Baricitinib alleviates lipopolysaccharide-induced human periodontal ligament stem cell injury and promotes osteogenic differentiation by inhibiting JAK/STAT signaling

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Abstract. Periodontitis is the chronic inflammation of the periodontal tissue. The present study aimed to investigate the role of baricitinib, a Janus kinase (JAK)1/2 inhibitor, in periodontitis by using a lipopolysaccharide (LPS)-induced human periodontal ligament stem cell (PDLSC) model. The viability of PDLSCs stimulated by LPS was assessed in the presence of baricitinib by Cell Counting Kit-8 assay. The induction of oxidative stress was evaluated by detecting the intracellular reactive oxygen species (ROS) levels, superoxide dismutase (SOD) activity and glutathione (GSH) content. ELISA and reverse transcription-quantitative PCR were used to determine the levels of inflammatory factors TNF-α, IL-1β and IL-6. Alkaline phosphatase (ALP) activity and alizarin red staining were used to assess the osteogenic differentiation of PDLSCs. The expression levels of osteogenic differentiation- and JAK/signal transducer and activator of transcription (STAT) signaling-associated proteins were estimated with western blotting. RO8191, an agonist of the JAK/STAT pathway, was used to treat PDLSCs to investigate the regulatory mechanism of baricitinib. The results indicated that baricitinib elevated the LPS-induced decrease in cell viability. LPS-triggered oxidative stress and inflammation were inhibited by baricitinib, as demonstrated by the decreased levels of ROS, TNF-α, IL-1β, IL-6 and increased levels of SOD and GSH. In addition, baricitinib caused a marked elevation in ALP activity and mineralization ability of PDLSCs, as determined by the upregulated osteocalcin and Runt-related transcription factor 2 expression. Moreover, the expression levels of phosphorylated (p)-JAK1, p-JAK2 and p-STAT3 were downregulated by baricitinib in a dose-dependent manner. Furthermore, addition of RO8191 restored the effect of baricitinib on the induction of oxidative stress, inflammation and osteogenic differentiation of PDLSCs exposed to LPS. Collectively, these findings suggested that baricitinib alleviated oxidative stress and inflammation and promoted osteogenic differentiation of LPS-induced PDLSCs by inhibiting JAK/STAT signaling.

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Introduction

Periodontitis, an infectious human chronic inflammatory disease of which the age-standardized prevalence rate increased by 8.44% (6.62-10.59%) worldwide from 1990 to 2019 (1), is caused by accumulation of subgingival plaque and the action of gram-negative anaerobic bacteria, such as *Porphyromonas gingivalis* and *Treponema denticola* (2,3). It is characterized by the progressive destruction of tooth-supporting tissue, eventually leading to tooth loss (4,5). A number of studies have demonstrated that periodontitis is associated with the initiation or progression of various systemic diseases, such as cardiovascular and chronic kidney disease and diabetes (6-8). Although progress has been achieved in the treatment of periodontitis, including skin flap curettage, scaling and root planting, the effects of the aforementioned therapies only control the development of this condition (9-11). Therefore, it is of clinical importance to investigate promising therapeutic agents for periodontitis to improve oral health and avert the development of systemic disease.

Periodontal ligament stem cells (PDLSCs), mesenchymal stem cells located in the periodontal ligament tissue, harbor osteogenic potential and regenerative capacity to recover lost/damaged periodontal tissue (12,13). PDLSCs have been shown to migrate to the site of periodontal disease and mediate periodontal regeneration (14,15). PDLSCs are considered an ideal cell source for periodontal tissue regeneration and repair (16,17). A body of evidence indicates that endotoxins

generated from periodontal pathogens, notably lipopolysaccharide (LPS) produced by *Porphyromonas gingivalis*, disrupt microenvironment homeostasis and damage periodontal tissue by inhibiting the viability of PDLSCs and causing disturbance of periodontal ligament cell differentiation (18,19). LPS is a potent stimulator of inflammation that produces proinflammatory cytokines. This compound has been widely used for construction of *in vitro* experimental models of periodontitis (20,21). It is crucial to control the inflammatory damage of PDLSCs for the restoration of the tissues and to inhibit progression of periodontitis.

Baricitinib is an oral drug commonly used in treatment of rheumatoid arthritis (22). A recent clinical study has suggested that baricitinib improves the periodontal health of patients with rheumatoid arthritis and decrease the inflammatory response in periodontal tissue (23). Baricitinib suppresses osteoclastogenesis by downregulating receptor activator of nuclear factor-κB ligand (RANKL) expression in osteoblasts (24). In addition, as a Janus kinase (JAK)1/2 inhibitor, baricitinib increases bone mass in physiological conditions and ameliorates pathological bone loss by stimulating osteoblast function (25). Meanwhile, the abnormal expression of STAT3, an important member of STAT protein family, has been reported to mediate the over-activation of JAK-STAT3 signaling pathway, which is related to the process of periodontitis (26). However, whether baricitinib affects inflammation and osteogenic differentiation of LPS-induced PDLSCs and its potential regulatory mechanisms remains to be elucidated.

Materials and methods

Cell culture. Human PDLSCs provided by Shanghai Chuntest Biotechnology Co., Ltd. were cultured in DMEM (Gibco; Thermo Fisher Scientific, Inc.) with 10% fetal bovine serum (FBS; HyClone; Cytiva) at 37˚C in an incubator with 5% CO_2. To induce osteogenic differentiation, PDLSCs were cultured in osteogenic differentiation medium, which was composed of α-MEM (Sigma-Aldrich; Merck KGaA) containing 10 mM β-glycerophosphate, 10% FBS, 50 mM ascorbic acid and 10 nM dexamethasone for 14 days in a 37˚C incubator with 5% CO_2, as previously described (27).

Treatment protocol. LPS (10 μg/ml; Sigma-Aldrich; Merck KGaA) was used to induce PDLSCs for 24 h at 37˚C to simulate the inflammatory microenvironment of periodontitis, as previously described (21). The biological source of LPS was *Porphyromonas gingivalis*. For baricitinib-treated groups, PDLSCs were treated with baricitinib (1.0, 2.5 and 5.0 μM; ChemScene) for 24 h at 37˚C in the presence or absence of LPS (24). In addition, PDLSCs were pretreated with RO8191 (20 μM; MedChemExpress), an agonist of the JAK/signal transducer and activator of transcription (STAT) pathway, for 1 h at 37˚C to investigate the regulatory effect of baricitinib on this signaling pathway, as previously described (28).

Cell viability assay. The viability of PDLSCs was evaluated by Cell Counting Kit-8 (CCK-8) assay (Shanghai Yeasen Biotechnology Co., Ltd.) A total of $5x10^3$ PDLSCs were seeded in a 96-well plate. LPS or baricitinib was employed to treat PDLSCs 24 h prior to addition of CCK-8 solution (10 μl). Following incubation for another 4 h, a microplate reader (Molecular Devices, LLC) was applied to record the optical density values at 450 nm.

Assessment of intracellular reactive oxygen species (ROS). The 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) probe is a commonly used probe for detecting intracellular ROS (29). PDLSCs were seeded in 96-well plates at a density of $2x10^4$ cells/well. Following treatment with LPS, baricitinib or RO8191 and PDLSCs were incubated with 10 μM DCFH-DA (Beyotime Institute of Biotechnology) for 20 min at 37˚C. An inverted fluorescence microscope (Olympus Corporation) (magnification, x400) was used to measure the fluorescence intensity corresponding to ROS levels from three random fields.

Determination of superoxide dismutase (SOD) activity and glutathione (GSH) content. The activity of SOD and content of GSH in the cell supernatant was evaluated by the SOD assay kit (cat. no. A001-3-2) and GSH assay kit (cat. no. A006-2-1) supplied by Nanjing Jiancheng Bioengineering Institute according to the manufacturer's protocol. The absorbance was detected using a microplate reader (Molecular Devices, LLC) at a wavelength of 450 and 420 nm.

Detection of inflammatory factors. The protein levels of TNF-α, IL-1β and IL-6 in cell supernatant was assessed by human TNF-α ELISA kit (cat. no. F02810), human IL-1β ELISA kit (cat. no. F01220) and human IL-6 ELISA kit (cat. no. F01310) (Shanghai Xitang Biotechnology Co., Ltd.) according to the manufacturer's instructions. The optical density values were determined at 450 nm by a microplate reader (Molecular Devices, LLC).

Assessment of alkaline phosphatase (ALP) activity. Following incubation at 37˚C in osteogenic medium for 14 days, ALP activity of the PDLSCs was assessed by an ALP assay kit (cat. no. A059-2-1; Nanjing Jiancheng Biotechnology Institute) according to the manufacturer's instructions. The optical density was detected at 520 nm using a microplate reader (Molecular Devices, LLC).

Alizarin red staining. The mineralization potential of PDLSCs was determined using alizarin red staining after cells were cultured at 37˚C seeded into a 24-well plate at a density of 250 cells/well in the osteogenic medium with or without LPS, baricitinib or RO8191 for 14 days. PDLSCs were incubated with 4% paraformaldehyde for 30 min at 4˚C, followed by staining with 1% alizarin red solution (Sigma-Aldrich; Merck KGaA) for 15 min at 37˚C. Following washing three times with PBS, the images were captured by a light microscope (magnification, x200; Olympus Corporation) with three fields for each group. To quantify mineralization, calcium deposits were desorbed using 10% cetylpyridinium chloride (Sigma-Aldrich; Merck KGaA) for 30 min at room temperature and the optical density value was detected using a plate reader at 562 nm (Molecular Devices, LLC).

Reverse transcription-quantitative PCR. Total RNA was isolated from PDLSCs with TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.). RT was performed to

generate complementary DNA by PrimeScript RT reagent kit (Takara Biotechnology Co., Ltd.) according to the manufacturer's instructions. The detection of mRNA expression was performed in the ABI 7500 system (Applied Biosystems; Thermo Fisher Scientific, Inc.) using the QuantiNova SYBR Green PCR kit (Qiagen GmbH). The thermocycling conditions were as follows: Initial denaturation at 95˚C for 5 min, followed by 40 cycles of denaturation for 15 sec at 95˚C and annealing for 30 sec at 60˚C. The primer sequences were as follows: TNF-α forward, 5'-CCTCTCTCTAATCAGCCC TCTG-3' and reverse, 5'-GAGGACCTGGGAGTAGAT GAG-3'; IL-1β forward, 5'-ATGATGGCTTATTACAGTGGC AA-3' and reverse, 5'-GTCGGAGATTCGTAGCTGGA-3'; IL-6 forward, 5'-ACTCACCTCTTCAGAACGAATTG-3' and reverse, 5'-CCATCTTTGGAAGGTTCAGGTTG-3' and GAPDH forward, 5'-GGAGCGAGATCCCTCCAAAAT-3' and reverse, 5'-GGCTGTTGTCATACTTCTCATGG-3'. The calculation of relative mRNA expression was performed with the $2^{-\Delta\Delta Cq}$ method (30). Primers were synthesized by Shanghai GenePharma Co., Ltd. GAPDH was utilized as an endogenous control.

Western blot analysis. PDLSCs were lysed in RIPA lysis buffer (Beyotime Institute of Biotechnology) to extract the total protein, which was quantified using a bicinchoninic protein assay kit (Beyotime Institute of Biotechnology). Protein separation was performed using 10% SDS-PAGE (30 μg/lane). The separated proteins were transferred to PVDF membranes. The blots were blocked with 5% non-fat dry milk at room temperature for 2 h, followed by incubation with primary antibodies including p-JAK1 (1:1,000; cat. no. ab138005; Abcam), JAK1 (1:1,000; cat. no. ab133666; Abcam), p-JAK2 (1:1,000; cat. no. ab32101; Abcam), JAK2 (1:5,000; cat. no. ab108596; Abcam), p-STAT3 (1:1,000; cat. no. ab68153; Abcam), STAT3 (1:1,000; cat. no. ab267373; Abcam), OCN (1:1,000; cat. no. ab133612; Abcam), RUNX2 (1:1,000; cat. no. ab236639; Abcam) and GAPDH (1:2,500; cat. no. ab9485; Abcam) overnight at 4˚C. Subsequently, membranes were labeled with a horseradish peroxidase-conjugated secondary antibody (1:5,000; cat. no. ab205718; Abcam) for 1 h at room temperature. The bands were visualized using an ECL kit (Beyotime Institute of Biotechnology) and detection system (MilliporeSigma). The band densities were evaluated using ImageJ software (v1.8.0; National Institutes of Health).

Statistical analysis. All experiments were repeated independently in triplicate and data are expressed as the mean ± standard deviation. GraphPad Prism 6.0 (GraphPad Software, Inc.) was used for data analysis. The level of significance was analyzed by one-way ANOVA followed by Tukey's post hoc test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Baricitinib increases viability of PDLSCs exposed to LPS. Firstly, cell viability was evaluated by CCK-8 assay following treatment of PDLSCs with baricitinib (1.0, 2.5 and 5.0 μM). Baricitinib treatment exhibited no significant effect on the viability of PDLSCs compared with the control group (Fig. 1A).

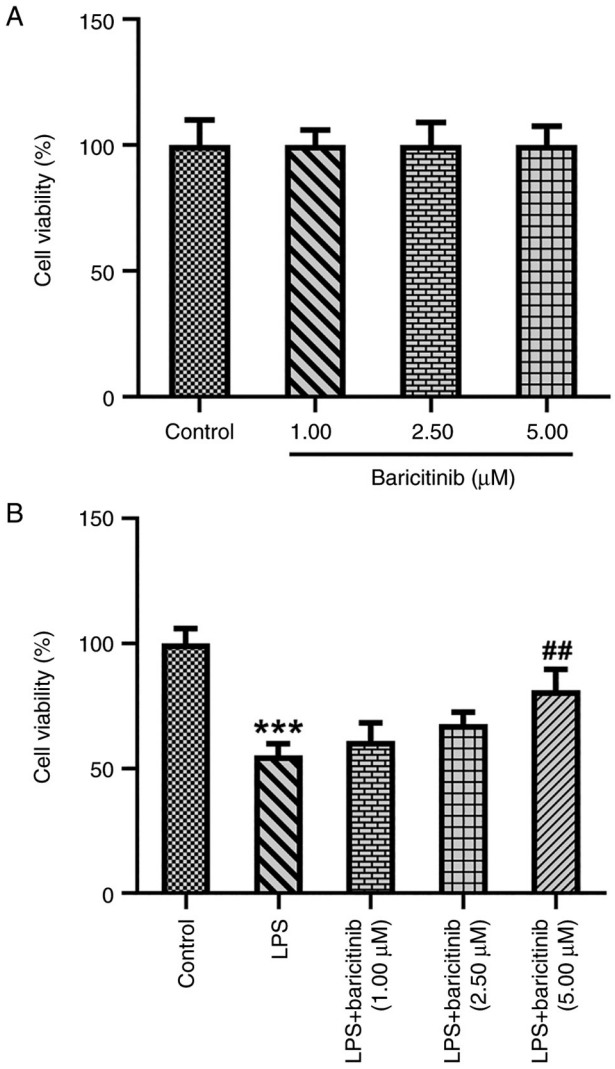

Figure 1. Baricitinib increases the viability of PDLSCs exposed to LPS. (A) Cell viability was assessed using CCK-8 assay in the presence or absence of baricitinib. (B) Viability of PDLSCs was assessed by the CCK-8 assay in the presence or absence of LPS and baricitinib. ***P<0.001 vs. control; ##P<0.01 vs. LPS. PDLSCs, periodontal ligament stem cells; LPS, lipopolysaccharide; CCK-8, Cell Counting Kit-8.

PDLSCs were stimulated by LPS with or without baricitinib. LPS treatment led to a significant decrease in PDLSC viability compared with the control group (Fig. 1B). Baricitinib dose-dependently increased the viability of PDLSCs following LPS treatment and PDLSCs viability was significantly exacerbated when treated by 5 μM baricitinib upon exposure to LPS. These results demonstrated that baricitinib reversed the LPS-induced decrease in viability of PDLSCs.

Baricitinib alleviates LPS-induced oxidative stress and inflammation in PDLSCs. The levels of intracellular ROS were evaluated using DCFH-DA as a probe; fluorescence intensity reflected the content of ROS. The fluorescence intensity in the LPS group was markedly enhanced compared with that of the control group (Fig. 2A). However, baricitinib decreased the fluorescence intensity induced by LPS in a concentration-dependent manner. Moreover, decreased SOD activity and GSH content caused by the LPS challenge gradually

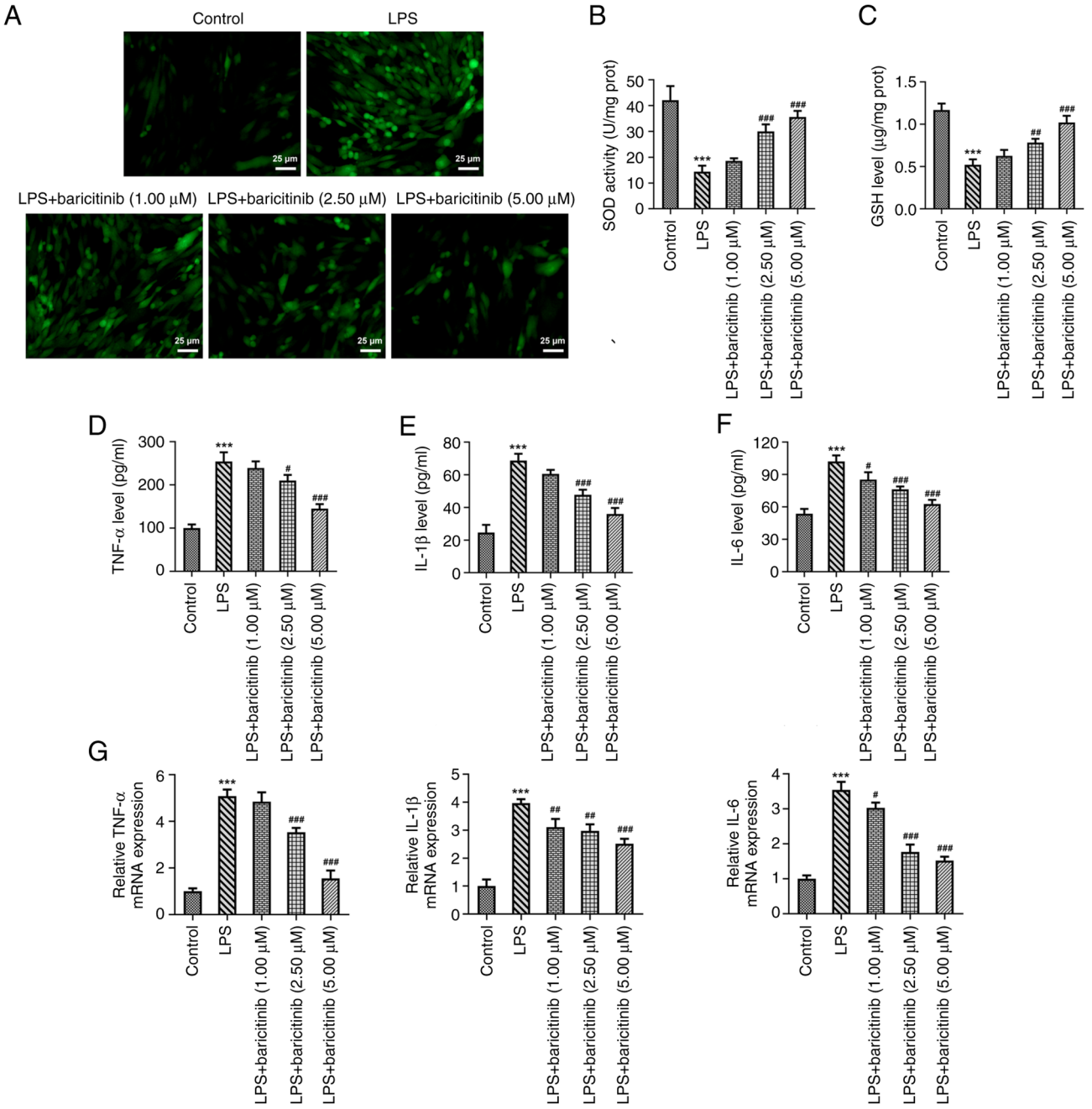

Figure 2. Baricitinib attenuates LPS-induced oxidative stress and inflammation in periodontal ligament stem cells. (A) Levels of intracellular reactive oxygen species were evaluated using 2,7-dichlorodihydrofluorescein diacetate as a probe. Measurement of (B) SOD activity and (C) GSH content with commercially available kits. ELISA kits were used for detection of (D) TNF-α, (E) IL-1β and (F) IL-6 levels. (G) mRNA expression levels of TNF-α, IL-1β and IL-6 were measured by reverse transcription-quantitative PCR. ***P<0.001 vs. control; $^{\#}$P<0.05, $^{\#\#}$P<0.01 and $^{\#\#\#}$P<0.001 vs. LPS. LPS, lipopolysaccharide; SOD, superoxide dismutase; GSH, glutathione; prot, protein.

recovered following baricitinib treatment (Fig. 2B and C). In addition, levels of TNF-α, IL-1β, and IL-6 were elevated following incubation of PDLSCs with LPS (Fig. 2D-F). Additional treatment with baricitinib reversed the impact of LPS on expression levels of the aforementioned inflammatory factors in a concentration-dependent manner. Consistently, elevated mRNA expression levels of these inflammatory factors (TNF-α, IL-1β and IL-6) induced by LPS were also decreased following baricitinib treatment (Fig. 2G). These data provide evidence that baricitinib alleviated LPS-induced oxidative stress and inflammation in PDLSCs.

Baricitinib promotes osteogenic differentiation in LPS-stimulated PDLSCs. The effect of baricitinib on osteogenic differentiation of PDLSCs challenged with LPS was investigated. ALP activity was significantly decreased in LPS group compared with that of the control group (Fig. 3A). By contrast, baricitinib dose-dependently increased ALP activity relative to the LPS group. The results of alizarin red staining indicated that the mineralization degree of PDLSCs was enhanced in the control group; this effect was reduced following stimulation of the cells with LPS (Fig. 3B and C). Furthermore, in the presence of baricitinib, the mineralization

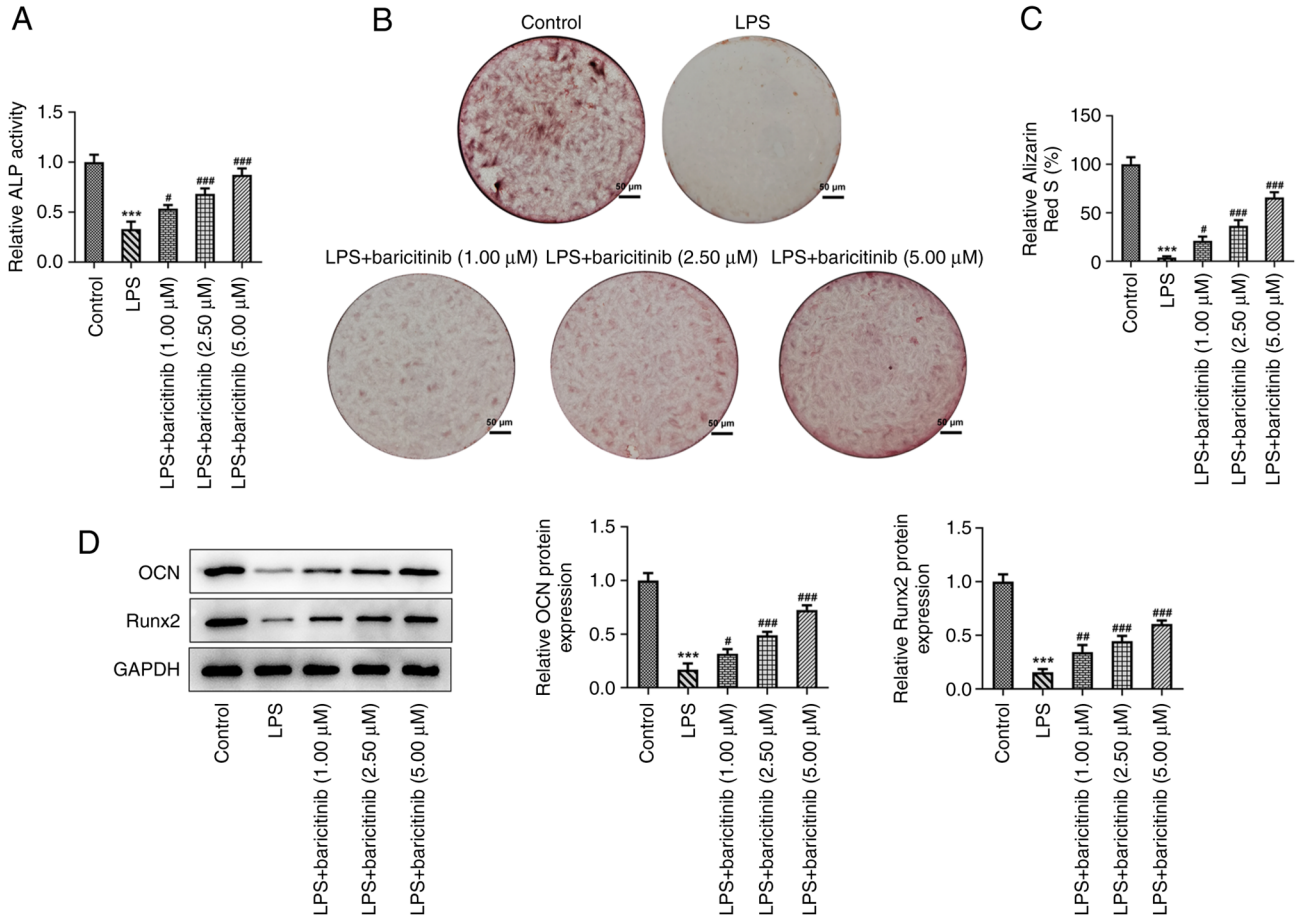

Figure 3. Baricitinib promotes osteogenic differentiation in LPS-induced PDLSCs. (A) ALP activity was examined using an ALP kit. (B) Alizarin red staining was used to evaluate (C) mineralization ability of PDLSCs. (D) Expression levels of OCN and Runx2 were assessed by western blotting. ***P<0.001 vs. control; $^{\#}$P<0.05, $^{\#\#}$P<0.01 and $^{\#\#\#}$P<0.001 vs. LPS. LPS, lipopolysaccharide; PDLSCs, periodontal ligament stem cells; ALP, alkaline phosphatase; OCN, osteocalcin; Runx2, Runt-related transcription factor 2; S, staining.

ability of PDLSCs increased in a dose-dependent manner. Moreover, the expression levels of proteins related to osteogenic differentiation, including osteocalcin (OCN) and Runt-related transcription factor (Runx) 2, were downregulated following treatment with LPS compared with the control group, as determined by western blot analysis. This effect was reversed and the levels of OCN and Runx2 were upregulated following subsequent treatment of the cells with baricitinib (Fig. 3D). These observations revealed that baricitinib promoted the osteogenic differentiation of PDLSCs stimulated by LPS.

Baricitinib inactivates the JAK/STAT signaling pathway in LPS-stimulated PDLSCs. To investigate the underlying mechanism by which baricitinib regulates the process of PDLSC injury and osteogenic differentiation triggered by LPS, expression of specific proteins involved in the JAK/STAT pathway was assessed using western blot analysis. The expression levels of phosphorylated (p)-JAK1, p-JAK2 and p-STAT3 were significantly increased in the LPS group compared with the control group (Fig. 4). By contrast, baricitinib caused a gradual downregulation in the expression levels of these proteins in a dose-dependent manner; the highest inhibitory effect was noted in the 5 μM-treated group. Thereafter, 5 μM baricitinib was applied to the subsequent experiments. Collectively, these data suggested that baricitinib suppressed the JAK/STAT signaling pathway in LPS-induced PDLSCs.

Baricitinib improves LPS-induced oxidative stress and inflammation in PDLSCs by inhibiting JAK/STAT signaling. To clarify the regulatory effect of baricitinib on the JAK/STAT signaling pathway in LPS-stimulated PDLSCs, RO8191, an agonist of the JAK/STAT pathway, was used to treat PDLSCs. RO8191 addition significantly upregulated p-JAK1, p-JAK2 and p-STAT3 expression in PDLSCs treated with LPS and baricitinib compared with the LPS +Baricitinib group (Fig. 5). Subsequently, levels of oxidative stress and inflammation were evaluated. RO8191 significantly increased ROS levels compared with those noted in the LPS + baricitinib group, as determined by the decreased SOD activity and GSH content (Fig. 6A-C). Moreover, the decline in the TNF-α, IL-1β and IL-6 levels induced by baricitinib was significantly reversed following RO8191 treatment (Fig. 6D-G). These findings confirmed that baricitinib exerted antioxidant and anti-inflammatory effects in LPS-stimulated PDLSCs by inhibiting JAK/STAT signaling.

Baricitinib promotes osteogenic differentiation of PDLSCs challenged with LPS by inhibiting JAK/STAT signaling. Subsequently, osteogenic differentiation of LPS-stimulated

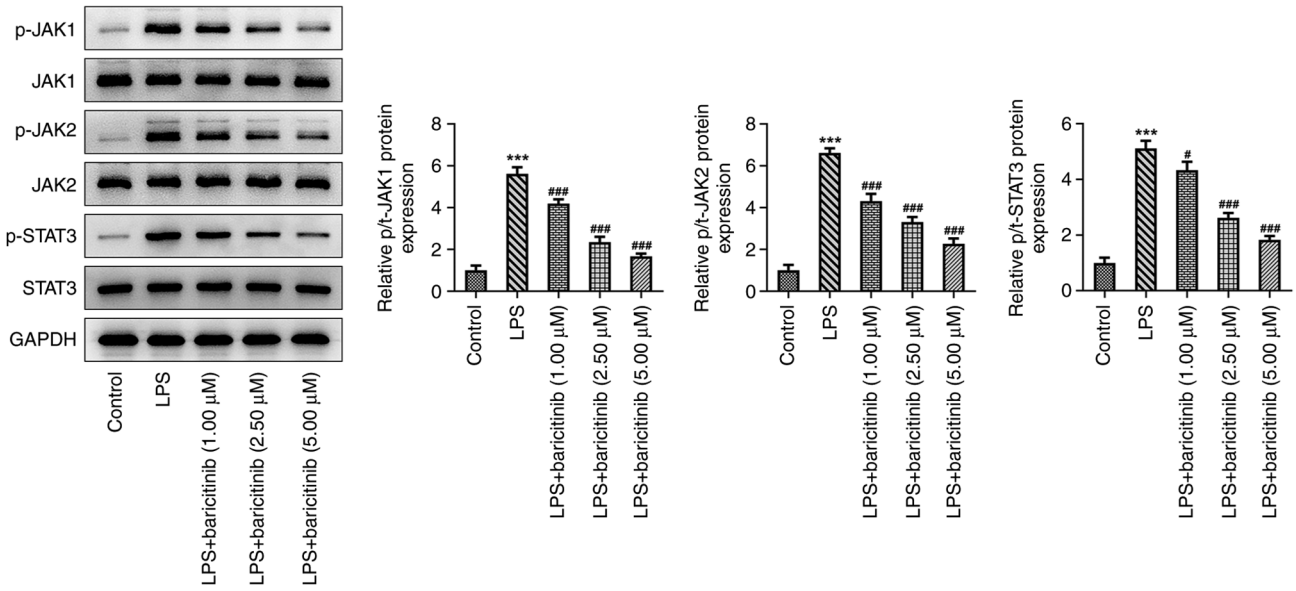

Figure 4. Baricitinib inactivates JAK/STAT signaling in LPS-induced periodontal ligament stem cells. Western blot analysis was used to evaluate the expression levels of p-JAK1, p-JAK2 and p-STAT3. ***P<0.001 vs. control; #P<0.05 and ###P<0.001 vs. LPS. JAK, Janus kinase; STAT, signal transducer and activator of transcription; LPS, lipopolysaccharide; p, phosphorylated.

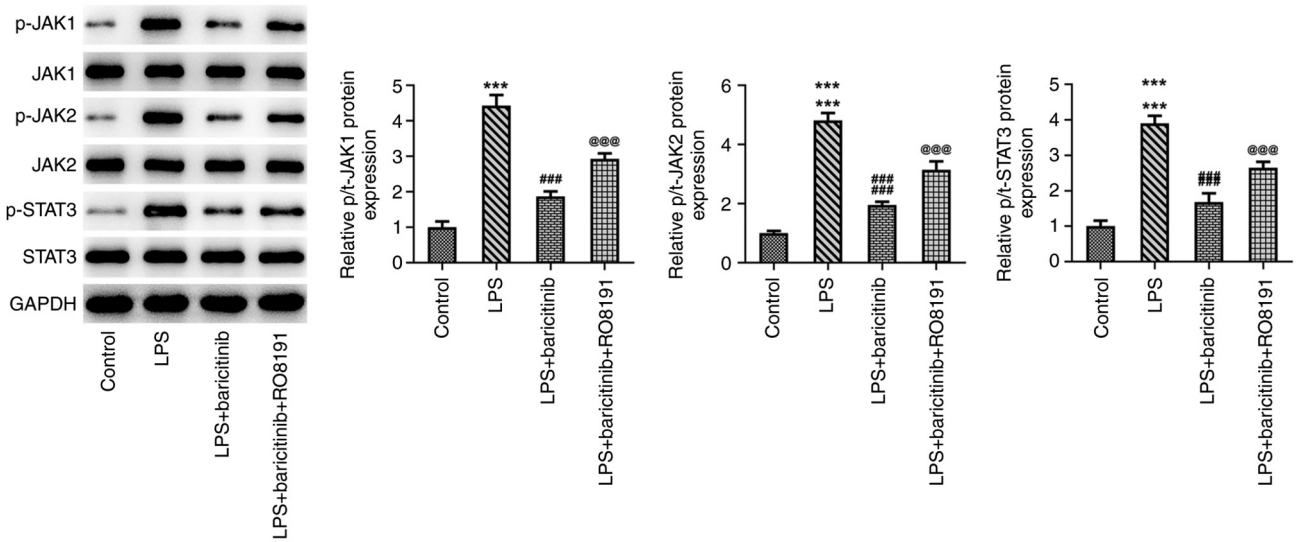

Figure 5. RO8191 activates JAK/STAT signaling in LPS- and baricitinib-induced periodontal ligament stem cells. Expression levels of p-JAK1, p-JAK2 and p-STAT3 were determined using western blot analysis. ***P<0.001 vs. control; ###P<0.001 vs. LPS; @@@P<0.001 vs. LPS + baricitinib. JAK, Janus kinase; STAT, signal transducer and activator of transcription; LPS, lipopolysaccharide; p, phosphorylated.

PDLSCs was assessed with or without baricitinib or RO8191. The results indicated that addition of RO8191 led to a significant decrease in ALP activity compared with that in the LPS + baricitinib group (Fig. 7A). Alizarin red staining suggested that the mineralization degree of PDLSCs was significantly decreased following addition of RO8191 in PDLSCs co-treated with LPS and baricitinib (Fig. 7B and C). Concomitantly, significant downregulation of OCN and Runx2 expression was observed in the LPS + baricitinib + RO8191 group compared with the LPS + baricitinib group (Fig. 7D). In summary, these results revealed that baricitinib promoted osteogenic differentiation of PDLSCs exposed to LPS by inhibiting JAK/STAT signaling.

Discussion

Periodontitis is a non-infectious chronic inflammatory disease that is primarily induced by microbial attack and affects tooth support structures. This condition is prevalent worldwide and affects large populations with age-standardized prevalence rate increased by 8.44% worldwide from 1990 to 2019 (1,31,32). Due to excellent osteogenic capacity, PDLSCs serve a crucial role in maintaining periodontal bone mass (33). However, the inflammatory microenvironment suppresses the osteogenic capacity of PDLSCs, which is also an important pathogenic feature of periodontitis (34). Therefore, it is critical to discover a novel drug that protects PDLSCs from

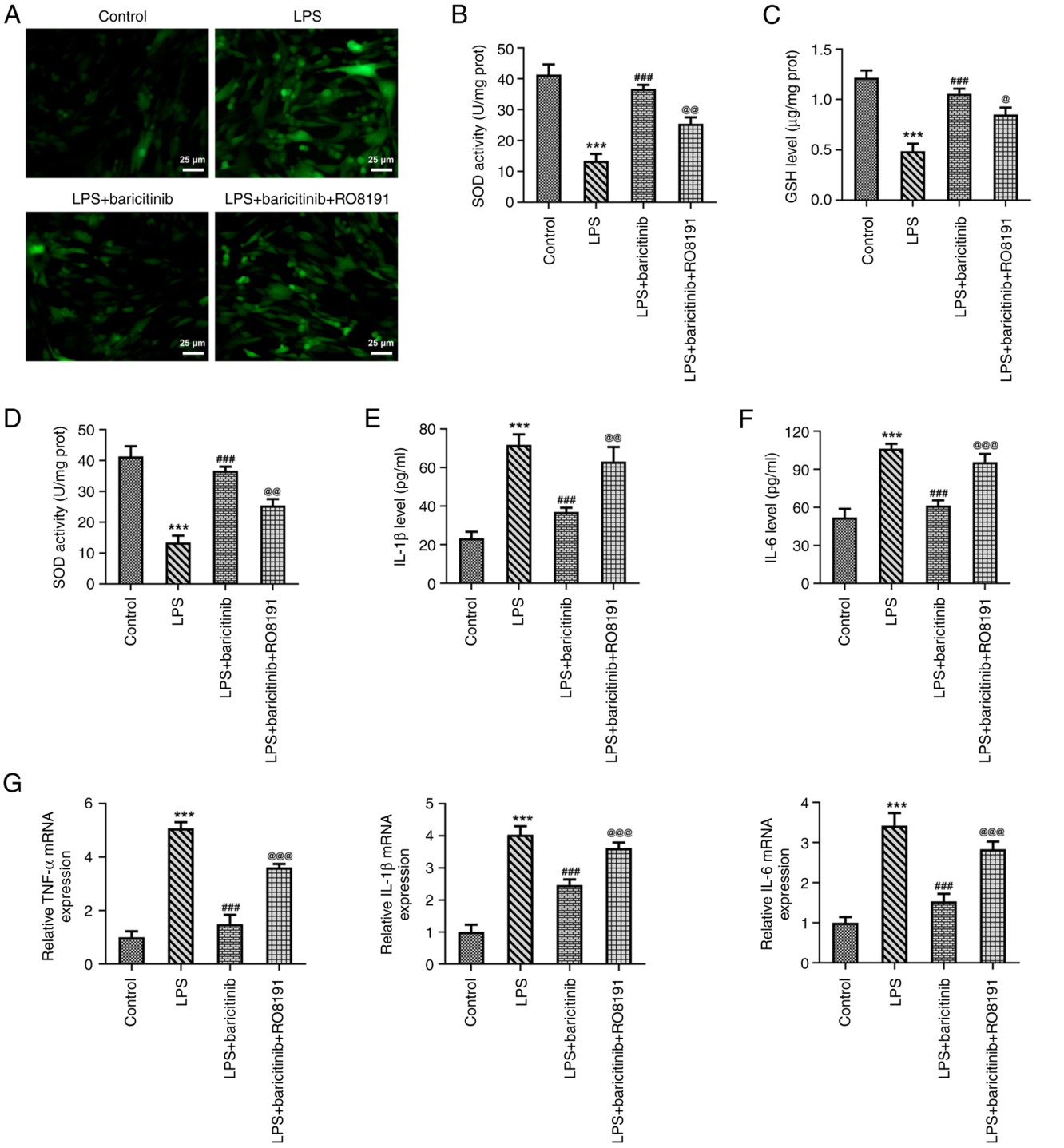

Figure 6. Baricitinib relieves LPS-induced oxidative stress and inflammation in periodontal ligament stem cells by inhibiting Janus kinase/signal transducer and activator of transcription signaling. (A) Levels of intracellular reactive oxygen species were evaluated using 2,7-dichlorodihydrofluorescein diacetate as a probe. (B) SOD activity levels and (C) GSH content were assessed by commercially available kits. ELISA kits were used for detection of (D) TNF-α, (E) IL-1β and (F) IL-6 levels. (G) Measurement of TNF-α, IL-1β and IL-6 mRNA expression by reverse transcription-quantitative PCR analysis. ***P<0.001 vs. control; $^{###}$P<0.001 vs. LPS; $^{@}$P<0.05, $^{@@}$P<0.01 and $^{@@@}$P<0.001 vs. LPS + baricitinib. LPS, lipopolysaccharide; SOD, superoxide dismutase; GSH, glutathione; prot, protein.

inflammation-associated injury and improves their functional characteristics. The present study demonstrated the effects of baricitinib on LPS-induced damage of PDLSCs and the decrease of osteogenic differentiation.

Periodontitis is an inflammatory disease mediated by immunization; LPS produced by dominant bacteria in gingival crevices serves an indispensable role in the occurrence and progression of this condition (35). Exposure to LPS leads to increased levels of certain inflammatory factors, including TNF-α, IL-1β and IL-6, in PDLSCs (36). Numerous studies have shown that LPS induces PDLSCs to produce and accumulate a large amount of ROS, which disrupts the balance between oxidative and antioxidant systems, resulting in cell and tissue damage (37,38). SOD and GSH are considered

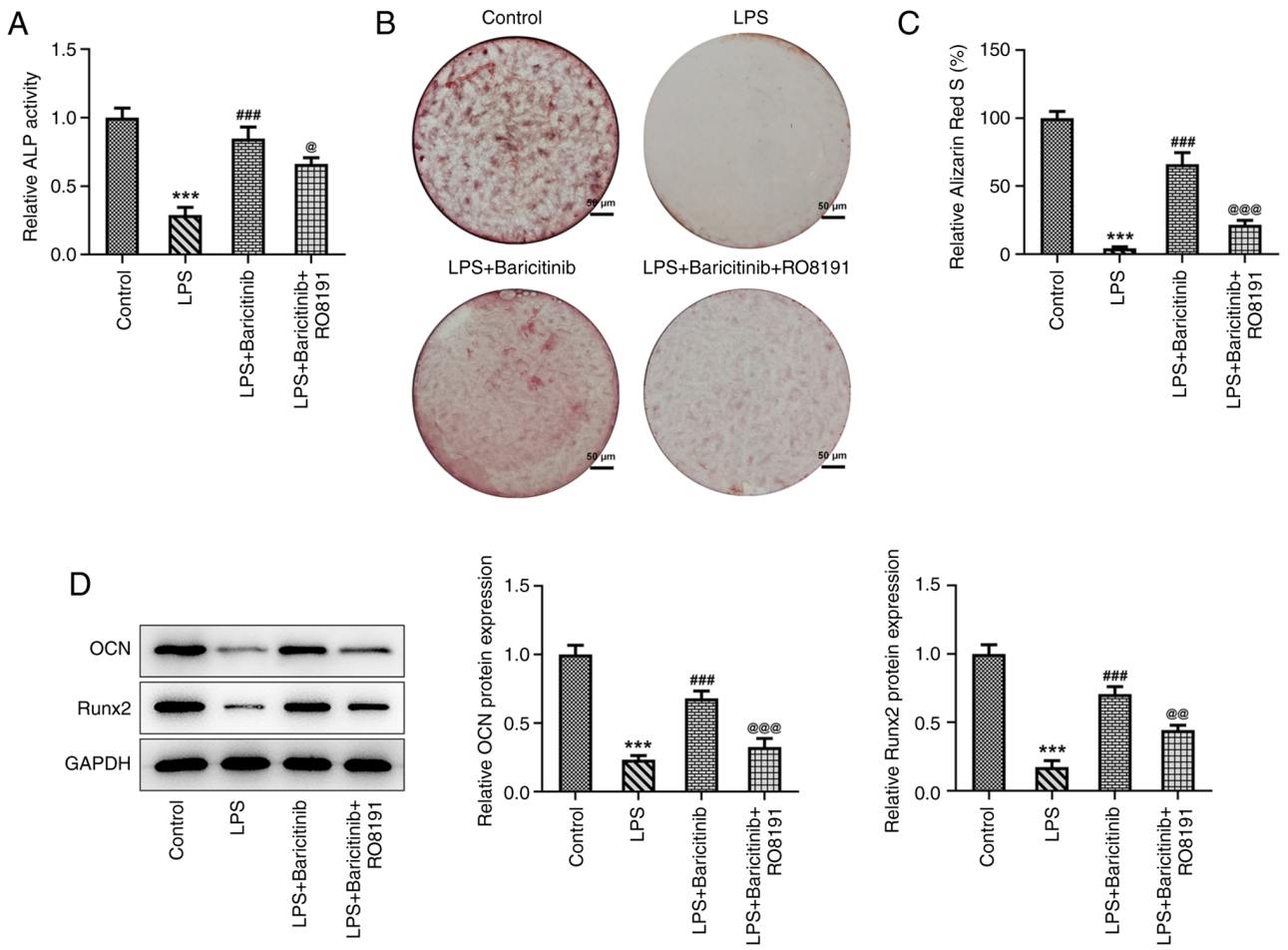

Figure 7. Baricitinib promotes osteogenic differentiation of PDLSCs challenged with LPS by inhibiting Janus kinase/signal transducer and activator of transcription signaling. (A) Evaluation of ALP activity using an ALP kit. (B) Analysis of (C) mineralization ability of PDLSCs using Alizarin red staining. (D) Determination of OCN and Runx2 expression by western blotting. ***P<0.001 vs. control; $^{###}$P<0.001 vs. LPS; $^{@}$P<0.05 vs. LPS + baricitinib. PDLSCs, periodontal ligament stem cells; LPS, lipopolysaccharide; ALP, alkaline phosphatase; OCN, osteocalcin; Runx2, Runt-related transcription factor 2; S, staining.

specific parameters of oxidative stress. A significant increase in the levels of ROS and expression levels of TNF-α, IL-1β and IL-6, and a decrease in the levels of SOD and GSH, were noted in PDLSCs following stimulation with LPS, which was consistent with previous studies (36-38). As a U.S Food and Drug Administration-approved oral JAK1/2 inhibitor, baricitinib has been used to treat moderate to extreme rheumatoid arthritis (39). A clinical study demonstrated that clinical manifestations and inflammatory biomarkers were notably improved in patients with autoinflammatory interferonopathy following baricitinib treatment (40). Baricitinib is reported to inhibit tetradecanoylphorbol-13-acetate-induced psoriasis and skin inflammation in a mouse model (41). Baricitinib improves the periodontal status of patients with rheumatoid arthritis and decreases the inflammatory response in the periodontal tissue (23). In the present study, the antioxidant and anti-inflammatory effects of baricitinib were verified in LPS-induced PDLSCs, suggesting the potential application of this compound in the treatment of periodontitis.

Subsequently, the effects of baricitinib on osteogenic differentiation of PDLSCs incubated with LPS were analyzed by evaluating the expression of several osteogenic differentiation markers and mineralization in PDLSCs. ALP is an early marker of osteogenic differentiation and a key hydrolase in cellular osteogenic differentiation (42). The increase in the activity of ALP reflects maturation of osteogenic differentiation (43). Alizarin red staining forms mineralized nodules through the specific binding of alizarin red S to calcium ions, which indicates mineralization capacity of cells (44). OCN, a non-collagen protein abundant in the bone tissue, is a specific indicator of bone metabolism and osteocyte activity; its expression levels reflect the rate of bone formation (45). Runx2 is a key gene of bone formation and an important indicator of osteogenic differentiation of mesenchymal stem cells (46). Baricitinib can increase bone mass in steady-state conditions and ameliorate pathological bone loss by stimulating osteoblast function (25). Murakami *et al* (24) demonstrated that baricitinib inhibits osteoclast formation *in vitro* by suppressing the expression of RANKL. The present results indicated that under the treatment of baricitinib, levels of ALP, Runx2 and OCN and mineralization degree were elevated, which confirmed that baricitinib promoted osteogenic differentiation of PDLSCs, highlighting its potential to treat periodontitis.

To investigate the underlying mechanism of baricitinib on the impact of LPS-stimulated PDLSCs, expression levels of proteins involved in the JAK/STAT pathway were assessed. A previous study revealed that *Porphyromonas gingivalis* activates the JAK/STAT signaling pathway and enhances

ROS accumulation, thereby promoting the occurrence and development of periodontitis (47). Downregulation of Toll-like receptor 4 expression and the JAK1/STAT3 signaling pathway can improve diabetic periodontitis in a mouse model (48). Zheng *et al* (26) revealed that inhibition of JAK/STAT signaling suppresses progression of chronic periodontitis. Here, upregulation of p-JAK1, JAK2 and STAT3 expression in PDLSCs induced by LPS was inhibited by baricitinib. Subsequent addition of RO8191, an agonist of the JAK/STAT pathway, abolished the protective effects of baricitinib on LPS-triggered oxidative stress, inflammation and loss of osteogenic differentiation, suggesting that this compound alleviated LPS-induced PDLSC injury and promoted osteogenic differentiation by inhibiting JAK/STAT signaling.

The present study demonstrated that baricitinib, with its antioxidant, anti-inflammatory and pro-osteogenic differentiation effects, may be a promising agent in protecting PDLSCs from periodontitis-induced injury. The present study may provide insight into the use of baricitinib for the treatment of periodontitis. Further *in vivo* animal studies will be performed in the future to confirm the effects of baricitinib on periodontitis.

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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

PY and FS designed the study. PY, FS and YZ performed the experiments and analyzed the data. PY drafted the manuscript and interpreted the data. YZ revised the manuscript for important intellectual content. All authors have read and approved the final manuscript. PY 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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