Protective Effects of Tyrosol against LPS-Induced Acute Lung Injury via Inhibiting NF-κB and AP-1 Activation and Activating the HO-1/Nrf2 Pathways

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Tyrosol (Tyr) is a natural antioxidant that displays anti-oxidant and anti-inflammatory properties. The present study aimed to investigate the effect and mechanism of Tyr on lipopolysaccharide (LPS)-induced acute lung injury (ALI). In a mouse model, we found that pretreatment with Tyr significantly improved survival rate, attenuated lung permeability, ameliorated histopathological alterations, reduced expression of the inflammatory mediators and improved expression of the antioxidant enzyme. Further study revealed that Tyr markedly inhibited nuclear factor-κB (NF-κB) and activator protein-1 (AP-1) activation at both in vivo and in vitro levels. To investigate the underlying mechanism, we examined the impact of Tyr on the heme oxygenase (HO)-1/nuclear factor erythroid-2 related factor 2 (Nrf2) pathway in vivo and in vitro. The results showed that Tyr significantly improved the expression of HO-1 and the activation of Nrf2. This study offers novel evidence to support the efficacy of Tyr against ALI, which helps to clarify the underlying causes of the therapeutic effects behind Tyr.

Key words acute lung injury (ALI); tyrosol(Tyr); nuclear factor (NF)-κB; activator protein (AP)-1; heme oxygenase (HO)-1; nuclear factor erythroid-2 related factor 2 (Nrf2)

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are common complications associated with both acute respiratory failure and multiple organ failure. A variety of acute severe illnesses can induce the occurrence of ALI/ARDS, including pneumonia, aspiration of gastric contents, sepsis, and major trauma, and the most major pathogenic condition of ALI/ARDS is sepsis induced by Gram-negative bacteria.1)

Lipopolysaccharide (LPS) is the predominant pathogen which induces ALI/ARDS.2) In ALI, LPS can activate nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) pathways, causing the overexpression of various inflammatory mediators, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), nitric oxide (NO) and prostaglandin E2 (PGE2).3,4) These inflammatory mediators activate and recruit peripheral polymorphonuclear neutrophils (PMNs) into lung, which initiate the inflammatory cascade and induce excessive oxidative stress. Eventually, these processes can cause alveolar capillary damage and alveolar epithelial cell damage, resulting in lung inflammatory injury.5)

Anti-oxidative enzymes, such as catalase, superoxide dismutase (SOD) and heme oxygenase (HO)-1, can protect lung tissues against oxidative damage.6) Among the anti-oxidative enzymes, HO-1, also called heat shock protein 32, is a phase II detoxifying enzyme and it is mediated by nuclear factor-erythroid-2 related factor 2 (Nrf2).7) Accumulating evidence has suggested that HO-1 improves LPS-induced ALI through counteracting inflammatory and oxidative damages.8,9) Therefore, up-regulation of the HO-1/Nrf2 pathway may be an effective method for preventing and treating ALI.

Tyrosol (Tyr), 2-(4-hydroxyphenyl) ethanol, is a kind of polyphenolic compound that exists abundantly in olive oil, white wine and many herbal extracts.9–11) Tyr has been reported to have many pharmacological properties, including cardio- protective, anti-oxidant and anti-inflammatory effects.9,10,12,13) Emerging evidence has shown that Tyr can also suppress the inflammatory response and oxidative stress by reducing the production of inflammatory mediators in vitro, probably via inhibition of the NF-κB and MAPKs signal pathways.14–16) Through rodent models, Tyr was demonstrated to attenuate the pulmonary edema induced by hypobaric hypoxia.17) However, the protective effect of Tyr on LPS-induced ALI has not yet been demonstrated in vitro or in vivo and the underlying mechanism remains to be defined. Thus, in this study, we explored the protective effect of Tyr on ALI and investigated the mechanisms related to inflammation and oxidative stress.

MATERIALS AND METHODS

Chemicals and Reagents LPS (Escherichia coli 055: B5) and Tyr (purity was 98%; 188255) were purchased from Sigma-Aldrich Co. LLC (St. Louis, MO, U.S.A.). Murine TNF-α, IL-6 and IL-1β enzyme-linked immunosorbent assay (ELISA) kits were purchased from Biolegend, Inc. (San Diego, CA, U.S.A.). Myeloperoxidase (MPO) and SOD activity assay kits were purchased from Jian Cheng Bioengineering Institute (Nanjing, China). Lactate dehydrogenase (LDH) Cytotoxic-
Care and Use of Laboratory Animals guide for the conditions. All procedures were in strict accordance with the survival rate was recorded for 7 d. After an i.p. injection of Dex or Tyr, LPS was administered by intratracheal instillation at a fatal dosage of 20 mg/kg. The lungs were harvested for further tests. The inferior lobe of the right lung was excised and weighed to obtain the ‘wet’ weight. The ratio of the wet lung to the dry lung was calculated to assess tissue edema.

**Histological Examination** The left lobes were fixed with a 10% neutral phosphate buffered formalin for 24 h, followed by dehydration with graded alcohol, and embedding in paraffin. After H&E staining, pathological changes of lung tissues were observed under a light microscope. The lung injury score was calculated as previously described.10

**Cytokine Content Measurement in the BALF and Plasma** The levels of the pro-inflammatory cytokines TNF-α, IL-6, IL-1β and IL-10 in BALF and plasma were measured by a sandwich ELISA kit.

**MPO and SOD Activities in Lung Tissues** After lung tissue homogenization, MPO and SOD activities in lung tissues were determined using relevant test kits according to the instructions.

**Cell Culture** The A549 cell line and HEK-293T cell line were purchased from the American Type Culture Collection (Manassas, VA, U.S.A.). The cells were cultured in Dulbecco’s modified Eagle’s medium (Gibco, Waltham, MA, U.S.A.) containing 10% fetal bovine serum (Kangyuan, Tianjing, China), 100 U/mL penicillin and 100 µg/mL streptomycin. Cells were maintained at 37°C with humidified air and 5% CO₂ and were passaged in a timely manner.

**Cell Viability Assay** The cytotoxicity effect of LPS or Tyr on A549 cells was assessed using a methylthiazol-tetrazolium (MTT) test. Cells were seeded in the exponential growth phase in 96-well culture plates for 24 h, then stimulated with various concentrations of LPS (10, 25, 50 and 100 µg/mL) for 6, 12 and 24 h or various concentrations of Tyr (100, 500 and 1000 µM) for 24 h. Following incubation, 20 µL of MTT solution (5 mg/mL) were added to each well. After 4 h, the resulting crystals were dissolved in dimethyl sulfoxide (DMSO). The absorbance was measured using a microplate reader (TECAN, Austria) at 570 nm. Cell viability (%)=(average absorbance of treated cells/average absorbance of control cells)×100.

The protective effect of Tyr for A549 cells was determined using a Cell Counting Kit-8 (CCK-8) Assay kit (Qihai, Shanghai, China). Cells were seeded in 96-well plates at 4×10³ cells/mL and were treated with diverse concentrations of Tyr (100, 500 and 1000 µM) for 12 h. Following incubation, the cells were treated with 25 µg/mL LPS. After 24 h of co-incubation, 20 µL of CCK-8 reagents were added to each well, and the cells were incubated for an additional 2 h. The optical density was evaluated with a microplate reader (TECAN, Austria) at a test wavelength of 450 nm and reference wavelength of 630 nm. Cell viability (%)=(average absorbance of treated cells/average absorbance of control cells)×100.

**LDH Lactate Dehydrogenase** Cytotoxicity was evaluated by measuring the activity of LDH in culture medium. A549 cells were pretreated with Tyr for 12 h and then stimulated with LPS for 24 h. Following co-incubation, the cultural supernatants were collected and processed with the LDH kit to measure the LDH activity.

**Transient Transfection and Luciferase Assay** HEK-293T cells were co-transfected with pAP-1-Luc or pNF-κB-Luc plasmid plus the phRL-TK plasmid using Lipofectamine 2000. Transient transfection of plasmids into cells was performed as described previously.99 Luciferase activity was measured using the luciferase assay system (Promega).

**Cell Treatment** A549 cells were seeded into 6-well
plates and maintained for 24 h. The A549 cells were incubated with Tyr (500 and 1000 µM) for 12 h and co-incubated with 25 µg/mL LPS for 6 h. Then the A549 cells were harvested for subsequent experiments.

**Western Blot Analysis** Following treatment with the test agents, the cells were washed twice in PBS and lysed in radioimmunoprecipitation assay (RIPA) lysis buffer (50 mM Tris (pH 7.4), 150 mM NaCl, 1% TritonX-100, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), protease inhibitors and phosphatase inhibitors). Lung tissues were homogenized in the corresponding RIPA lysis buffer. Cytosolic or nuclear extractions were performed on the cells and harvested lung tissues. Protein concentrations were determined with a BCA protein assay. The protein samples were separated by SDS-polyacrylamide gel electrophoresis (PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes, which were subsequently blocked for 1 h with 5% skim milk and incubated overnight at 4°C with primary antibodies in TBST. Next, the membranes were incubated with secondary antibodies in TBST for 2 h and observed with a chemiluminescence kit. Quantitative analysis of immunoblots was performed using Image J software (NIH).

![Fig. 1. Effects of Tyr on LPS-Induced Survival Rate in Mice (n=15)](image)
The survival rates of all the groups were recorded for 7 d (Kaplan–Meier method, log-rank test), *p<0.05 vs. LPS group.

![Fig. 2. Tyr Pretreatment Reduced Pulmonary Edema and Microvascular Permeability in LPS-Induced ALI](image)
BALF were collected at 12 h after LPS challenge (n=5 in each group). (A) The accumulation of total cells in BALF. (B) The accumulation of neutrophils in BALF. (C) The accumulation of macrophages in BALF. (D) The lung W/D ratio. (E) Total protein concentration in BALF. Data represents mean±S.D. *p<0.05 vs. control group, *p<0.05 vs. LPS group.
NF-κB and AP-1 Activity  The transcriptional activity of NF-κB and AP-1 were determined with NF-κB and AP-1 filter plate assay kits. Nuclear extractions from mice lung were performed using a commercial kit and NF-κB and AP-1 filter assays were performed according to the instructions of the kits.

Statistical Analysis  The survival rate was determined using the Kaplan–Meier method and comparisons were made with a log-rank test. Other data are expressed as the mean±standard deviation (S.D.) of at least 3 independent experiments. To determine the differences between the groups, the ANOVA was used. p<0.05 was considered statistically significant.

RESULTS

Tyr Improved the Survival Rate  As shown in Fig. 1, pretreatment with Tyr significantly improved the survival rate of mice with ALI. The survival rate in the control group was 100% at day 7. The LPS group was 13.33%. While the Dex pretreatment group improved to 73.33%, the survival rates of the middle dosage group and high dosage group were 53.33 and 60%, which were obviously higher compared to that of LPS group (p<0.05). The low dosage group were not statistically different from the LPS group.

Tyr Attenuated the LPS-Induced Inflammatory Cells Infiltration  Twelve hours after the LPS challenge, the BALFs were collected. The number of total cells and inflammatory cells (neutrophils and macrophages) in the BALF were sig-
nificantly increased in the LPS group. Pretreatment with 70, 140 and 280 mg/kg of Tyr significantly reduced the number of total cells (p < 0.05), neutrophils (p < 0.05) and macrophages (p < 0.05) compared to those in the LPS group (Figs. 2A, B, C).

**Tyr Reduced LPS-Induced Lung Permeability** We next examined lung permeability by calculating the lung W/D ratio and the total protein concentration in the BALF. The lung W/D ratio (Fig. 2D) and the BALF protein concentration (Fig. 2E) of the LPS group were significantly increased compared to those of the control group (p < 0.05). Meanwhile, Tyr significantly decreased the lung W/D ratio (p < 0.05) and the total protein concentration (p < 0.05) in the BALF in a dose-dependent manner.

**Tyr Ameliorated the Histopathological Changes in ALI Lung** Normal pulmonary histology was observed in the control group (Fig. 3A). In the LPS group (Fig. 3A), lung tissues exhibited marked inflammatory damage including tissue interstitial edema, pulmonary hemorrhage, thickening of the alveolar wall and increases in inflammatory cell infiltration. However, these histopathological changes were ameliorated in the Tyr groups (Fig. 3A). As shown in Fig. 3B, the ameliorations in histopathological changes were revealed in a dose-dependent manner. It showed a downward trend in the lung injury score with gradually increases in the doses of Tyr (p < 0.05).

**Tyr Reduced the Secretion of Pro-Inflammatory Cytokines and Inhibited iNOs and COX-2 Expression in Vivo** To assess the anti-inflammatory effects of Tyr, the levels of pro-inflammatory cytokines in the BALF and plasma were examined. The level of TNF-α, IL-1β and IL-6 were all significantly elevated in the BALF and plasma upon LPS challenge (p < 0.05; Figs. 4A–F). In contrast, administration of diverse concentrations of Tyr effectively reduced the production of pro-inflammatory cytokines (p < 0.05) in a dose-dependent fashion (Figs. 4A–F). Meanwhile, we determined the levels of

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**Fig. 4.** Tyr Pretreatment Reduced the Production of Inflammatory Cytokines in LPS-Induced ALI

(A–C) The level of TNF-α, IL-1β and IL-6 in BALF. (D–F) The level of TNF-α, IL-1β and IL-6 in plasma. (G) Tyr pretreatment reduced the expression of iNOs and COX-2 in the lung tissues. Data represents mean±S.D. *p<0.05 vs. control group, *p<0.05 vs. LPS group.
iNOS and COX-2 proteins in the lung tissues using immunoblotting. As shown in Fig. 4G, the levels of iNOS and COX-2 proteins in the Tyr group compared with the LPS group were significantly decreased \((p<0.05)\).

**Effects of Tyr on MPO Activity and Antioxidative Enzyme Expression in LPS-Induced Mice** Activated PMNs are recognized as a critical factor in the development of ALI. The accumulation of PMNs in lungs can be evaluated by measuring MPO activity. Anti-oxidative enzymes can ameliorate inflammatory activities in ALI. Therefore, the expression level of SOD and catalase were investigated.\(^{20}\) As shown in Fig. 5A, in the LPS group, MPO activity was obviously increased, whereas the Tyr pretreated groups presented a trend toward lower levels. The expression of SOD and catalase were significantly reduced in the LPS group \((p<0.05)\), but Tyr pretreatments improved the expression of these enzymes (Figs. 5B, C; \(p<0.05)\).

**Cytotoxicity of LPS and Tyr on Alveolar Epithelial Cells (A549 Cells)** Apoptosis of alveolar epithelial cells is thought to be crucial for the occurrence of ALI.\(^{21}\) To investigate the protective effects of Tyr on alveolar epithelial cells, we investigated the cytotoxicity of LPS and Tyr at first. A549 cells were treated with 10, 25, 50 or 100 \(\mu\)g/mL LPS at 6, 12 and 24h or were treated with 100, 500 and 1000 \(\mu\)M Tyr at 24h. Cell viability was observed using an MTT assay. When the cells were stimulated with 25 \(\mu\)g/mL LPS, the survival rate was approximate 70\% after 6h, 50\% after 12h and 45\% after 24h \((p<0.05)\) (Fig. 6A). As Fig. 6B shows, treatment with various concentrations of Tyr (100, 500 and 1000 \(\mu\)M) did not have any effect on the proliferation of A549 cells.

**Protective Effects of Tyr on LPS-Insulted Alveolar Epithelial Cell (A549 Cell)** Then we examined the protective effect of Tyr on A549 cells using LDH assays and a CCK-8 assay. As shown in Fig. 6C, LPS significantly increased LDH activity in the cell supernatant compared with that of the control group \((p<0.05)\). The treatments with 500 and 1000 \(\mu\)M Tyr decreased the LDH activity in LPS-insulted A549 cells \((p<0.05)\). The CCK-8 assay results showed that LPS decreased cell viability, while Tyr dose-dependently improved cell viability after the LPS injury (Fig. 6D; \(p<0.05)\).

**Effects of Tyr on NF-\(\kappa\)B and AP-1 Activation in A549 Cells and HEK-293T Cells** To investigate the effects of Tyr on NF-\(\kappa\)B and AP-1 activation \textit{in vitro}, we analyzed NF-\(\kappa\)B and AP-1 activation in A549 cells using immunoblotting and in HEK-293T cells using a Luciferase assay. In LPS-induced inflammation, NF-\(\kappa\)B, consisting of p50/p65 subunits, and AP-1, mainly consisting of c-Jun and c-Fos subunits, translocated to the nucleus and induced the transcription of pro-inflammatory genes.\(^{22,23}\) We therefore determined the expression levels of p65, c-Jun and c-Fos proteins in cell nucleus. As shown in Figs. 7A and B, LPS treatment significantly increased the expression of p65, c-Jun and c-Fos in the nucleus, while the presence of Tyr significantly reduced the expression of p65, c-Jun and c-Fos in the nucleus in LPS-induced cells \((p<0.05)\). The Luciferase assay results showed that pretreatment with Tyr can significantly inhibit the activation of NF-\(\kappa\)B and AP-1 in LPS-induced HEK-293T cells in a dose dependent manner \((p<0.05)\); Fig. 7C).
**Effects of Tyr on HO-1 and Nrf2 Nuclear Translocation in A549 Cells**

HO-1 is a kind of antioxidant enzyme that ameliorates LPS-induced ALI through the inhibition of oxidative stress and the MAPK and NF-κB pathways. We investigated the effect of Tyr on HO-1 expression in vitro using Western blotting. As shown in Fig. 7D, LPS stimulation significantly improved HO-1 expression, and pretreatment with Tyr further enhances LPS-induced HO-1 expression in a dose dependent manner ($p<0.05$). HO-1 is mediated by Nrf2 activation. Therefore, we further determined whether Tyr enhanced Nrf2 activation using Western blotting. In parallel with HO-1 expression, the results showed that pretreatment with Tyr dose-dependently exhibited a trend toward higher nuclear levels of Nrf2 in A549 cells ($p<0.05$; Fig. 7E).

**Tyr Inhibited NF-κB and AP-1 Activation in LPS-Induced ALI Mice**

To further explore the potential mechanism of Tyr on ALI in vivo, we performed Western blotting and transcription factor (TF) filter plate assays to investigate NF-κB and AP-1 activation. As shown in Figs. 8A and B, LPS induced a significant increase in NF-κB and AP-1 activation ($p<0.05$), which was inhibited by pretreatment with Tyr ($p<0.05$). The TF filter plate assay further confirmed that pretreatment with Tyr markedly blocked NF-κB and AP-1 activation in lung tissue (Fig. 8C). The data suggested that Tyr may exert a protective effect against LPS induced ALI via inhibition of NF-κB and AP-1 activity ($p<0.05$).

**Tyr Up-Regulated HO-1 through the Nrf2 Pathway in LPS-Induced ALI Mice**

To further confirm the effect of Tyr on HO-1 expression and Nrf2 activation in vivo, we investigated HO-1 expression and Nrf2 activation in ALI mice by Western blotting. The results showed that, in the LPS group, there is a considerable increase in HO-1 expression and Nrf2 activation ($p<0.05$; Figs. 8D, E). This suggests that Tyr up-regulated HO-1 through the Nrf2 pathway in LPS-induced ALI.

**DISCUSSION**

ALI is an inflammatory disease process of the lungs. Inflammatory disorders and excessive oxidative stress play important roles in the development of ALI. Therefore, the agent that inhibits inflammation and oxidative stress may be a potential candidate to prevent or treat ALI. Tyr is a natural antioxidant that displays various properties, including anti-oxidant and anti-inflammatory properties. In the present study, we found that Tyr had a protective effect on LPS-induced ALI and identified some of the underlying mechanisms for this.

Corticosteroid are the most widely used medications for ALI/ARDS. They display potent anti-inflammatory and anti-fibrotic properties in ALI/ARDS. In the study, Dex was used as a positive control drug to confirm the protective effect of Tyr in vivo. Pretreatment with Tyr reduced LPS-induced death and prolonged the survival as dexamethasone. This suggested that Tyr could protect the mice from injury induced by LPS.
Fig. 7. Tyr Pretreatment Suppressed NF-κB and AP-1 Activation and Activated HO-1/Nrf2 Pathway in Vitro

Western blot results showed that Tyr pretreatment inhibit NF-κB (A) and AP-1 (B) activation in LPS-induced A549 cells. (C) Luciferase reporter assay showed that Tyr suppressed NF-κB and AP-1 activation in a dose-dependent manner. Western blot showed HO-1 expression (D) and Nrf-2 activation (E) in A549 cells. Data represents mean±S.D. *p<0.05 vs. control group, **p<0.05 vs. LPS group.
Fig. 8. Tyr Pretreatment Suppressed NF-κB and AP-1 Activation and Up-Regulated HO-1/Nrf2 Pathway in ALI Mice

Western blot showed that Tyr suppressed NF-κB (A) and AP-1 (B) activation in ALI mice induced by LPS. (C) NF-κB and AP-1 activity were analyzed by NF-κB and AP-1 filter plate assay respectively. (D) Tyr improved HO-1 protein expression in the homogenates of whole lung tissues from ALI mice. (E) Tyr increased Nrf2 nuclear translocation in ALI mice. Data represents mean±S.D. ※p<0.05 vs. control group, *p<0.05 vs. LPS group.
Activation of inflammatory cells is thought to play a critical role for the occurrence of ALI. The activated neutrophils and macrophages release a variety of inflammatory mediators to recruit additional inflammatory cells and magnify the production of cytotoxic mediators.\(^{28}\) In the present study, mice exposed to LPS exhibit a massive migration of neutrophils and macrophages to the airways. In contrast, Tyr significantly decreased the numbers of total cells, neutrophils and macrophages in the BALF in a dose-dependent manner.

The increase in lung tissue permeability and pulmonary edema induced by alveolar epithelial cell damage is a typical complication of inflammation in ALI.\(^{20}\) Compared with the LPS group, Tyr groups showed a significant decrease in the lung W/D ratio and the total protein concentration in the BALF in a dose-dependent fashion. The results showed that Tyr pretreatment effectively alleviated lung tissue permeability and pulmonary edema in ALI. Additionally, in lung histological examination, we can observe directly that Tyr markedly ameliorated the damage of pulmonary alveolar walls and decreased neutrophil infiltration. The lung injury score also confirmed that Tyr dose-dependently alleviated the lung tissue injury \textit{in vivo}.

LPS is a kind of pro-inflammatory reaction factor. When LPS is administered, the main inflammatory cells, PMNs, are activated and infiltrated into lung tissues to release massive inflammatory mediators, such as TNF-\(\alpha\), IL-\(\beta\), IL-6, NO and PGE2. These cytokines amplify and perpetuate the inflammatory response, damaging the vascular endothelial cells and alveolar epithelial cells in ALI.\(^{29}\) In contrast, suppressing the production of inflammatory mediators may have a protective effect on inflammatory responses.\(^{30}\) In this study, we found that Tyr pretreatment significantly decreased the concentrations of TNF-\(\alpha\), IL-1\(\beta\) and IL-6 and decreased iNOS and COX-2 expressions in ALI mice. On the other hand, antioxidant enzymes can ameliorate inflammatory activities and oxidative damage through reducing superoxide anions and hydrogen peroxide.\(^{31,32}\) We demonstrated that Tyr pretreatment significantly decreased the level of lung MPO activity and improved the expressions of SOD and catalase in ALI mice. The data indicated that Tyr is able to reduce lung damage through suppressing the production of inflammatory mediators and increasing antioxidant enzymes. In LPS-induced inflammation, NF-\(\kappa\)B and AP-1 translocate into the nucleus and induce the expression of various inflammatory genes, such as pro-inflammatory cytokines, iNOS, COX-2, and chemokines.\(^{33,34}\)

To further clarify the mechanism by which Tyr decreases the production of inflammatory mediators, we determined the activation of NF-\(\kappa\)B and AP-1. The present study showed that pre-treatment with Tyr markedly inhibited the activation of NF-\(\kappa\)B and AP-1 in LPS-induced A549 cells. A similar result was obtained in LPS-induced ALI mice. These results confirmed that Tyr decreased inflammatory mediator production through suppressing NF-\(\kappa\)B and AP-1 activation.

HO-1 is an anti-inflammatory and anti-oxidative enzyme that is regulated by Nrf2.\(^{7}\) The activation of the HO-1/Nrf2 pathway is closely related to oxidative stress. Several reports have suggested that HO-1 played a pivotal protective role in LPS-induced ALI through suppressing the production of inflammatory mediators.\(^{8}\) Hence, we speculated whether HO-1 was involved in the beneficial effect of Tyr on LPS-induced ALI. To verify the hypothesis, we first determined the expression of HO-1 and the activation of Nrf2 in LPS-induced A549 cells. The results showed that pretreatment with Tyr significantly improved HO-1 expression and Nrf2 compared with those of the LPS group. Based on the outcome, we further investigated HO-1 expression and Nrf2 activation in LPS-induced ALI mice. Our findings demonstrated that Tyr pretreatment enhanced HO-1 expression and Nrf2 activation in ALI mice. These data indicated that Tyr inhibited the activation of NF-\(\kappa\)B and AP-1, which can, in part, be associated with activation of the Nrf2/HO-1 pathway \textit{in vivo} and \textit{in vitro}.

In conclusion, the present study demonstrated that Tyr could improve the survival rate of mice with ALI induced by LPS and reduce lung injury \textit{via} suppressing inflammatory reaction and oxidative stress. The underlying mechanism was possibly associated with the inhibition of AP-1 and NF-\(\kappa\)B activation and up-regulation of the HO-1/Nrf2 pathway (Fig. 9). The evidence suggested that Tyr may be a potential therapeutic drug against ALI.

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Conflict of Interest The authors declare no conflict of interest.

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