RETRACTED ARTICLE: Endoplasmic reticulum stress potentiates the autophagy of alveolar macrophage to attenuate acute lung injury and airway inflammation

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\textbf{ABSTRACT}

Endoplasmic reticulum (ER) stress has been reported to play a role in acute lung injury (ALI), yet the in-depth mechanism remains elusive. This study aims to investigate the effect of ER stress-induced autophagy of alveolar macrophage (AM) on acute lung injury (ALI) and airway inflammation using mouse models. ALI models were induced by intranasal instillation of lipopolysaccharide (LPS). The lung weight/body weight (LW/BW) ratio and excised lung gas volume (ELGV) in each group were measured. Mouse bronchoalveolar lavage fluid (BALF) was collected for cell sorting and protein concentration determination. Expression of tumor necrosis factor-\textalpha{} (TNF-\textalpha{}) and interleukin-6 (IL-6) in lung tissues and BALF was also detected. Mouse AMs were isolated to observe the autophagy. Expression of GRP78, PERK, LC3I, LC3II and Beclin1 was further determined. The results indicated that tunicamycin (TM) elevated GRP78 and PERK expression of AMs in ALI mice in a dose-dependent manner. Low dosage of TM abated LC3I expression, increased LC3II and Beclin1 expression, triggered ER stress and AM autophagy, and alleviated pathological changes of AMs in ALI mice. Also, in ALI mice, low dosage of TM attenuated goblet cell proliferation in tracheal wall, and declined LW/BW ratio, ELGV, total cells and neutrophils, protein concentrations in BALF, and IL-6 and TNF-\textalpha{} expression in lung tissues and BALF. Collectively, this study suggests that a low dosage of TM-induced ER stress can enhance the autophagy of AM in ALI mice models, thus attenuating the progression of ALI and airway inflammation.

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\textbf{Introduction}

Acute lung injury (ALI) is a major disease responsible for high morbidity and mortality, as well as for heavy healthcare burden in critically ill patients [1,2]. The progression of ALI may eventually lead to its worst form, acute respiratory distress syndrome (ARDS), which was usually investigated together with ALI [3,4]. ALI/ARDS is characterized by pulmonary infiltrates, hypoxemia and lung edema due to increased permeability of the alveolar-capillary barrier, which will eventually result in subsequent impairment of arterial oxygenation [3]. The well-established risk factors for ALI include sepsis, trauma or multiple traumatic injuries, and other factors such as genetic, age, living habit (smoking, alcohol abuse) may also lead to ALI [5]. In addition, several noninfectious causes also may trigger ALI, for instance, lipopolysaccharide (LPS), a glycolipid of the outermost membrane of gram-negative bacteria, is a common approach to induce ALI in animal studies [6]. Interestingly, the most common way to eradicate bacteria in the airway is an inflammatory response, whereas the excessive air inflammation may damage lung tissues or lead to ARDS [7]. Evidence indicated that macrophages also contribute to the regulation of inflammatory responses and lung injuries [8,9]. Macrophages are found to be a master of fibrosis and regulate fibrogenesis by secreting chemokines that recruit fibroblasts and other inflammatory cells, which stimulate fibroblast activity and extracellular matrix (ECM) deposition [10]. Alveolar macrophages (AMs), serving as sentinels of a healthy state, are different from other macrophages due to their unique tissue location and function in the lung, and can adapt to accommodate the ever-changing needs of the tissue [11]. Macrophages have been classified into two classic activated (M1) and alternative activated (M2)

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Macrophages, in which the former can be induced by LPS and is characterized by secretion of pro-inflammatory factors, including interleukin (IL)-1β, IL-12, tumor necrosis factor (TNF)-α [12,13]. The Unfolded Protein Response (UPR) is an adaptive survival pathway which will be activated by accumulation of misfolded proteins in response to a variety of cell insults that result in endoplasmic reticulum (ER) stress and previous research has indicated that ER stress has been causally associated with macrophage apoptosis in advanced atherosclerosis [14]. Although the previous study suggested that alterations in the balance and function of macrophages could lead to ALI [15,16], whether the ER stress-induced autophagy of AM is casually associated with ALI and airway inflammation remains to be elucidated. Accordingly, this study was conducted with the aim to explore the exact role of ER stress-induced autophagy of AMs in ALI and airway inflammation, with the expectation to provide the least potential theoretical basis for ALI treatment.

**Methods and materials**

**Ethics statement**

Animal experiments were conducted in strict accordance with the approved animal protocols and guidelines established by the Medicine Ethics Review Committee for animal experiments of North China University of Science and Technology.

**Animals for experiments**

Seventy-two BALB/c female mice (6–8 wk, 18–20 g) were purchased from the animal center of Perking University. Before experiments, all mice were fed in the animal house under 19–22°C for 3 d to adapt to the environment with free access to pellet feed. The animal house shall have a light exposure time of 12 h a day and shall be subjected to regular disinfection and ventilation.

**Animal grouping**

LPS (Sigma-Aldrich, St. Louis, MO, USA) was used to induce ALI model [17] and 72 mice were grouped into six groups with 12 mice in each group: (1) Blank group (no treatment); (2) Phosphate buffered saline (PBS) group (mice were administered with PBS through nose); (3) LPS group (mice were administered with LPS through nose); (4) LPS + normal saline (NS) group (mice were administered with LPS through nose and injected intraperitoneally with normal saline); (5) LPS + 0.3 Tunicamycin (TM) group (mice were administered with LPS through nose and injected intraperitoneally with 0.3 mg/kg TM); (6) LPS + 3.0 TM group (mice were administered with LPS through nose and injected intraperitoneally with 3.0 mg/kg TM). Mice in the LPS + NS group, LPS + 0.3 TM group and LPS + 3.0 TM group were daily intraperitoneally injected with NS or TM (Sigma-Aldrich, St. Louis, MO, USA) 3 d before model establishment. One hour after the final time of injection, mice were anesthetized using pentobarbital sodium (Sigma-Aldrich, St. Louis, MO, USA). Except for mice in the blank group and the PBS group, mice in the PBS group were administered with 0.5 mg/kg LPS, while mice in the PBS group were administered with 0.5 mg/kg PBS. About 12 h later, mice were euthanized and the sample was gathered.

**Lung weight (LW)/body weight (BW) ratio and excised lung gas volume (ELGV)**

After mice were euthanized and before the chest was opened, the trachea was separated from the neck and ligated with 3–0 surgical suture on the 2–3 cartilaginous rings of the cricoid cartilage. The chest was open up from the processus xiphoideus to take out the whole trachea, lung and pericardium. The fibrous connective tissues around the pericardium and the lung were removed. A density instrument (Mettler Toledo, Zurich, Switzerland) was applied for measuring the LW/BW ratio and the ELGV.

**Pathological observation of lung tissues**

Once ELGV and LW/BW ratio were measured, a catheter was inserted to the trachea and 4% paraformaldehyde was injected through the catheter at a height of 20 cm. Then, the trachea was ligated and the whole lung was fixed in 4% paraformaldehyde for 24 h. After that, the lung tissues were subjected to routine dehydration, paraffin embedding and slicing (5 μm). The infiltration, edema and damage to inflammatory cells in lung tissues and the secretion...
of airway mucus were observed under a light microscope (Olympus, Tokyo, Japan) by performing hematoxylin-eosin (HE) staining and Alcian blue-periodic acid Schiff (AB-PAS) staining.

**Bronchoalveolar lavage fluid (BALF)**

The pulmonary alveoli were lavaged with PBS twice (0.5 mL each time) and the BALF (0.8 mL) was collected for centrifugation at 1200 rpm for 10 min under 4°C. The cell precipitates were re-suspended using 0.2 mL PBS, after which 20 µL solution was adopted for cell counting. The rest solution was centrifuged at 1200 rpm for 15 min. Certain cells in BALF were evenly distributed on the slides, which was then air-dried and counted under a high-power microscope for 200 cells after Wright’s staining. The supernatant of BALF was stored at −80°C for determination of the expressions of relative proteins and cytokines. Bicinchoninic acid (BCA) protein quantitative kit (Pierce, Rockford, IL, USA) was used to measure protein expressions.

**Isolation and purification of AMs**

The BALF was made into cell suspension under the sterile operation of filtration, centrifugation and rinse. The suspension was diluted to count cells and to calculate cell mass concentration. Appropriate cell suspension (about 5 × 10⁶) was added into 2 mL of DMEM (GibcoBRL, Grand Island, NY, USA) culture flask containing 10% fetal bovine serum (FBS, Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 2-h incubation at a 5% CO₂ incubator with 84% humidity. The cells adhered to walls were purified AMs, and Trypan blue staining suggested that cell viability could reach 90%.

**Autophagosome by monodansylcadaverine (MDC) staining**

AMs from each group were inoculated in a six-well plate, subject to PBS washing for three times and fixation of paraformaldehyde for 15 min at 4°C. MDC (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was diluted with PBS at a ratio of 1:1000 until a final volume of 50 µM solution was obtained. The paraformaldehyde was removed and AMs were washed with PBS for another three times and added with the prepared solution at 37°C for a reaction for 60–90 min without light exposure. The reaction time may be adjusted based on the degree of staining. Prior to observation under a confocal microscope (HITACHI, Tokyo, Japan), AMs were washed with PBS for three times to get rid of the staining solution. The staining of AMs was photographed and recorded. Autophagosomes were presented in forms of green spots or granules.

**Reverse transcription quantitative polymerase chain reaction (RT-qPCR)**

Trizol method (Invitrogen Technology, Carlsbad, CA, USA) was applied to extract total RNA from AMs in each group. The high-quality RNA was then identified by ultraviolet spectrophotometer and electrophoresis on a gel containing formaldehyde. RNA (1 µg) was reversed into cDNA using AMV reverse transcriptase (Thermo Fisher Scientific, Massachusetts, USA). PCR primers were designed and synthesized by Invitrogen (Carlsbad, CA, USA) (Table 1). Glyceraldehyde phosphate dehydrogenase (GAPDH) was considered as a loading control. PCR products were electrophoresed with sepharose gel and then analyzed using OpticonMonitor3 software (BioRad Labs Inc., Hercules, CA, USA). The threshold cycle (Ct) was analyzed using 2⁻ΔΔCt method. The 2⁻ΔΔCt was used to test the relative transcriptional levels of IL-6 and TNF-α mRNA:

\[ \Delta \Delta C_t = \Delta C_t \text{ experimental group} - \Delta C_t \text{ control group}, \quad \Delta C_t = C_t \text{ target gene} - C_t \text{ internal reference} [18]. \]

Experiments were conducted for three times to obtain the average value.

**Western blot analysis**

Proteins from AMs in each group were extracted and BCA kit (Pierce, Rockford, IL, USA) was used to identify the protein concentration. After

| Table 1. Sequences of primers. |
|--------------------------------|
| **Gene** | **Sequences** |
| IL-6 | F: 5’- TGGAGTCACAGAAGGAGTGGCTAAG -3’<br>R: 5’- TCTGACCACTGAGGAAATGTCCAC -3’ |
| TNF-α | F: 5’- CTGGGACAGTGACCTGGACT -3’<br>R: 5’- GCACCTCAGGGAAGAGTCTG -3’ |
| GAPDH | F: 5’- CAAGTTCAACGGGCAATGG -3’<br>R: 5’- CCCCATTGATGTATAGGGG-3’ |

F, forward; R, reverse.
adding with lading buffer, the proteins were boiled at 95°C for 10 min. Then, 30 µg of solution was added in each well in the plates, and the proteins were separated by 10% polyacrylamide gel (Beijing Liuyi Biotechnology Co., Ltd., Beijing, China) and transferred to polyvinylidene difluoride (PVDF) membrane followed by blocking with 5% bovine serum albumin at ambient temperature for 1 h. Primary antibodies GRP78 (1:1000, Abcam, Cambridge, MA, USA), PERK, LC3I, LC3II, Beclin1, Bcl-2 and caspase-3 (1:1000, Cell Signaling Technology, Inc., Beverly, MA, USA) and primary antibody β-actin (loading control, 1:3000, BD Pharmingen, San Jose, CA, USA) were added for overnight at 4°C. The membrane was then washed three times with PBS for 5 min and incubated with secondary antibodies at ambient temperature. Chemiluminescence reagents were used for color development. Gel Doc EZ imager (Bio-rad, California, USA) was used for color observation. Image J software (National Institutes of Health, Maryland, USA) was used to analyze the gray value of target band. Experiments were conducted for three times to obtain the average value.

**Enzyme-linked immunosorbent assay (ELISA)**

IL-6 and TNF-α kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) were used to detect the contents of IL-6 and TNF-α according to the instruction of the manufacturer. The expressions of IL-6 and TNF-α were calculated based on optical density (OD) value and standard curve.

**Statistical analysis**

SPSS version 21.0 (IBM Corp. Armonk, NY, USA) was applied for data analysis. Measurement data were displayed as mean ± standard deviation. Data which comply with normal distribution between groups were compared with the t-test, comparison among multiple groups were compared using one-way analysis of variance (ANOVA) and pairwise comparison was conducted using the least significance difference (LSD) method. \( P < 0.05 \) was considered as statistically significant.

**Results**

**TM elevates GRP78 and PERK expression of AMs in ALI mice in a dose-dependent manner**

Western blot analysis was used to detect the expressions of ER stress-related protein GRP78 and PERK of mouse AMs in each group. As shown in Figure 1, in comparison to the blank group and PBS group, the expression of GRP78 and PERK was notably increased in the LPS group and LPS + NS group (all \( P < 0.05 \)). Among the six groups, LPS + 3.0 TM group had the highest expression of GRP78 and PERK, followed by the LPS + 0.3 TM group (\( P < 0.05 \)) and then the LPS group and LPS + NS group (both \( P < 0.05 \)) accordingly.

**Figure 1. TM elevates GRP78 and PERK expression of AMs in ALI mice in a dose-dependent manner.** (a) Expression of ER stress-related proteins GRP78 and PERK in AMs of mice in each group. (b) Protein bands of ER stress-related proteins GRP78 and PERK in AMs of mice in each group. *, compared with the blank group, \( P < 0.05 \); #, compared with the LPS group, \( P < 0.05 \). Measurement data were displayed as mean ± standard deviation. Comparison among multiple groups was conducted by one-way ANOVA followed with LSD-t. \( N = 12 \).
Low dosage of TM abates LC3I expression and increases LC3II and Beclin1 expressions of AMs in ALI mice

The expression of autophagy marker proteins (LC3I, LC3II and Beclin1) of mouse AMs in each group was detected by Western blot analysis. Compared with the blank group and PBS group, the mice in the LPS group and LPS + NS group had decreased expression of LC3I, but increased expression of LC3II and Beclin1 (all \( P < 0.05 \)). Mice in the LPS + 0.3 TM group had lower expression of LC3I, but higher expressions of LC3II and Beclin1 than those in the LPS group and LPS + NS group (all \( P < 0.05 \)). A significant transformation of LC3I to LC3II was observed. No significant difference on the expression of LC3II and Beclin1 was detected among the LPS + 3.0 TM group, LPS group and LPS + NS group (all \( P > 0.05 \)) (Figure 2).

Low dosage of TM triggers ER stress and AM autophagy of AMs in ALI mice

Autophagosomes were presented in the LPS group, LPS + 0.3 TM group and LPS + 3.0 TM group, which indicated the occurrence of AM autophagy. Moreover, the autophagosome in the LPS + 0.3 TM group was much larger than those in the LPS + 3.0 TM group and LPS group (both \( P < 0.05 \)), suggesting that low dosage of TM can trigger ER stress and AM autophagy and high dosage of TM can activate ER stress but also inhibit AM autophagy (Figure 3).

Low dosage of TM alleviates pathological changes of AMs in ALI mice

HE staining was performed to observe the pathological changes of mouse AMs. No alveolar thickness and neutrophil infiltration were found in the blank group and PBS group. HE staining showed that mice in the LPS group and LPS + NS group had typical ALI manifestations, such as serious neutrophil infiltration, damaged alveolar structure and alveolar thickness. Mice in the LPS + 0.3 TM group had slightly attenuated ALI manifestations than those in the LPS group and LPS + NS group, while mice in the LPS + 3.0 TM group had similar ALI manifestations with those in the LPS group and LPS + NS group (Figure 4(a)).

Low dosage of TM attenuates goblet cell proliferation of tracheal wall in ALI mice

AB-PAS staining was performed to observe the airway mucus secretion of mice in each group. The results showed that the blank group and PBS group had little PAS-positive epithelial cells, while the LPS group and LPS + NS group had increased epithelial cells and goblet cell proliferation. The LPS + 0.3.0TM group had less PAS-positive epithelial cells than that in the LPS group, which indicates that a small dosage of TM can remarkably decrease goblet cell proliferation in mice with LPS-induced ALI. The positive expression of PAS-positive epithelial cells and goblet cell proliferation in the LPS + 3.0 TM group was consistent with those in the LPS group and LPS + NS group (Figure 4(b)).
Low dosage of TM declines LW/BW ratio in ALI mice

We determined LW/BW of mice in each group. No significant difference was detected between the PBS group and blank group regarding LW/BW ratio ($P > 0.05$). The mice in the LPS group and LPS + NS group had a higher LW/BW ratio than those in the blank group and PBS group (all $P < 0.05$). Mice in the LPS + 0.3 TM group had a lower LW/BW ratio than those in the LPS group and LPS + NS group (all $P < 0.05$).
3.0 TM group had a similar LW/BW ratio with the LPS group and LPS + NS group (all \( P > 0.05 \)) (Figure 5(a)).

**Low dosage of TM declines ELGV to activate ER stress in ALI mice**

We further determined ELGV of mice in each group. The ELGV was significantly increased in the LPS group and LPS + NS group in contrast to the PBS group and blank group (all \( P < 0.05 \)). ELGV was reduced in the LPS + 0.3 TM group versus the LPS group and LPS + NS group (\( P < 0.05 \)), which indicated that a small dosage of TM can activate ER stress, thus protecting ALI mice. No difference in ELGV expression was detected between the LPS + 3.0 TM group and the LPS group (\( P > 0.05 \)) (Figure 5(b)).

**Low dosage of TM diminishes total cells and neutrophils in BALF in ALI mice**

In contrast to the blank group and PBS group, the total cells and neutrophils in BALF were up-regulated in the LPS group and LPS + NS group (all \( P < 0.05 \)). When compared with the LPS group and LPS + NS group, the total cells and neutrophils in BALF were decreased in the LPS + 0.3 TM group (\( P < 0.05 \)). No significant difference in the total cells and neutrophils in BALF was found among the LPS + 3.0 TM group, LPS group and LPS + NS group (all \( P > 0.05 \)) (Figure 6(a)).

**Low dosage of TM diminishes protein concentrations in BALF in ALI mice**

To investigate the effect of ER stress on LPS-induced ALI, we examined the protein concentrations in BALF of mice in each group. Compared with the LPS group and LPS + NS group, the protein concentrations in BALF were decreased in the blank group and LPS + 0.3 TM group (all \( P < 0.05 \)). The comparisons on the protein concentrations in BALF among the LPS + 3.0 TM group, LPS group and LPS + NS group showed no statistical significance (all \( P > 0.05 \)), as well as between the PBS group and blank group (Figure 6(b)).

**Low dosage of TM down-regulates IL-6 and TNF-\( \alpha \) expression in lung tissues and BALF in ALI mice**

The mRNA expression and protein contents of inflammatory cytokines (IL-6 and TNF-\( \alpha \)) in lung tissues and BALF of mice in each group were detected by RT-qPCR and ELISA. The mRNA expression and protein contents of IL-6 and TNF-\( \alpha \) in the blank group were not different from those in the PBS group (all \( P > 0.05 \)). In contrast to the PBS group and blank group, mice in the LPS group and LPS + NS group had increased mRNA expression and protein contents

![Figure 5. Low dosage of TM declines LW/BW ratio and ELGV in ALI mice. (a) Determination of LW/BW ratio of mice in each group. (b) Changes in ELGV value in each group of mice. *, compared with the blank group, \( P < 0.05 \); #, compared with the LPS group, \( P < 0.05 \). Measurement data were displayed as mean ± standard deviation. Comparison among multiple groups was conducted by one-way ANOVA followed with LSD-t. N = 12.](image-url)
of IL-6 and TNF-α (all $P < 0.05$). Mice in the LPS + 0.3 TM group had reduced mRNA expression and protein contents of IL-6 and TNF-α relative to the LPS group and LPS + NS group (all $P < 0.05$). No significant difference in mRNA expression and protein contents of IL-6 and TNF-α was found among the LPS group, LPS + NS group and LPS + 3.0 TM group (all $P > 0.05$) (Figure 7).

**Discussion**

The low dosage of TM was used in our study to induce ER stress which was a trigger that leads to autophagy of AM; meanwhile, LPS was applied in a mouse model to induce ALI. Conclusively, our study supported that ER stress could enhance the autophagy of AM, thus attenuating ALI in mouse models.

The results in our experiments showed that the mice in the LPS + 3.0 TM group and LPS + 0.3TM group had high expression of GRP78 and PERK, both of which were indicators for ER stress. GRP78 is mainly responsible for controlling the binding and activation of PERK, IRE1α and ATF6α [19,20]. ER stress generally refers to dysfunction of the ER which may be caused by pathogenic stress signals, resulting in accumulating of misfolded and unfolded proteins [21,22]. To overcome the adverse effect of ER stress and adapt to the various cell microenvironment, cells may operate an adaptive response, namely unfolded protein...
response (UPR) [23]. However, if the misfolded and unfolded proteins remain to be accumulated, or the adaptive response fails, ER stress can be a disaster for cells and resulting in ER stress-induced apoptosis [19]. Moreover, the successful detection of GRP78 and PERK also indicated that ER stress was successfully triggered [24]. In addition, our study also found that the LPS + 0.3 TM group and LPS + 3.0 TM group had substantial differences compared with other groups regarding the expression of LC3I, LC3II and Beclin1, which suggested that cell autophagy occurred. Consistent with our study, a previous study suggested that TM, irrespective of the dosage, can induce ER stress, as evidenced by the increased phosphorylation or activation of eIF2α and PERK which enhance the cell autophagy, as supported by the increased expression of LC3-II, beclin-1 and Atg5 [25]. Interestingly, we also found that the low dosage of TM can trigger ER stress but also activate the autophagy of AMs, while the high dosage of TM can also trigger ER stress but somehow can inhibit the autophagy of AMs. Those results support that the ER stress induced autophagy in a TM dose-dependent manner. As the TM relies on protein synthesis on ER stress [23], it is possible that the dosage of TM may have a certain role in protein synthesis.

In addition, ALI and airway inflammation in the mouse model were successfully established using LPS, as evident by infiltration of neutrophil, alveolar structure damage and thickness of alveolar in LPS, as well as the measurement of levels of IL-6 and TNF-α. LPS-induced ALI is essentially an inflammation in the lung resulted from the initiation of macrophages and over-activation of neutrophils. Inflammation, a host response to protect the body against harmful stimuli, can be initiated by complex processes triggered by microbial pathogen LPS, which can directly activate macrophages [26]. In our study, mice treated with LPS were presented with autophagosomes under the observation of a microscope. As the autophagosome is the hallmark of autophagy, our results supported that AM autophagy occurred in the ALI mouse models. AM is of significant importance in clearing bacteria from the alveolar surface and preventing microbe-induced infections [27]. Moreover, it is evidenced that lung cells may adapt pro-survival mechanisms in case of injury or inflammation, including autophagy [28,29]. Meanwhile, in order to regain the ER homeostasis in lung diseases, the UPR may operate adaptive mechanisms involving the stimulation of autophagy [30]. Altogether, our results may be explained in a way that the UPR in ALI or airway inflammation may restore the ER function by enhancing the AM autophagy, thus attenuates the disease progression. Although our study was conducted with the aim to shed light on providing the least theoretical basis for the treatment of ALI and airway inflammation, our study still has several limitations that are worth mention. Firstly, the sample size in this current study was not large enough to provide objective and solid experimental results. Then, since we mentioned that the dosage of TM may have certain effect on AM autophagy, it is required to explore its potential mechanism in a more detailed way and, therefore, caution should be exercised on handling the TM during experiments in future studies.

In summary, our study showed that TM-induced ER stress can potentiate the autophagy of AM, thus contributing to the attenuation of ALI and airway inflammation. Given what we know that the effect of TM on AM autophagy was in a dosage-dependent manner, the possible mechanism or exploration shall be one of the major challenges in this field.

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Authors’ contributions

Guarantor of integrity of the entire study: Qingzeng Qian
Study design: Xiangke Cao; Fumin Feng
Literature research: Bin Wang, Xiaoliu Dong
Experimental studies: Jian Pei; Ling Xue

Disclosure statement

No potential conflict of interest was reported by the authors.

Ethics statement

Animal experiments were conducted in strict accordance with the approved animal protocols and guidelines
established by the Medicine Ethics Review Committee for animal experiments of North China University of Science and Technology. The procedures for animal experiments were in accordance with requirements in the Declaration of Helsinki.

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