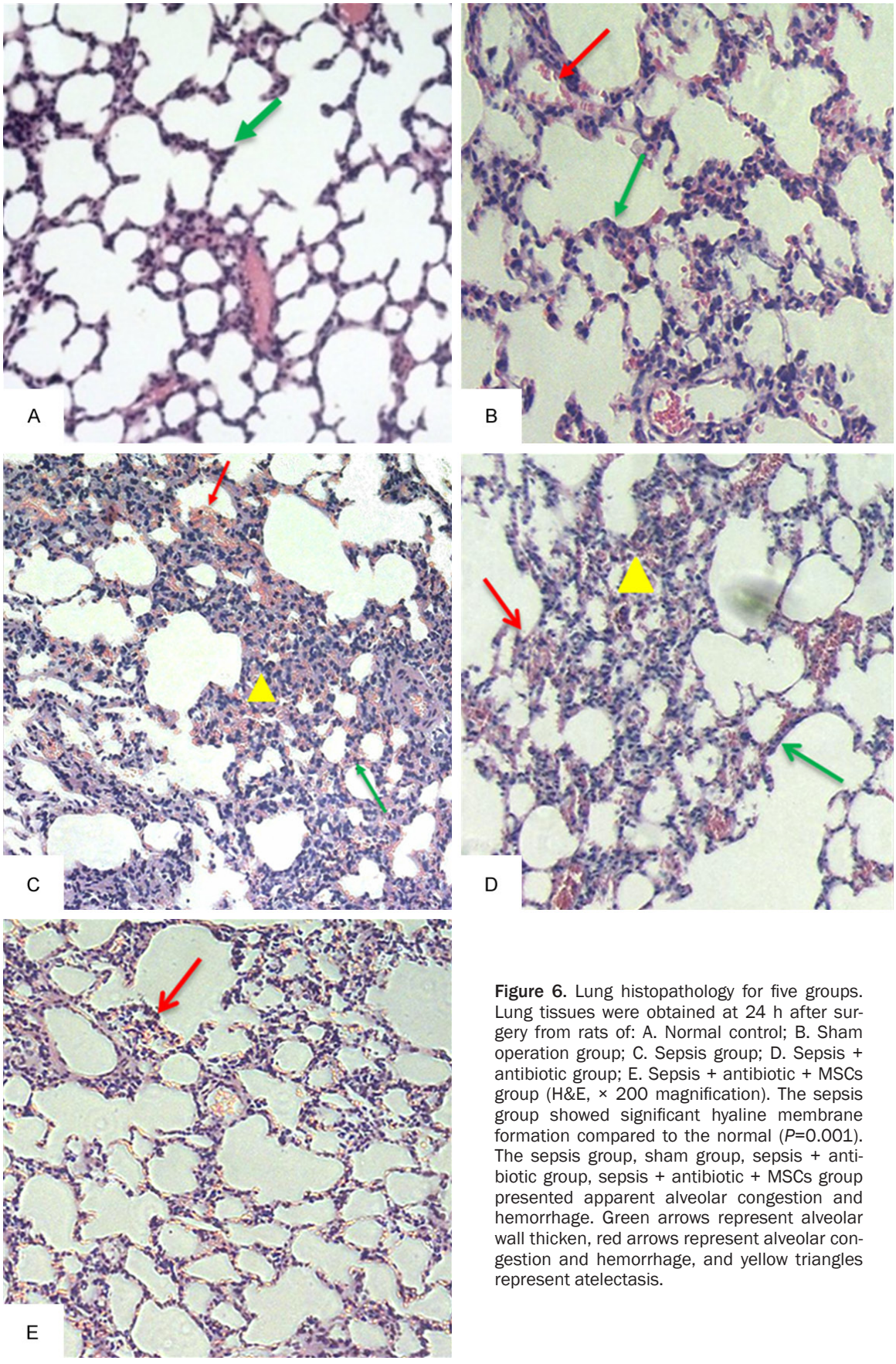


**Figure 6.** Lung histopathology for five groups. Lung tissues were obtained at 24 h after surgery from rats of: A. Normal control; B. Sham operation group; C. Sepsis group; D. Sepsis + antibiotic group; E. Sepsis + antibiotic + MSCs group (H&E, × 200 magnification). The sepsis group showed significant hyaline membrane formation compared to the normal ($P$=0.001). The sepsis group, sham group, sepsis + antibiotic group, sepsis + antibiotic + MSCs group presented apparent alveolar congestion and hemorrhage. Green arrows represent alveolar wall thicken, red arrows represent alveolar congestion and hemorrhage, and yellow triangles represent atelectasis.

**Table 1.** Lung histopathology of different subgroups

| Groups | | | Thickness of alveolar wall | Aggregation of inflammatory cells | Hyaline membrane | Atelectasis | Congestion and hemorrhage |
|---|---|---|---|---|---|---|---|
| Sepsis | 12 h | n=3 | 0.7 (0.5, 0.7) | 1.7 (1.7, 1.7) | 0.1 (0.1, 0.1) | 1.2 (0.9, 1.2) | 1.6 (0.9, 1.6)* |
| | 18 h | n=3 | 1.2 (1, 1.2) | 1.5 (1.4, 1.5) | 0.1 (0.1, 0.1) | 0.3 (0.3, 0.3) | 1.3 (1.2, 1.3)* |
| | 24 h | n=9 | 0.9 (0.35, 1.4) | 1.7 (1.3, 2) | 0.6 (0.35, 0.65)* | 0.9 (0.6, 1.1) | 1.4 (1.3, 1.75)* |
| | 48 h | n=5 | 1 (0.25, 1.25) | 1.8 (1.45, 2.05) | 0.1 (0, 0.6) | 1.4 (1.05, 2.25)* | 1.5 (1.15, 2.3)* |
| Sham | 12 h | n=8 | 0.9 (0.33, 1.6) | 1.5 (1.3, 1.73) | 0.05 (0, 0.18) | 1.25 (0.85, 2.05)* | 0.55 (0.15, 1.20) |
| | 18 h | n=4 | 0.35 (0.23, 0.93) | 1.2 (1.05, 1.5) | 0.05 (0, 0.25) | 0.95 (0.75, 1.68) | 0.75 (0.33, 2) |
| | 24 h | n=4 | 0.45 (0.18,0.73) | 1.18 (1.03, 1.36) | 0.2 (0.03, 0.45) | 1.35 (1, 1.6) | 1.65 (1.6, 1.80)* |
| | 48 h | n=2 | 0.65 (0.6, 0.65) | 1.3 (1.3, 1.3) | 0.1 (0, 0.1) | 1.3 (1.3, 1.3) | 1.85 (1.8, 1.85)* |
| Sepsis + | 12 h | n=4 | 0.4 (0.08, 0.8) | 1.2 (1.05, 1.65) | 0.1 (0, 0.35) | 1.2 (0.65, 1.3) | 1.2 (1.03, 1.75)* |
| Antibiotic | 18 h | n=8 | 0.7 (0.4, 0.8) | 1.5 (1.33, 1.8) | 0.35 (0.13, 0.55)* | 1.05 (0.78, 1.18) | 1.25 (1.03, 1.8)* |
| | 24 h | n=4 | 0.15 (0.1, 1.25) | 1.4 (1.15, 2.03) | 0.4 (0.175, 0.48) | 0.7 (0.7, 1.6) | 1.5 (1.3, 2.15)* |
| | 48 h | n=3 | 0.5 (0, 0.5) | 1.5 (1.1, 1.5) | 0 (0, 0) | 0.6 (0.5, 0.6) | 1.1 (1.1, 1.1) |
| Sepsis + | 12 h | n=3 | 0.9 (0.9, 0.9) | 1.4 (1.4, 1.4) | 0 (0, 0) | 1.4 (1.3, 1.4) | 1.1 (0.8, 1.1) |
| Antibiotic | 18 h | n=3 | 1.2 (0.6, 1.2) | 1.5 (1.4, 1.5) | 0 (0, 0) | 1.1 (0.8, 1.1) | 1.4 (1.2, 1.4)* |
| + MSCs | 24 h | n=4 | 1.5 (1.2, 1.58) | 1.85 (1.63, 2) | 0.15 (0.03, 0.28) | 1.65 (1.12, 1.88)* | 1.9 (1.15, 2.28)* |
| | 48 h | n=3 | 0.6 (0.1, 0.6) | 1.4 (0.9, 1.4) | 0 (0, 0) | 1 (0.5, 1) | 1.4 (1, 1.4) |
| Normal | | n=5 | 0.8 (0.45, 1.15) | 1.6 (1.1, 1.7) | 0 (0, 0) | 0.5 (0.3, 0.65) | 0 (0, 0.6) |

The data are expressed as the median ($P_{25}$, $P_{75}$); *Significant difference compared with normal controls (P<0.05), #Significant difference between groups (P<0.05).

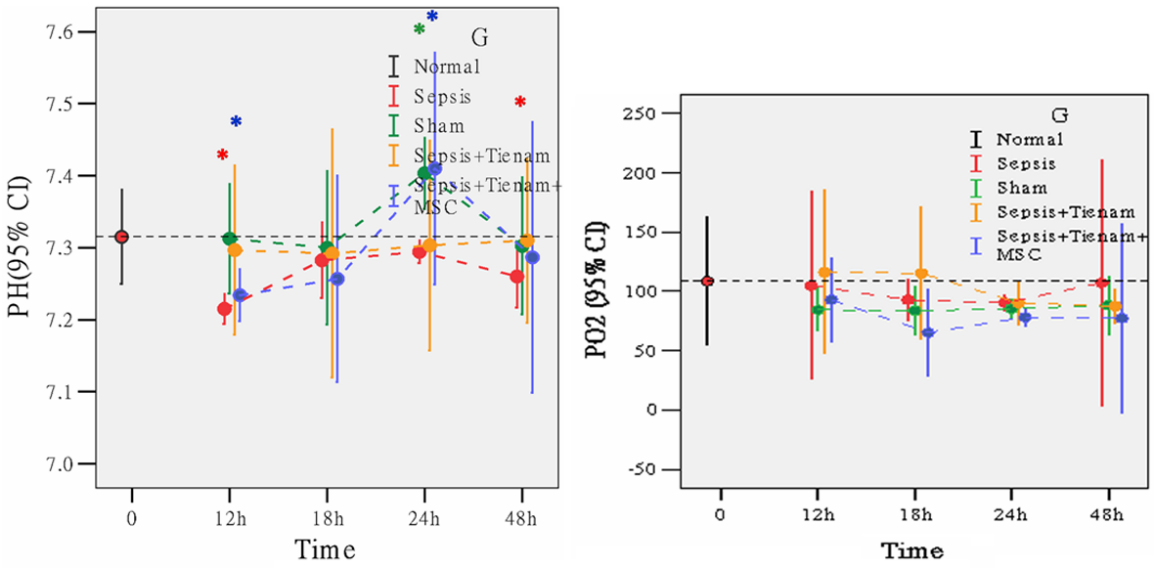


**Figure 7.** Inferential error bars for PH and $PaO_2$. Error bars indicate 95% confidence interval. Asterisks indicate statistical significance.

within 48 h in sham group. In the sepsis + antibiotic group, obvious alveolar congestion and hemorrhage was detected in 12 h, 18 h, 24 h subgroups, significant hyaline membrane formation in 18 h subgroup compared to normal control, ($P$=0.01, 0.001, 0.001 and $P$=0.041). In the sepsis + antibiotic + MSCs group, apparent alveolar congestion and hemorrhage was observed at 18 h compared to normal control ($P$=0.03) (**Figure 6** and **Table 1**).

At 12 h after surgery, PH in normal control was 7.315±0.062, as compared with 7.215±0.013 in the sepsis subgroup ($P$=0.003) and 7.234±0.029 in the sepsis + antibiotic + MSCs subgroup ($P$=0.009). At 18 h, $SaO_2$ in the sepsis +

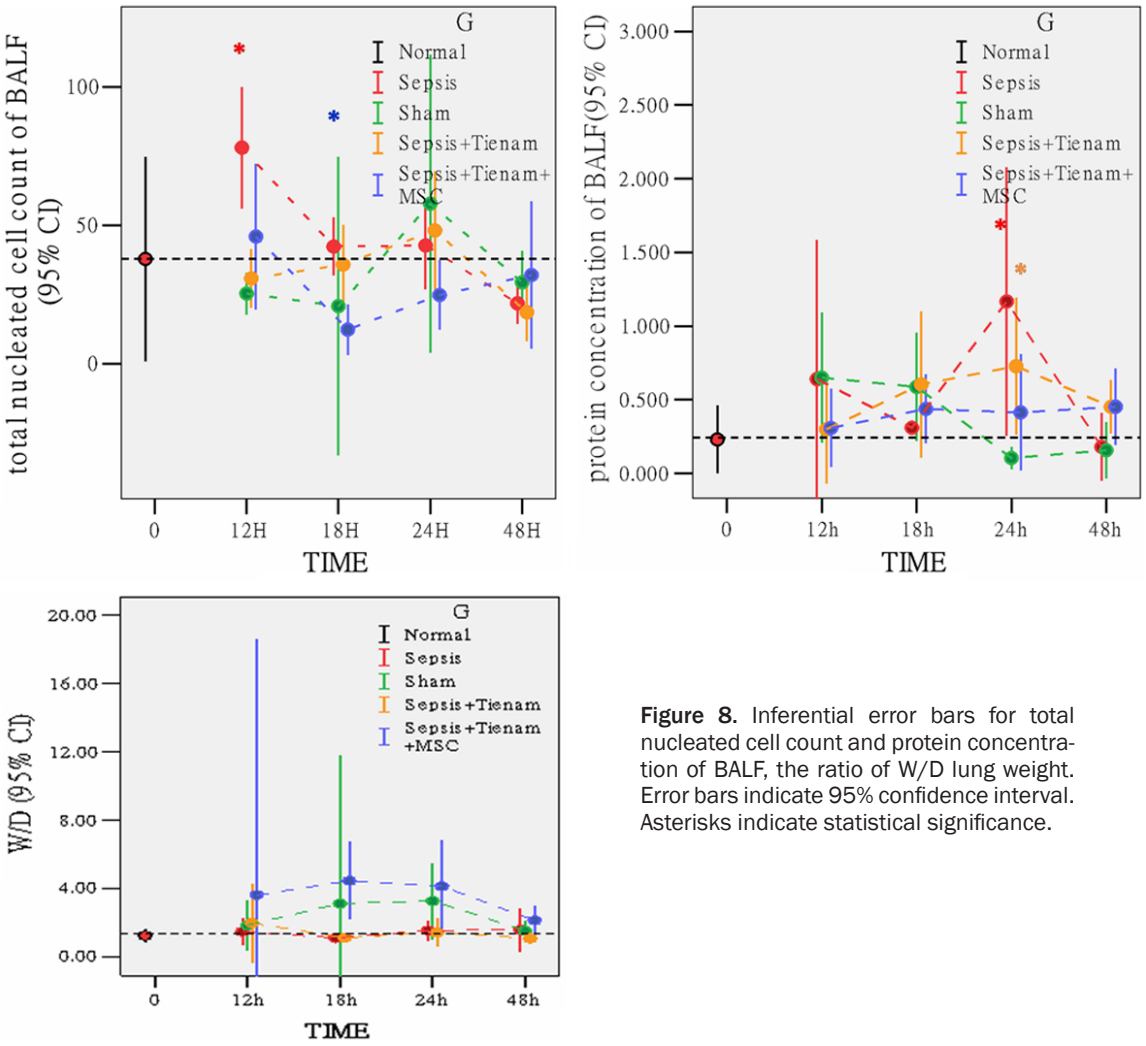


**Figure 8.** Inferential error bars for total nucleated cell count and protein concentration of BALF, the ratio of W/D lung weight. Error bars indicate 95% confidence interval. Asterisks indicate statistical significance.

antibiotic + MSCs subgroup (81.1±18.63%) was lower than the sepsis subgroup (95.75±1.26%, *P*=0.022), sepsis + antibiotic subgroup (94.4±7.73%, *P*=0.028), sham subgroup (94.5±2.52%, *P*=0.034) and normal control (96.33±2.34%, *P*=0.012) (**Figure 7**).

At 12 h after surgery, total nucleated cell count of BALF in the sepsis subgroup (78.05±8.68) was significantly higher than normal control (37.80±22.96, *P*=0.001), sham subgroup (25.3±8.61, *P*<0.001), sepsis + antibiotic subgroup (30.78±9.80, *P*<0.001) and sepsis + antibiotic + MSCs subgroup (45.88±16.28, *P*=0.005). At 18 h, cell count was 12.29±7.03 in sepsis + antibiotic + MSCs subgroup, as compared with 42.3±8.18 in sepsis subgroup (*P*=0.007), 35.77±16.80 in sepsis + antibiotic subgroup (*P*=0.016) and 37.80±22.96 in normal controls (*P*=0.025) (**Figure 8**).

Protein concentration of BALF did not differ among the four subgroups (0.64±0.38 mg/ml in sepsis subgroup, 0.65±0.52 mg/ml in sham subgroup, 0.30±0.34 mg/ml in sepsis + antibiotic subgroup, 0.31±0.16 mg/ml in sepsis + antibiotic + MSCs subgroup) and normal control (0.23±0.09 mg/ml) at 12 h. At 24 h, protein concentration in the sepsis subgroup (1.17±0.57 mg/ml) and the sepsis + antibiotic subgroup (0.73±0.37 mg/ml) were higher than normal control (*P*=0.001, 0.047, respectively), the sepsis subgroup higher than sepsis + antibiotic + MSCs subgroup (0.41±0.24 mg/ml, *P*=0.004). At 48 h, the protein concentration of the sepsis + antibiotic subgroup (0.45±0.11 mg/ml) and sepsis + antibiotic + MSCs subgroup (0.45±0.20 mg/ml) were higher than that of the sepsis subgroup (0.18±0.18 mg/ml, *P*=0.025, 0.019, respectively) and the sham

subgroup (0.16±0.18 mg/ml, *P*=0.013, 0.009, respectively) (**Figure 8**).

No significant difference in the ratio of W/D lung weight was detected among subgroups (1.47±0.47 in sepsis subgroup, 1.84±1.37 in sham subgroup, 1.96±1.86 in sepsis + antibiotic subgroup and 3.61±1.67 in sepsis + antibiotic + MSCs subgroup) and normal control (1.21±0.39) 12 hours after surgery. At 18 h and 24 h, the W/D ratios were 4.45±1.41 and 4.12±2.16 in the sepsis + antibiotic + MSCs subgroups respectively, as compared with 1.09±0.03 and 1.53±0.66 in the sepsis subgroups (*P*=0.002 and 0.005, respectively), 1.10±0.03 and 1.41±0.62 in the sepsis + antibiotic subgroups (*P*=0.001 and 0.008, respectively) (**Figure 8**).

## Discussion

Acute Respiratory Distress Syndrome (ARDS) is well defined in humans. However, no agreed-upon diagnostic criteria of different lung injury entities (ALI vs. ARDS) in animal models of lung injury have been made [15]. It is obviously not practical to use the Berlin definition in animal studies. For this consideration, ALI was adopted in the whole study to keep consistent with previous research.

In this study, we induced polymicrobial acute diffuse peritonitis by establishing a surgical connection between the cecum and the peritoneal cavity (by placing a tube with specific diameter across the cecum), allowing the leakage of faeces into the peritoneal cavity. We made several modifications to the previously described Colon Ascendens Stent Peritonitis (CASP) model in which the tube is placed in the ascending colon, the diameter of which is much narrower than cecum, leading to obstruction rather than diffuse peritonitis. The Cecum Stent Peritonitis model applied in our study constructed acute diffuse peritonitis stably and induced lung injury in rats successfully. In addition, operation, foreign body stimulation and other stimulations may also play important roles in the pathophysiological process of injury. The facts that sham group in our experiment presented signs of peritonitis including peritoneal hyperemia and edema, fibrous exudation, intestinal dilatation and as cites formation, and demonstrated obvious alveolar congestion and hemorrhage at 24 h after surgery are in support of this standpoint. A potential explanation is that stimulations of operation, trauma, circulatory disorder, hypoxia and foreign body have the potential to induce gastrointestinal disorders, mucosal barrier damage, followed by intestinal bacteria and endotoxin translocation, SIRS and organ damage [16].

Sepsis-induced ARDS is a major cause of morbidity and mortality in critically ill patients in spite of appropriate antimicrobial therapy [17] and significant progress in intensive care therapy. Innovative therapeutic strategies for sepsis-induced ARDS are needed to improve clinical outcome. MSCs have received increasing interest for ARDS treatment [11]. To adequately mimic the actual clinical practice, as recommended by Deitch [18], new therapeutic approaches must be administered in combination with standard clinical therapies, antibiotic as a critical component of sepsis therapy is the basic therapy in our experiment. Our findings indicate that MSCs administrated in early stage of sepsis improve alveolar inflammatory cells infiltration and protein exudation, as well as alveolar congestion and hemorrhage, which are in line with previous research [19].

The mechanisms of different risk factors inducing ARDS remain largely unknown. It's well accepted that ARDS is a type of acute diffuse inflammatory lung injury associated with different predisposing risk factors. Pulmonary and extra-pulmonary insults lead to alveolar epithelial and vascular endothelial injury, resulting in increased permeability of the endothelial and epithelial barriers, and then accumulation of protein rich and highly cellular edema fluid in interstitium and alveoli, coupled with coagulation cascade activation and inflammatory mediators' release. Profound imbalance in systemic and local coagulation and fibrinolysis systems, combined with inflammatory and anti-inflammatory reactions result in lung injury [2, 3, 7-10]. Given this, it is perhaps not surprising that strategies targeted at one aspect of the disease process have been unsuccessful [12]. Most current researches demonstrate that beneficial effects of MSCs are based on a variety of mechanisms [11, 20], among which paracrine soluble factors appear to play a major role [11]. One potential mechanism is that circulating or systemically administered MSCs are recruited to lung by passive retention and che-

mokine induced migration, then interact with injured host cells directly and secrete multiple paracrine factors including anti-inflammatory cytokines, growth factors and antimicrobial peptides, which potentially improve major abnormalities on ARDS [11, 20]. Further researches suggest that MSCs may enhance repair by mitochondrial transfer of material from one cell to another (transport of intracellular substances including protein, nucleic acid, cytoplasmic organoids to damaged tissue cells) [21-24].

MSCs have been widely accepted as a potential therapy for numerous diseases [25]. Meanwhile, security issues attract considerable attention, including transfusion safety, delayed toxicity and oncogenicity etc. At present, most of the experiments and clinical studies regarding use of autologous or allogeneic MSCs present no obvious delayed toxicity. The most controversial safety concern with MSCs is tumorigenicity. Novelty of this research field, coupled with scarcity of clinical long-term follow-up, lead to uncertainty on this issue. Obtained data neither confirm nor exclude the risk for tumorigenicity in patients [26]. In aspect of infusion security, researches focus on hemodynamic changes during and within a short time after MSCs transplantation. In spite of size variation associated with sources, culture conditions, number of generation and other factors, the diameters of MSCs have been shown generally larger than capillaries and precapillary vessels of receptors. Therefore, some systemically delivered MSCs may be entrapped in lung [27]. In addition, as Kohei Tatsumi [28] reported, tissue factor as a triggering factor in procoagulative cascade is highly expressed at the level of mRNA and localized to cell surface of cultured mouse or human adipose-derived MSCs. The possibility exists that when dose, frequency and other infusion conditions meet particular condition, entrapment of MSCs in lung microvascular and subsequent microthrombosis formation may result in significant hemodynamic changes. Rats administrated with MSCs in our study presented a decrease in oxygen saturation and PH, accompanied with an increase of lung water within 24 h after MSCs transplantation. One possible explanation is that a large number of infused MSCs detained in pulmonary microcirculation and related microthrombosis formation result in respiratory and circulatory changes. S. Schrepfer etc [29] also observed episodes of tachypnea, apnea, and hemodynamic alterations characteristic of pulmonary embolism after MSCs transplantation in their study. Additional studies are needed to confirm the safety of MSCs for patients with ARDS and determine an optimal infusion strategy.

We recognize several limitations of this study. First, the failure to surgically treat the focus of infection results in this model clinically resembling incompletely treated peritonitis. Moreover, efforts to provide organ support and recreate the clinical environment of patients with sepsis are limited, creating a gap between this model of bacterial peritonitis and septic humans. Second, we cannot determine with certainty whether a particular sepsis model has been successfully modeled without sacrificing it, which weakens the efficiency of survival analysis. Third, differentiation assays and other biological properties identification of cultured cells will be done to further confirm viability and functioning of these cells. Additionally, some therapeutic effects of MSCs reversed with time. The fact that we prescribed just one dose of MSCs in the early stage of disease prevents us from being able to identify the exact cause of this reverse effect. One possibility is that therapeutic effects of MSCs attenuate 24 hours after transplantation.

In summary, our findings indicate that intravenous injection of ex vivo cultured allogeneic MSCs is safe for rats with sepsis induced acute lung injury. MSCs administrated in early stage of sepsis improve alveolar inflammatory cells infiltration and protein exudation, as well as alveolar congestion and hemorrhage. However, some therapeutic effects reverse with time. In addition, there is a potential risk of oxygenation impairment and lung water increase with intravenous injection of MSCs. Further studies are needed to confirm the therapeutic potential and safety of MSCs in sepsis-induced ARDS.

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## Disclosure of conflict of interest

None.

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