Tranilast attenuates lipopolysaccharide-induced lung injury via the CXCR4/JAK2/STAT3 signaling pathway

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Abstract. It has been reported that the expression of C-X-C motif chemokine receptor 4 (CXCR4) is increased in patients with lung injury, while CXCR4 downregulation can improve sepsis-induced lung injury. Previous studies have shown that tranilast can inhibit CXCR4 mRNA expression. Therefore, the present study aimed to investigate whether tranilast could protect against lipopolysaccharide (LPS)-induced lung injury via the CXCR4/Janus kinase 2 (JAK2)/STAT3 signaling pathway. A Cell Counting Kit-8 assay was performed to evaluate the effect of different concentrations of tranilast on the viability of LPS-induced BEAS-2B cells. The mRNA and protein expression levels of the inflammatory factors, TNFα, IL-1β, IL-6, cytochrome c oxidase subunit II and inducible nitric oxide synthase were detected using reverse transcription-quantitative PCR and western blot analysis, respectively. In addition, the cell apoptosis rate and the expression levels of apoptosis-related proteins were analyzed using a TUNEL staining assay and western blot analysis, respectively. The expression levels of the CXCR4/JAK2/STAT3 signaling pathway-related proteins were also determined using western blot analysis. Furthermore, the effects of tranilast on cell viability, inflammation and apoptosis were also evaluated in LPS-stimulated BEAS-2B cells following CXCR4 overexpression, which were pre-treated with tranilast. The results demonstrated that tranilast could alleviate LPS-induced cell viability, the secretion of inflammatory cytokines and cell apoptosis. In addition, cell treatment with tranilast inhibited the expression of CXCR4/JAK2/STAT3 signaling-related proteins in LPS-induced BEAS-2B cells. Following CXCR4 overexpression, the alleviating effect of tranilast on cell viability, inflammatory response and apoptosis was notably attenuated. Overall, the current study suggested that

tranilast could attenuate LPS-induced lung injury via the CXCR4/JAK2/STAT3 signaling pathway, suggesting that tranilast could be considered as a promising agent for treating sepsis-induced acute lung injury.

Introduction

Sepsis, a systemic inflammatory syndrome, which is induced in response to infection, is considered to be a significant clinical issue in intensive and critical care medicine (1). It is estimated that ~14,000 individuals die from sepsis-induced multiple organ dysfunction every day in the world (2). Acute lung injury (ALI), one of the most severe complications of sepsis, is characterized by high complication and mortality rates, while its treatment is considered to be challenging (3). In response to endotoxins, such as lipopolysaccharides (LPS), chemokines and inflammatory mediators, the overactivation of inflammatory responses in the lungs may lead to lung dysfunction, which in turn promotes oxidative stress injury, the main pathological mechanism of ALI in sepsis (4-6).

Tranilast was developed and launched to the market in the 1980s. It is used as an allergic mediator by promoting the secretion of blockers that stabilize mast cell membranes and basal cells to prevent the release of allergic reaction-related substances, such as histamine and 5-hydroxymycin, eventually protecting against acute asthma (7,8). In addition, tranilast was originally used to treat type-I allergic diseases, such as bronchial asthma and allergic rhinitis, while it was later found to be effective against other inflammatory diseases, such as acute colitis and atherosclerosis (9-11). It has been reported that tranilast regulates the inflammatory response by upregulating the anti-inflammatory factors IL-4, IL-10 and heme oxygenase 1 and inhibiting the levels of the pro-inflammatory cytokines, IL-1β, IL-6 and TNF-α in serum (10,12,13). Tranilast can improve the effect of lung injury induced by cyclophosphamide (14) and decrease TGFβ-induced pulmonary fibrosis (15), indicating that tranilast has an important protective effect on lung injury. However, whether tranilast could reduce lung injury caused by sepsis, as well as the underlying mechanism, remains unknown. A recent study demonstrated that pre-treatment of Ldlr$^{-/-}$ mice with tranilast, fed on a high-fat and high-sugar diet, could prevent the progression of atherosclerosis and vascular inflammation (16). The aforementioned study also reported that tranilast could suppress the activation

of Nod-like receptor family pyrin domain-containing 3 inflammasome in LPS-induced murine macrophages. In addition, another study showed that treatment of breast cancer cell lines with tranilast could inhibit C-X-C motif chemokine receptor 4 (CXCR4) signaling, a chemokine receptor involved in lung cancer and sepsis-induced ALI (17-19). Based on the aforementioned findings, the present study aimed to investigate whether tranilast could also ameliorate sepsis-induced ALI by attenuating the LPS-induced secretion of pro-inflammatory cytokines via the CXCR4 signaling pathway.

Materials and methods

Cell culture and treatment. The human non-cancerous pulmonary epithelial cell line BEAS-2B (Procell Life Science and Technology Co., Ltd.) was maintained in 55% bronchial epithelial growth medium (BEGM; Scienbio; Beijing Sunbio Biotech Co., Ltd.), supplemented with 40% FBS (Alphacell) and 5% DMSO (Beijing Solarbio Science and Technology Co. Ltd.) in a humidified incubator with 5% CO_2 at 37˚C. The cells were pre-treated with tranilast at different concentrations (50, 100 or 200 μM) for 8 h prior to cell stimulation with 10 mg/l LPS (both from Abmole Bioscience Inc.) for 16 h.

Cell transfection. The CXCR4 overexpression plasmid (ov-CXCR4) and the corresponding negative control plasmid (ov-NC) with lentiviral expression vector GV 493 were synthesized by Shanghai GenePharma Co., Ltd. The BEAS-2B cells were transfected with 20 nM ov-CXCR4 or ov-NC using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) at 37˚C with 5% CO_2 followed by incubation in serum-free medium for 4 h and then in complete culture medium for 16 h. The transfection efficacy was determined using reverse transcription-quantitative PCR (RT-qPCR) after 48 h.

Cell Counting Kit (CCK)-8 assay. The BEAS-2B cells in the logarithmic growth phase were seeded into 96-well plates, at a density of $1x10^5$ cells/well and cultured in the presence of 100 μl/well complete culture medium. Each well was then supplemented with 10 μl CCK-8 solution (Beijing Solarbio Science and Technology Co., Ltd.) and the cells were then incubated for 3 h. The optical density was measured at 450 nm using a microplate reader.

RT-qPCR analysis. Total RNA was extracted from cells using the RNAMolPure® Cell RNA kit (Shanghai Yeasen Biotechnology Co., Ltd.), then reverse transcribed into cDNA using the TransScript® First-Strand cDNA Synthesis SuperMix (Beijing Transgen Biotech Co., Ltd.), according to the manufacturer's instructions . cDNA amplification was performed using the TransStart® Green qPCR SuperMix (Beijing Transgen Biotech Co., Ltd.) according to the manufacturer's recommendations. The following thermocycling conditions were used for qPCR: Initial denaturation at 95˚C for 10 min; followed by 40 cycles at 95˚C for 10 sec and 60˚C for 60 sec. mRNA expression levels were quantified using the $2^{-\Delta\Delta Cq}$ method and normalized to the internal reference gene GAPDH (20). The following primers were used: TNF-α forward, 5'-CGAGTGACAAGCCTGTAGCC-3' and reverse, 5'-TTGAAGAGGACCTGGGAGTAG-3'; IL-1β forward,

5'-AAAAGCTTGGTGATGTCTGG-3' and reverse, 5'-TTT CAACACGCAGGACAGG-3'; IL-6 forward, 5'-GGAGAC TTGCCTGGTGAAA-3' and reverse, 5'-CTGGCTTGTTCC TCACTACTC-3'; CXCR4 forward, 5'-AGCTGTTGGTGA AAAGGTGGTCTATG-3' and reverse, 5'-GCGCTTCTG GTGGCCCTTGGAGT-3'; GAPDH forward, 5'-TGACTTCA ACAGCGACACCCACT-3' and reverse, 5'-GACTGAGTG TGGCAGGGACT-3'.

Western blot analysis. The cells from each group were lysed on ice for 30 min using RIPA lysis buffer (Beyotime Institute of Biotechnology). The supernatant was then collected following centrifugation at 300 x g for 15 min at 4˚C. The concentration of the extracted proteins was measured using a BCA kit. Subsequently, a total of 20 μg protein/sample were mixed with 12% SDS buffer (Shanghai Uteam Biotechnology Co., Ltd.) and incubated at 100˚C in a water bath for 10 min for protein denaturation. The protein samples were then separated using SDS-PAGE and electrophoresed until the bromophenol blue reached the bottom of the separating gel. The proteins were transferred to PVDF membranes, then washed three times. Following blocking with 5% skimmed milk for 2 h at room temperature, the membranes were first incubated with diluted primary antibodies overnight at 4˚C, then with secondary HRP-conjugated anti-rabbit antibody (1:5,000; cat. no. ab205718; Abcam) at room temperature for 1 h. Protein bands were visualized using an enhanced chemiluminescence detection system (EMD Millipore). The gray values of the proteins were quantified using ImageJ software (v146; National Institutes of Health). The following primary antibodies were used: cytochrome *c* oxidase subunit II (COX2; 1:1,000; cat. no. ab179800), inducible nitric oxide (iNOS; 1:1,000; cat. no. ab178945), Bax (1:1,000; cat. no. ab32503), cleaved caspase 3 (1:1,000; cat. no. ab32042), cleaved caspase 3 (1:1,000; cat. no. ab32351), Bcl2 (1:1,000; cat. no. ab32124), CXCR4 (1:1,000; ab124824), phosphorylated (p)-Janus kinase 2 (JAK2; 1:1,000; cat. no. ab32101), p-STAT3 (1:1,000; cat. no. ab76315), JAK2 (1:1,000; cat. no. ab108596), STAT3 (1:1,000; cat. no. ab68153) and GAPDH (1:1,000; cat. no. ab8245) (all from Abcam).

TUNEL assay. The cells were seeded onto a glass slide (dimensions, 1x1 cm), then placed into a 24-well plate. After transfection of LPS induced cells, the cells were washed three times with PBS and fixed in 4% paraformaldehyde for 15 min at 37˚C. The TUNEL Detection kit (Shanghai Yeasen Biotechnology Co., Ltd.) was utilized to stain the apoptotic cells for 1 h at 37˚C. The cell nuclei were counterstained with DAPI for 10 min at room temperature. Five random fields of views were selected for analysis and the apoptotic cells were observed under a fluorescence microscope (magnification, x200). The percentage of apoptotic cells was calculated as the ratio of the number of TUNEL-positive nuclei to that of DAPI-stained cells.

Statistical analysis. SPSS v22.0 statistical software (IBM Corp.) was used to analyze and process the experimental data. The results are expressed as the mean ± SD. To compare the differences among multiple groups, one-way ANOVA followed by Tukey's post hoc test was used, while comparisons between
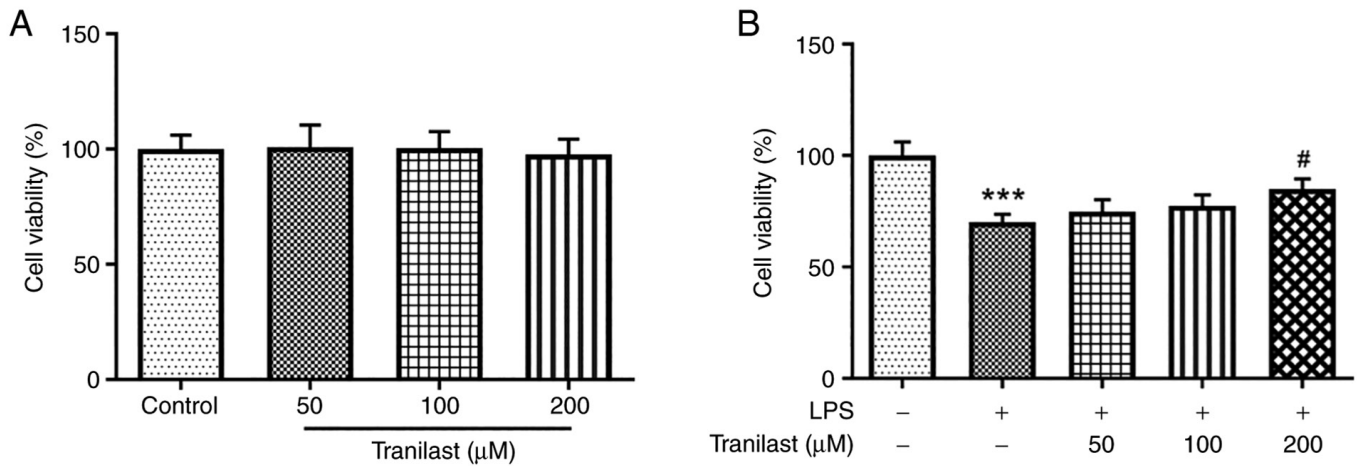
Figure 1. Tranilast reduces LPS-induced reduced viability in the BEAS-2B cells. The viability of the BEAS-2B cells treated with (A) different concentrations of tranilast and (B) different concentrations of tranilast then stimulated with LPS was detected using Cell Counting Kit-8 assay. ***P<0.001 vs. control; #P<0.05 vs. LPS. LPS, lipopolysaccharide.
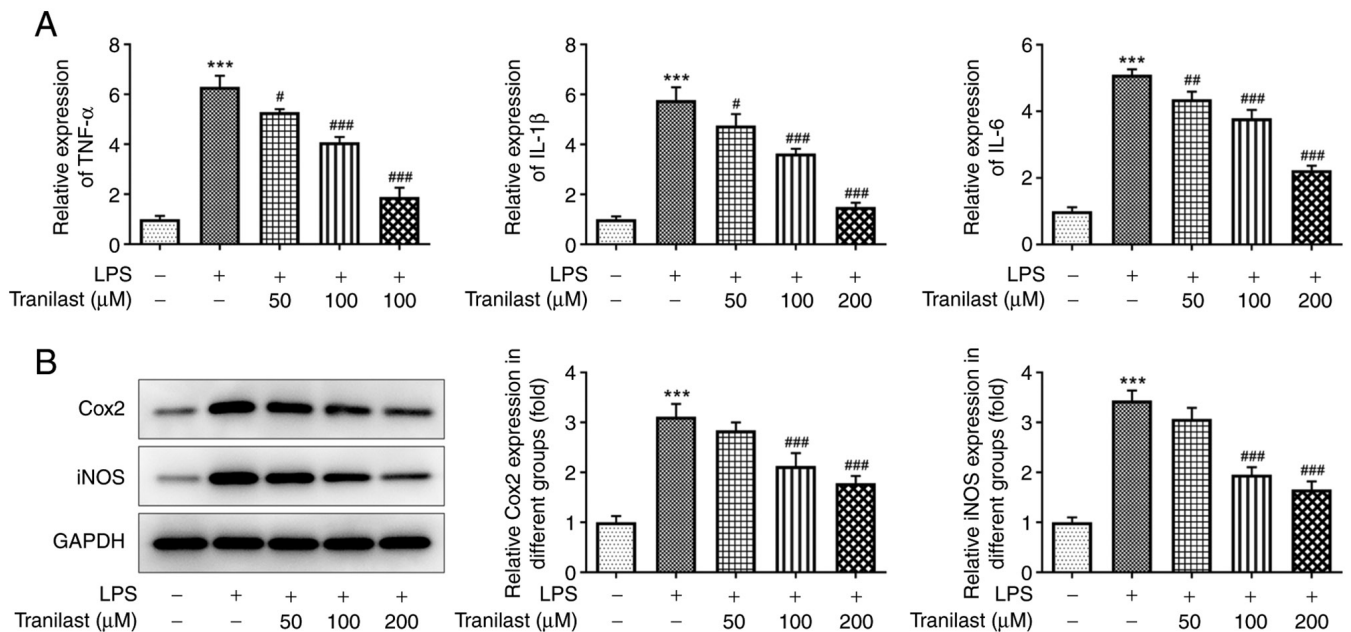

Figure 2. Tranilast reduces LPS-induced pro-inflammatory cytokine release in the BEAS-2B cells. The mRNA and protein expression level of TNF-α, IL-1β, IL-6, COX2 and iNOS in LPS-stimulated BEAS-2B cells pretreated with different concentrations of tranilast was detected using (A) reverse transcription-quantitative PCR and (B) western blot analysis, respectively. ***P<0.001 vs. control; #P<0.05, ##P<0.01, ###P<0.001 vs. LPS. LPS, lipopolysaccharide; COX2, cytochrome c oxidase subunit II; iNOS, inducible nitric oxide.

two groups were assessed using unpaired Student-t test. P<0.05 was considered to indicate a statistically significant difference. Each experiment was repeated three times.

Results

Tranilast attenuates LPS-induced BEAS-2B cell viability. Treatment with different concentrations of tranilast exerted no significant effect on untreated BEAS-2B cells with respect to cell viability (Fig. 1A). However, pre-treatment of the LPS-induced BEAS-2B cells with tranilast significantly increased cell viability in a dose-dependent manner (Fig. 1B). Therefore, these findings indicated that tranilast could attenuate LPS-induced BEAS-2B cell viability.

Tranilast attenuates the release of pro-inflammatory cytokines in LPS-induced BEAS-2B cells. As shown in Fig. 2A, treatment with LPS increased the expression levels of TNF-α (P<0.001), IL-1β (P<0.001) and IL-6 (P<0.001) in the BEAS-2B cells, whereas pre-treatment with 50 µM tranilast reduced them (P=0.0104, P=0.0271 and P=0.00521, for TNF-α, IL-1β and IL-6 respectively). In addition, pre-treatment with 100 and 200 µM tranilast restored TNF-α, IL-1β and IL-6 expression levels (P<0.001 in all cases). Similarly, western blot analysis revealed that LPS increased the protein expression levels of COX2 and iNOS (P<0.001). When the cells were pre-treated with 100 µM tranilast, the protein expression levels of COX2 (P=0.0009) and iNOS (P<0.001) were decreased (Fig. 2B). Similar results were found with 200 µM. The aforementioned
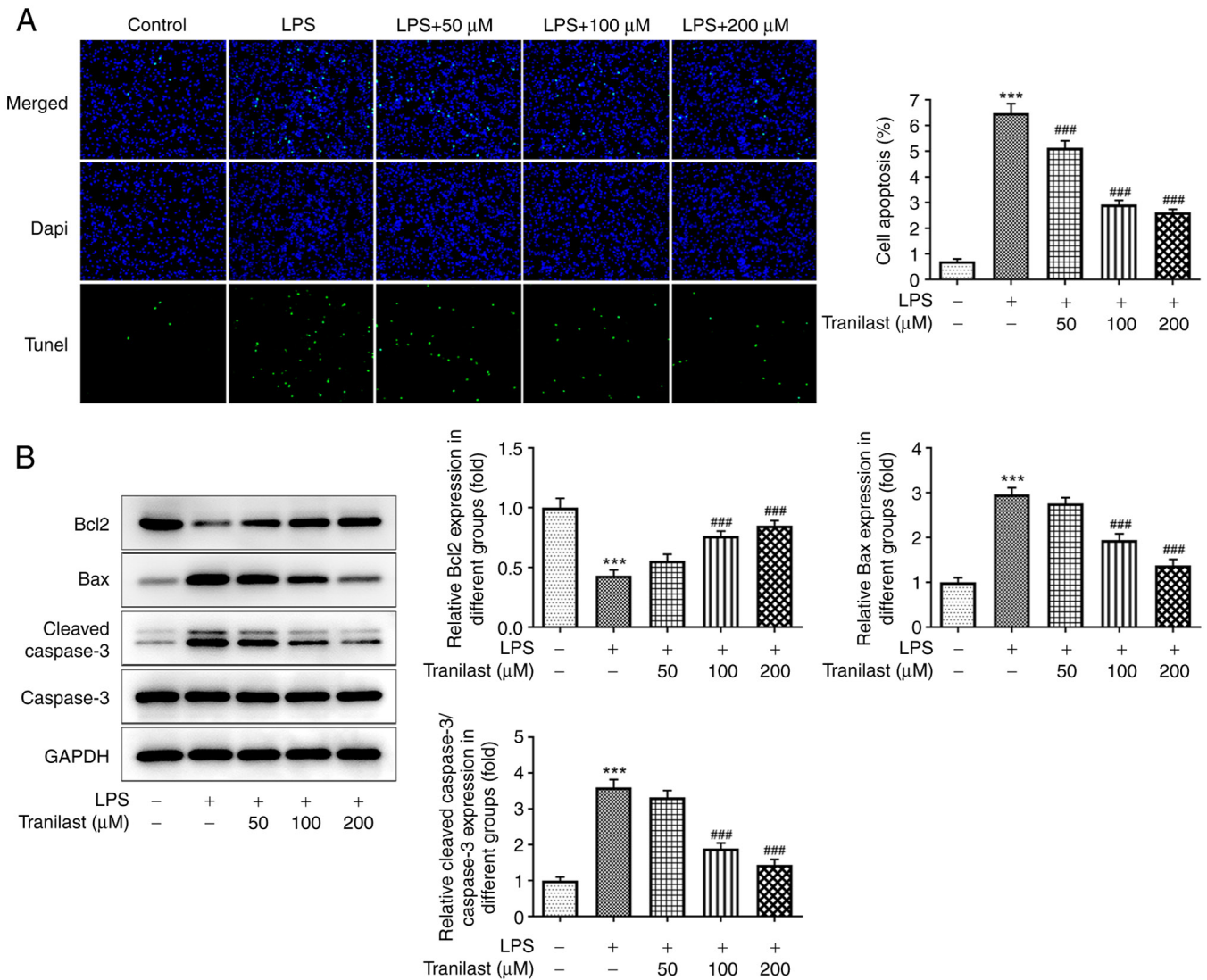
Figure 3. Tranilast alleviates LPS-induced apoptosis in the BEAS-2B cells. (A) TUNEL analysis of apoptosis in the BEAS-2B cells treated with or without LPS and/or tranilast at different concentrations. (B) Western blot analysis was used to determine the protein expression level of the apoptosis-related proteins, Bcl2, Bax and cleaved caspase-3 in the BEAS-2B cells treated with or without LPS and/or tranilast at different concentrations. ***P<0.001 vs. control; ###P<0.001 vs. LPS. LPS, lipopolysaccharide.

results suggested that tranilast could reduce the LPS-induced secretion of pro-inflammatory cytokines in the BEAS-2B cells.

Tranilast alleviates LPS-induced BEAS-2B cell apoptosis. As shown in Fig. 3A, the number of TUNEL-positive apoptotic cells was increased in the LPS group compared with that in the control group (P<0.001). However, the number of apoptotic cells was reduced in the tranilast pre-treatment group with 50 (P=0.0002), 100 (P<0.001) and 200 μM (P<0.001) compared with that in the LPS group. Consistently, western blot analysis further confirmed the TUNEL assay results. Pre-treatment of the BEAS-2B cells with tranilast reversed the LPS-mediated decreased Bcl2 and increased Bax and cleaved caspase-3 protein expression levels (Fig. 3B), indicating that tranilast could alleviate LPS-induced BEAS-2B cell apoptosis.

Tranilast suppresses LPS-induced activation of the CXCR4/JAK2/STAT3 signaling pathway. To confirm the association between tranilast and CXCR4 in ALI, the protein

expression levels of CXCR4 and those of its downstream targets, JAK-2 and STAT3 were evaluated using western blot analysis. The results showed that CXCR4, p-JAK2 and p-STAT3 (P<0.001) levels were upregulated in the LPS group compared with those in the control group. The protein expression level of CXCR4 was not significantly different following treatment with 50 μM tranilast (P=0.2226), while the protein expression level of CXCR4 was decreased following treatment with 100 and 200 μM tranilast (both P<0.001). Compared with that in the LPS group, the protein expression level of p-JAK2 decreased following treatment with 50 (P=0.0318), 100 (P=0.0318) and 200 μM tranilast (P<0.001). Compared with that in the LPS group, the protein expression level of p-STAT3 decreased with 50 (P=0.0346), 100 (P=0.0002) and 200 μM tranilast (P<0.001) (Fig. 4). The improvement of LPS-induced lung injury was significant when 200 μM tranilast was used; therefore, this concentration was used for subsequent experiments. These results suggested that tranilast could attenuate LPS-induced
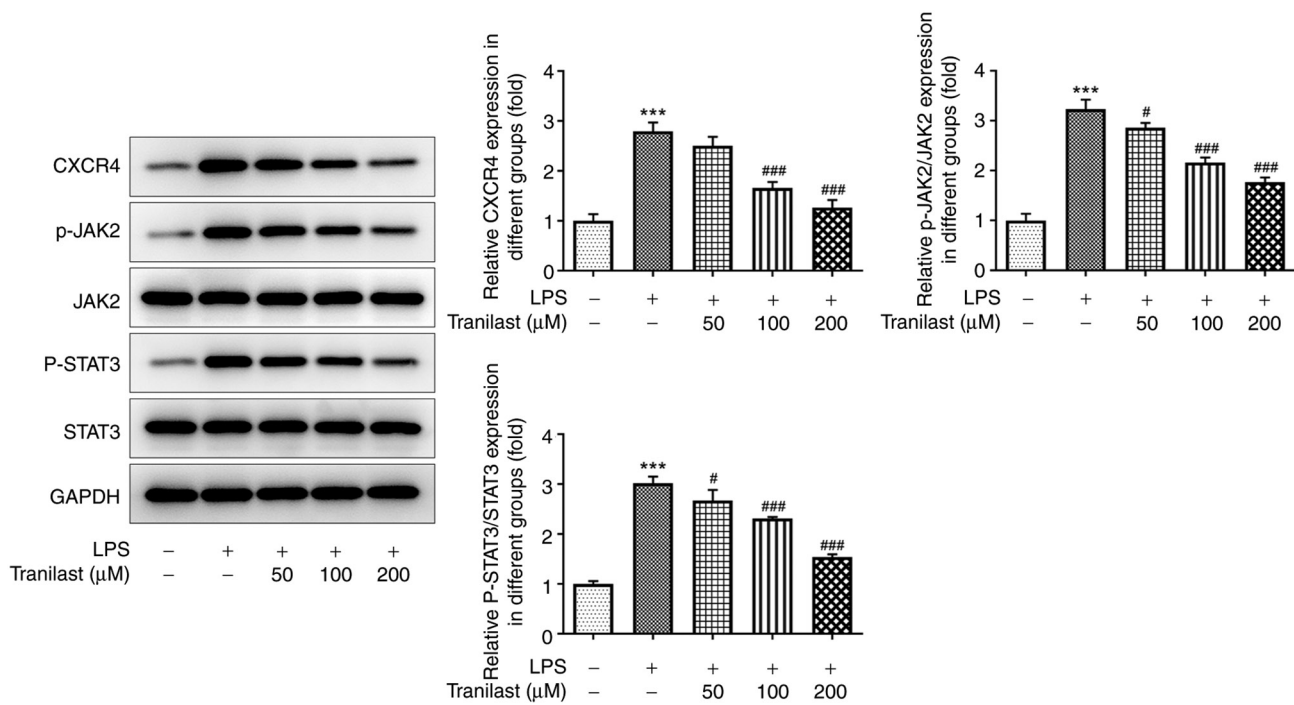
Figure 4. Tranilast inhibits LPS-induced activation of the CXCR4/JAK2/STAT3 signaling pathway. The protein expression level of CXCR4, p-JAK2/JAK2 and p-STAT3/STAT3 in the BEAS-2B cells treated with or without LPS and/or tranilast at different concentrations. ***$P<0.001$ vs. control; #$P<0.05$, ###$P<0.001$ vs. LPS. LPS, lipopolysaccharide; p, phosphorylated; JAK2, Janus kinase 2; CXCR4, C-X-C motif chemokine receptor 4.

activation of the CXCR4/JAK2/STAT3 signaling pathway in BEAS-2B cells.

CXCR4 overexpression diminishes the alleviative effect of tranilast on LPS-induced BEAS-2B cell viability. The transfection efficiency of the BEAS-2B cells with ov-CXCR4 was confirmed using RT-qPCR. The results confirmed that CXCR4 was successfully overexpressed in the BEAS-2B cells transfected with ov-CXRC4 ($P<0.001$) (Fig. 5A). As shown in Fig. 5B, LPS notably elevated the protein expression levels of CXCR4, p-JAK2 and p-STAT3, while pre-treatment with tranilast (200 μM) significantly reduced their expression levels ($P<0.001$). However, CXCR4 overexpression upregulated the protein expression level of CXCR4, p-JAK2 and p-STAT3 in LPS-stimulated BEAS-2B cells pre-treated with tranilast. In addition, CXCR4 overexpression attenuated cell viability in LPS-stimulated BEAS-2B cells pre-treated with tranilast (Fig. 5C). These results suggested that tranilast could attenuate LPS-induced cell viability by inhibiting the CXCR4/JAK2/STAT3 signaling.

CXCR4 overexpression abrogates the suppressive effect of tranilast on LPS-induced secretion of inflammatory cytokines in the BEAS-2B cells. Cell pre-treatment with tranilast could decrease the mRNA expression level of TNF-α, IL-1β and IL-6 in LPS-stimulated BEAS-2B cells; however, the results showed that CXCR4 overexpression could reverse this effect (all $P<0.001$) (Fig. 6A). Compared with that in the LPS + 200 μM + ov-NC group, the protein expression level of COX2 ($P=0.0315$) and iNOS ($P=0.0019$) was increased in the LPS + 200 μM + ov-CXCR4 group (Fig. 6B). This finding suggested that tranilast could suppress LPS-induced

pro-inflammatory cytokine release by inhibiting the activation of CXCR4/JAK2/STAT3 signaling.

CXCR4 overexpression attenuates the alleviative effect of tranilast on LPS-induced BEAS-2B cell apoptosis. TUNEL assay results revealed that CXCR4 overexpression reversed the LPS-mediated increased apoptosis rate in the BEAS-2B cells pre-treated with tranilast ($P<0.001$) (Fig. 7A). Western blot analysis also demonstrated that the protein expression levels of Bcl2 were reduced, while those of Bax and cleaved caspase-3 were enhanced following CXCR4 overexpression in LPS-stimulated BEAS-2B cells pre-treated with tranilast (Fig. 7B). Therefore, tranilast could alleviate LPS-induced cell apoptosis via inhibiting CXCR4/JAK2/STAT3 signaling.

Discussion

Sepsis, a common clinical complication of severe infectious diseases, is characterized by multi-organ failure, as a result of LPS-induced systemic inflammatory responses (21). Furthermore, ALI is a serious complication that can develop in the early stages of sepsis, with an incidence rate of 70% and a mortality rate of 50-70%, without timely treatment (22,23). Therefore, it is crucial to identify novel agents for protecting the lungs from exacerbated sepsis-induced inflammatory injury.

The anti-allergic drug tranilast could inhibit the release of chemical mediators, such as histamine, thereby exerting an anti-inflammatory effect (24,25). Emerging evidence suggests that tranilast exhibits a broad range of anti-inflammatory properties. Previous studies have shown that tranilast preconditioning can attenuate the
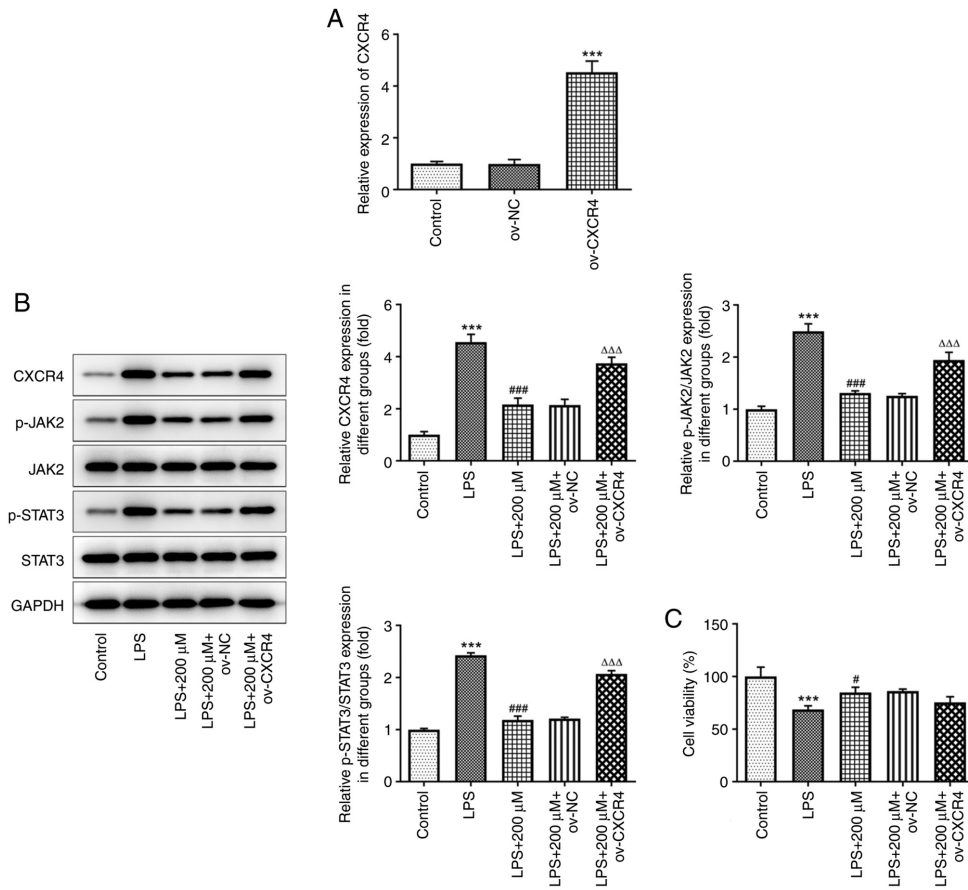
Figure 5. CXCR4 overexpression reduces the alleviative effect of tranilast on LPS-induced decreased viability via the CXCR4/JAK2/STAT3 pathway. (A) CXCR4 mRNA expression level in the BEAS-2B cells transfected with or without ov-NC or ov-CXCR4 was detected using reverse transcription-quantitative PCR. ***P<0.001 vs. ov-NC. (B) Western blot analysis revealing the protein expression level of p-JAK2/JAK2, p-STAT3/STAT3 and CXCR4 in LPS-stimulated BEAS-2B cells pretreated with tranilast (200 μM) and transfected with ov-NC or ov-CXCR4. (C) The viability of the BEAS-2B cells stimulated with LPS, pretreated with tranilast (200 μM) and transfected with ov-NC or ov-CXCR4. ***P<0.001 vs. control; #P<0.05, ###P<0.001 vs. LPS; ΔΔΔP<0.001 vs. ov-NC. LPS, lipopolysaccharide; ov, overexpression; NC, negative control; JAK2, Janus kinase 2; CXCR4, C-X-C motif chemokine receptor 4; p, phosphorylated.
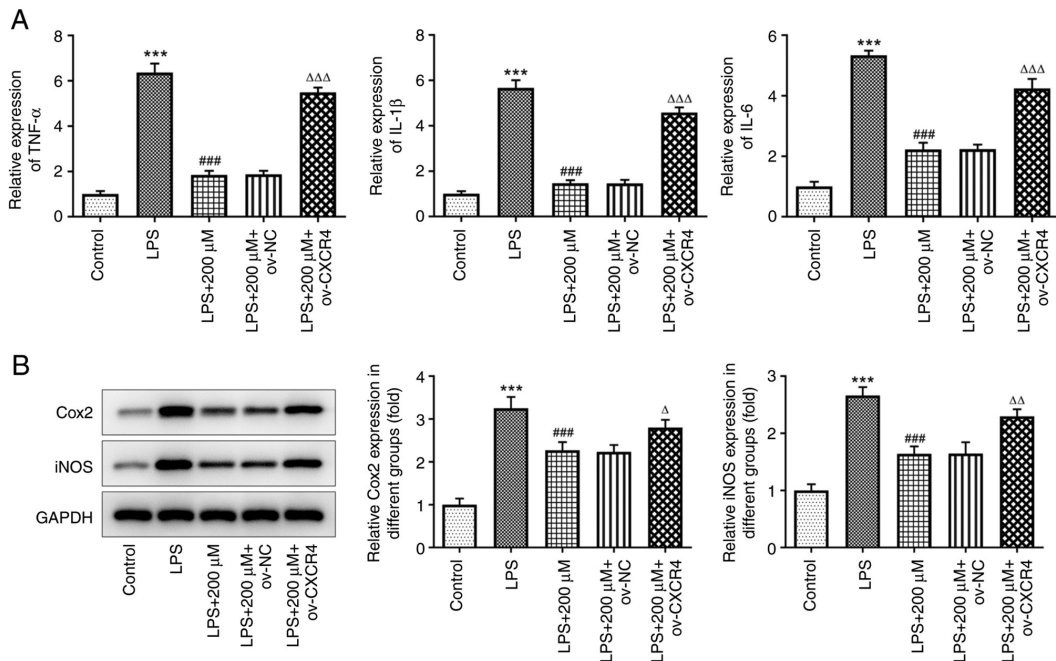

Figure 6. CXCR4 overexpression reduces the suppressive effect of tranilast on LPS-induced inflammatory cytokine release. (A) mRNA expression level of TNF-α, IL-1β, IL-6, and (B) protein expression level of COX2 and iNOS in LPS-stimulated BEAS-2B cells, pretreated with tranilast (200 μM) and transfected with ov-NC or ov-CXCR4. ***P<0.001 vs. control; ###P<0.001 vs. LPS; ΔP<0.05, ΔΔP<0.01, ΔΔΔP<0.001 vs. ov-NC. LPS, lipopolysaccharide; ov, overexpression; NC, negative control; CXCR4, C-X-C motif chemokine receptor 4; COX2, cytochrome *c* oxidase subunit II; iNOS, inducible nitric oxide.
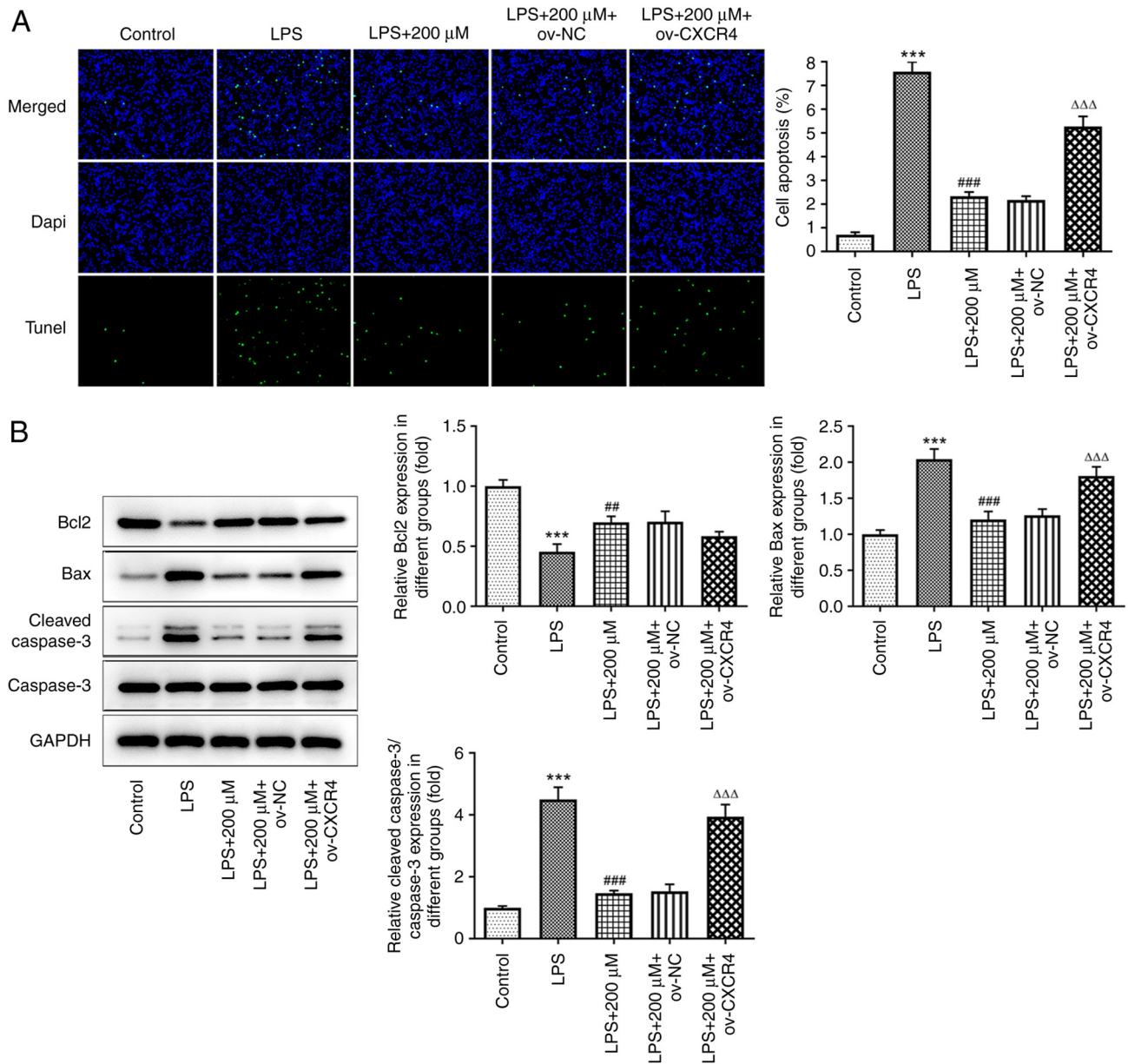
Figure 7. CXCR4 overexpression reduces the alleviative effect of tranilast on LPS-induced apoptosis. (A) TUNEL analysis of apoptosis in the LPS-stimulated BEAS-2B cells, pretreated with tranilast (200 μM) and transfected with ov-NC or ov-CXCR4. (B) Western blot analysis of the apoptosis-related proteins, Bcl2, Bax and cleaved caspase-3 in LPS-stimulated BEAS-2B cells, pretreated with tranilast (200 μM) and transfected with ov-NC or ov-CXCR4. ***$P<0.001$ vs. control; ##$P<0.01$, ###$P<0.001$ vs. LPS; $\Delta\Delta\Delta$$P<0.001$ vs. ov-NC. LPS, lipopolysaccharide; ov, overexpression; NC, negative control; CXCR4, C-X-C motif chemokine receptor 4.

LPS-induced release of pro-inflammatory cytokines from macrophages (12,26). An *in vivo* study also demonstrated that tranilast effectively attenuated inflammation in rats with spinal cord injury (27). In addition, tranilast was shown to improve inflammasome-triggered gouty arthritis by directly targeting NLRP3 (28). Importantly, tranilast was shown to exert a significant protective effect against lung injury by ameliorating cyclophosphamide-induced lung injury and TGF-β-mediated pulmonary fibrosis (14,15). In the present study, preliminary experiments revealed a dose-dependent improvement in cell viability in LPS-stimulated BEAS-2B cells following tranilast preconditioning. To the best of our knowledge, the results confirmed, for the first time, the potential protective effect of tranilast on LPS-induced lung injury.

LPS acts as the main 'mediator' of sepsis-induced multi-organ failure by stimulating neutrophils and macrophages to release large quantities of inflammatory cytokines, such as TNF-α, IL-1β and IL-6, as well as inflammation-related enzymes, such as COX2 and iNOS (29,30). TNF-α not only directly damages vascular endothelial cells in the lungs, but also induces immune stress by binding to TNF receptors of other inflammatory cells, such as macrophages and mast cells, thus promoting the secretion of inflammatory cytokines, leading to the initiation of the inflammatory cascade (31). IL-1β can further promote immune stress and inflammation, while IL-6 leads to the release of oxygen-derived free radicals, such as reactive oxygen species. COX2 and iNOS have been associated with the inflammatory response in several

pulmonary diseases, including pulmonary fibrosis, ALI and lung cancer (32-34). The results of the current study showed that pre-treatment of LPS-induced BEAS-2B cells with tranilast notably downregulated the mRNA expression levels of TNF-α, IL-1β, IL-6, COX2 and iNOS, suggesting that tranilast could exert an anti-inflammatory effect on sepsis-induced ALI.

To further verify the aforementioned results, the mechanism underlying the effects of tranilast on ALI was investigated. CXCR4, a chemokine receptor, is a highly conserved 7-pass transmembrane G-protein-coupled receptor, that plays a vital regulatory role in morphogenesis, angiogenesis and immune responses (35,36). A previous study revealed an aberrant upregulation of CXCR4 in an *in vitro* ALI model (37). In addition, a study showed that the reduced expression levels of CXCR4 mRNA and the decreased number of neutrophils could largely improve ALI in sepsis, thus suggesting that CXCR4 knockdown could be used in ALI treatment (18). A previous study also showed that combination treatment of tamoxifen and tranilast could downregulate CXCR4 mRNA expression in breast cancer cell lines (17). Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis also predicted that chemokine signaling could activate JAK2/STAT3 signaling, thereby affecting cell survival and apoptosis (38). In the present study, pre-treatment with tranilast attenuated LPS-induced activation of the CXCR4/JAK2/STAT3 signaling pathway, while CXCR4 overexpression diminished the alleviative effect of tranilast on LPS-induced BEAS-2B cell viability, the inflammatory response and apoptosis, which is the novelty of the present study. Those were what make our paper innovative.

The present study examined the effect of tranilast on LPS-induced lung injury at the cellular level; however, further studies using animal models are required to confirm the results.

In conclusion, the present study showed that tranilast alleviated LPS-induced BEAS-2B cell viability, inflammatory response and apoptosis by attenuating CXCR4/JAK2/STAT3 signaling. Therefore, the results confirmed the curative potential of tranilast in treating sepsis-induced ALI and partly uncovered its underlying mechanism of action. However, more experiments are required to verify the results of the current study *in vivo*.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YL and YZ wrote the manuscript and analyzed the data. ZH and HW performed experiments, supervised the present study, searched the literature and revised the manuscript. All authors have read and approved the final manuscript. YL and YZ confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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