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Original Article

AR-A014418, a glycogen synthase kinase-3β inhibitor, mitigates lipopolysaccharide-induced inflammation in rat dental pulp stem cells via NLR family pyrin domain containing 3 inflammasome impairment

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KEYWORDS
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NLRP3 inflammasome

Abstract *Background/purpose:* Cell pyroptosis and gingival inflammation have been implicated in periodontitis progression. Our previous study revealed that AR-A014418, a pharmacological inhibitor of glycogen synthase kinase-3β (GSK-3β), can enhance the migratory and osteogenic differentiation abilities of rat dental pulp stem cells (rDPSCs). The present study aimed to explore the effect of AR on the inflammation of rDPSCs.

Materials and methods: The primary rDPSCs were isolated and identified by flow cytometry, as well as Oil red O and Alizarin Red S staining. The rDPSCs were cultured and exposed to lipopolysaccharide (LPS) before treating them with different concentrations of AR-A014418. The cell viability was detected using the CCK-8 assay. The generation and secretion of pro-inflammatory cytokines (IL-18, TNF-α, L-1β, and IL-6) were examined by qPCR and ELISA, respectively. To investigate the activation of the NLRP3 inflammasome, the expression levels of pro-caspase 1, cleaved caspase 1, as well as NLRP3 were analyzed by western blotting and immunofluorescence, respectively.

Results: In the rDPSCs, LPS prohibited cell viability and enhanced the generation and secretion of pro-inflammatory cytokines. LPS upregulated NLRP3 and cleaved caspase-1 protein levels and promoted ASC speck formation in the rDPSCs. AR-A014418 administration effectively blocked the LPS-induced inflammation of the rDPSCs in a dose-dependent way. Mechanistically, AR-A014418 significantly restrained the up-regulation of NLRP3 and cleaved caspase-1 in LPS-treated rDPSCs.

Conclusion: Collectively, our findings suggest that AR-A014418 significantly mitigates LPS-induced inflammation of rDPSCs by blocking the activation of the NLRP3 inflammasome.

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Introduction

As a widespread disease with chronic inflammation in the tooth-surrounding tissues, periodontitis mainly resulted from a mixed population of bacteria found in the oral biofilm.[1] Many periodontitis results in periodontium damage, which in turn causes the pathologic loss of teeth. Inflammation in the dental region is mainly related to increased microvascular density, and finally causes the activation of the stem cells that are in the cell-rich zone of the pulp to chemokine secretion.[2] It has been reported that the dental pulp recognizes molecular patterns involving chemokines and cytokines via cell surface receptors typical to microbes to trigger defense against bacterial aggression.[3] Moreover, increasing reports support that periodontitis is an important cause to aggravate inflammation, which is closely related to the development of cardiovascular diseases.[4] Basic therapy of periodontitis mainly focuses on inhibiting inflammation via the management of the oral biofilm.[5] However, there are still no clinically effective treatment methods specific to periodontitis. Therefore, it is an urgent need to develop therapeutic strategies that effectively improve disrupted periodontium.

It's widely accepted that mesenchymal stem cell populations contribute to cell self-renewal and respond to damage in diverse types of tissue.[6] The dental pulp stem cells (DPSCs) in dental pulp tissue are not only related to multipotential differentiation and self-renewal but also involved in pulp repair and regeneration by regulating the secretion of inflammation-related factors and inhibiting the immune response.[7,8] Dental caries and trauma usually lead to an immune response and the infiltration of inflammatory cells, rendering to the injury of the dental pulp tissue. The balance between regeneration and inflammation is a determinant factor for the consequence of pulp injury.[9] In general, moderate inflammation is able to facilitate tissue regeneration by inducing the proliferation, differentiation, as well as migration of DPSCs, while prolonged or excess inflammation usually causes destructive effects on vital pulp and ultimately results in the necrosis of total tissue.[10] Hence, during the treatment of periodontitis, in order to preserve the remaining vital pulp and repair the damaged pulp, it's important to enhance the self-repair capacity of the dental pulp and resolve the inflammatory response in time.

Over the past decade, a type of large multimolecular complex defined as "inflammasome" has been proven to be critical in the innate immune response during its early stages.[11] Among multiple inflammasomes, the NLRP3 inflammasome has been reported to participate in the innate immune response[12] of various types of inflammatory disorders, such as periodontitis.[13] During subclinical stages of periodontitis, NLRP3 has been revealed as an independent predictor of disease risk in several reports,[14,15] highlighting the important role of NLRP3 inflammasome activation in periodontitis. Moreover, several studies revealed that NLRP3 inflammasome may represent a promising target for periodontitis treatment.[16,17]

It has been reported that glycogen synthase kinase-3β (GSK-3β) participates in several physiological processes including inflammation and bone homeostasis as a negative regulator,[18,19] such as periodontitis, which hinited that inhibitors of GSK3β has a potential of exerting therapeutic effects for periodontitis.[20] Our recent study has shown that AR-A014418, a GSK3β inhibitor, could promote migration and osteogenic differentiation of rat DPSCs (rDPSCs).[21] However, whether it could mitigate the inflammation of DPSCs remains unknown.

In this study, lipopolysaccharide (LPS) was utilized to induce the inflammatory response of rDPSCs to mimic periodontitis *in vitro*. Based on this, the effects and underlying mechanisms of AR-A014418 on the inflammation of rDPSCs were explored.

Materials and methods

Isolation and identification of rDPSCs

Three months old Sprague-Dawley rats with an SPF grade supplied by Experimental Animal Center of Fujian Medical University (Fuzhou, China) were exploited to harvest the dental pulp of incisors for rDPSCs isolation, as previously described.[21] The obtained rDPSCs were maintained in DMEM containing 10% FBS and 1% penicillin/streptomycin and cultured in a 5% CO_2 incubator at 37 °C. The rDPSCs at the third passage were subjected to identification by identifying the cell surface markers and investigating the multidirectional differentiation of rDPSCs. All procedure regarding rats was approved by the Animal Care and Use Committee of our hospital.

Osteogenic and adipogenic differentiation

The rDPSCs with a density of 5×10^4 were subjected to osteogenic and adipogenic differentiations by respectively maintaining in osteogenic and adipogenic media for 14 days. During the cultivation, the media were renewed three times every week.

Alizarin red S (ARS) staining

To investigate the osteogenic differentiation of rDPSCs based on calcium-rich deposits assessment, ARS staining was carried out after 14 days of osteogenic induction. Briefly, after finishing osteogenic induction, rDPSCs were subjected to 15 min fixation with 4% paraformaldehyde (PFA) and subsequent washing with PBS. Next, the fixed rDPSCs were incubated with

2% ARS solution for 15 min. Finally, the stained rDPSCs were observed with a microscope.

Oil Red O staining

Oil Red O is a fat-soluble diazo dye that can stain lipid droplets in cells, which is often exploited to verify the adipogenic differentiation of DPSCs. In brief, rDPSCs were stained with Oil Red O solution in isopropanol for 15 min after fixation with 4% PFA. Afterward, the stained rDPSCs were observed with a microscope.

Flow cytometry

200 mL cell suspension of passage 3 DPSCs (1×10^6 cells/mL) was then stained with polyethyleneglycol (PE)-conjugated antibodies to CD34, CD45, CD90, and CD29 for 15 min in the dark. After removing the unbound antibodies, 0.01 M PBS containing 0.5% PFA was added for preparing cell resuspension prior to detection by flow cytometry.

3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay

rDPSCs were maintained in media that supplemented with LPS or/and a series of concentrations (1, 2.5, 5, 10, and 20 μM) AR-A014418 (Sigma-Aldrich; A3230) for 48 h. Next, 10% of MTT (v/v) was added to each well for additional 4 h cultivation. Finally, the absorbance was measured under a microplate reader (Bio-Rad, Hercules, CA, USA) at 450 nm.

Annexin-V/propidium iodide double staining

rDPSCs from each group were collected and washed twice with PBS, and then 5 μL of Annexin-V dye solution and 10 μL of propidium iodide dye solution (Sigma-Aldrich, Burlington,

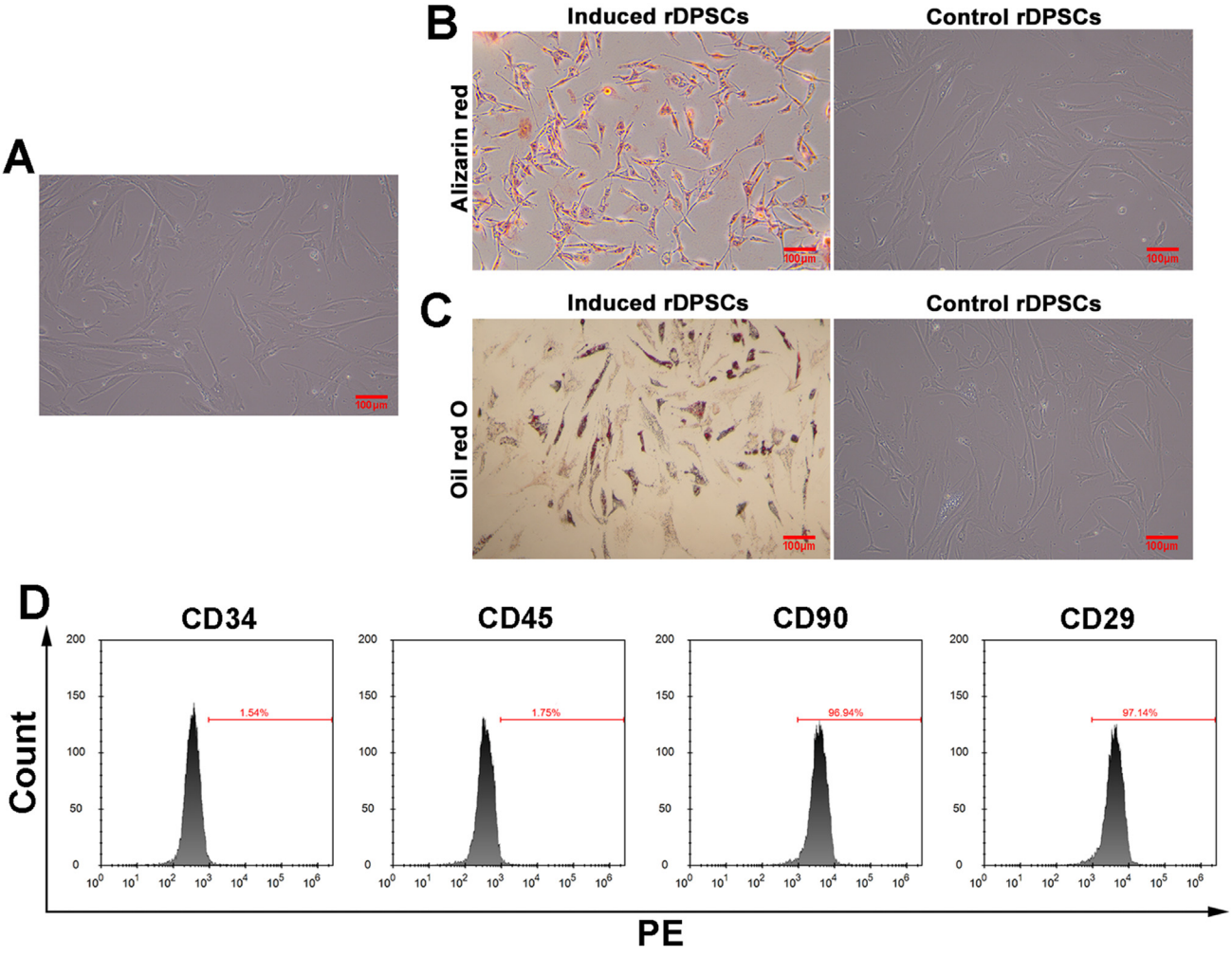

Figure 1 The characterization of rDPSCs. (A) Morphology of rDPSCs (Scale bar: 100 μm). (B) Alizarin red staining of rDPSCs with (left) or without (right) induction of osteogenic differentiation (Scale bar: 100 μm). (C) Oil red O staining of rDPSCs with (left) or without (right) induction of adipogenic differentiation (Scale bar: 100 μm). (D) Flow cytometry identified the expression of stem cell markers (CD34, CD45, CD 90, and CD29) in rDPSCs.

MA, USA) were added to stain for 30 min in the dark at 4 °C. The apoptosis rate was checked by using flow cytometry.

Reverse transcription polymerase chain reaction (RT-PCR)

By using TRIzol reagent (Life Technologies, Carlsbad, CA, USA), total RNA was extracted from rDPSCs and subsequently subjected to RT-PCR analysis with the following primer sequences: TNF-α (F: 5′-ATGGGCTCCCTCTCATCAGT-3′, R: 5′-GCTTGGTGGTTTGCTACGAC-3′), IL-6 (F: 5′-AGAGACTTCCAG CCAGTTGC-3′, R: 5′-TGCCATTGCACAACTCTTTTC-3′), CXCL1 (F: 5′-CCCAAACCGAAGTCATAGCCA-3′, R: 5′-ACGCCATCGGTG CAATCTAT-3′), IL-1β (F: 5′-TGGCAACTGTCCCTGAACTC-3′, R: 5′- AGGGCTTGGAAGCAATCCTTA-3′), NLRP3 (F: 5′-GGGACT-CAAGCTCCTCTGTG-3′, R: 5′-GAGGCTCTGGTTATGGGTCA-3′), Caspase 1 (F: 5′-AAGGTGGCGCATTTCCTGGAC-3′, R: 5′-GGGCACTTCAATGTGTTCATC-3′), GADPH (F: 5′-CTCTCTG CTCCTCCCTGTTC -3′, R: 5′-CGATACGGCCAAATCCGTTC-3′). Then, on a 7500 Real-Time PCR System, the quantification was conducted using the QuantiTect SYBR Green PCR Kit (Toyobo, Osaka, Japan), which selected GAPDH as an internal control for normalization.

Western blot

Total protein extracted from rDPSCs using RIPA buffer (Abcam, Boston, MA, USA) on ice was separated by SDS-PAGE gels and electrophoretically transferred onto PVDF membranes. Afterward, the membranes were subjected to blocking with 12% of skimmed milk for 2 h, followed by incubation overnight at 4 °C with primary antibodies: *p*-GSK3β (44-604G, ThermoFisher, Waltham, MA, USA), NLRP3 (PA5-79740, ThermoFisher), Pro caspase 1 (ab179515, Abcam), Caspase 1 (PA5-87536, ThermoFisher), GAPDH (AF1186, Beyotime, Nantong, China). Subsequently, membranes were rinsed three times prior to incubation with

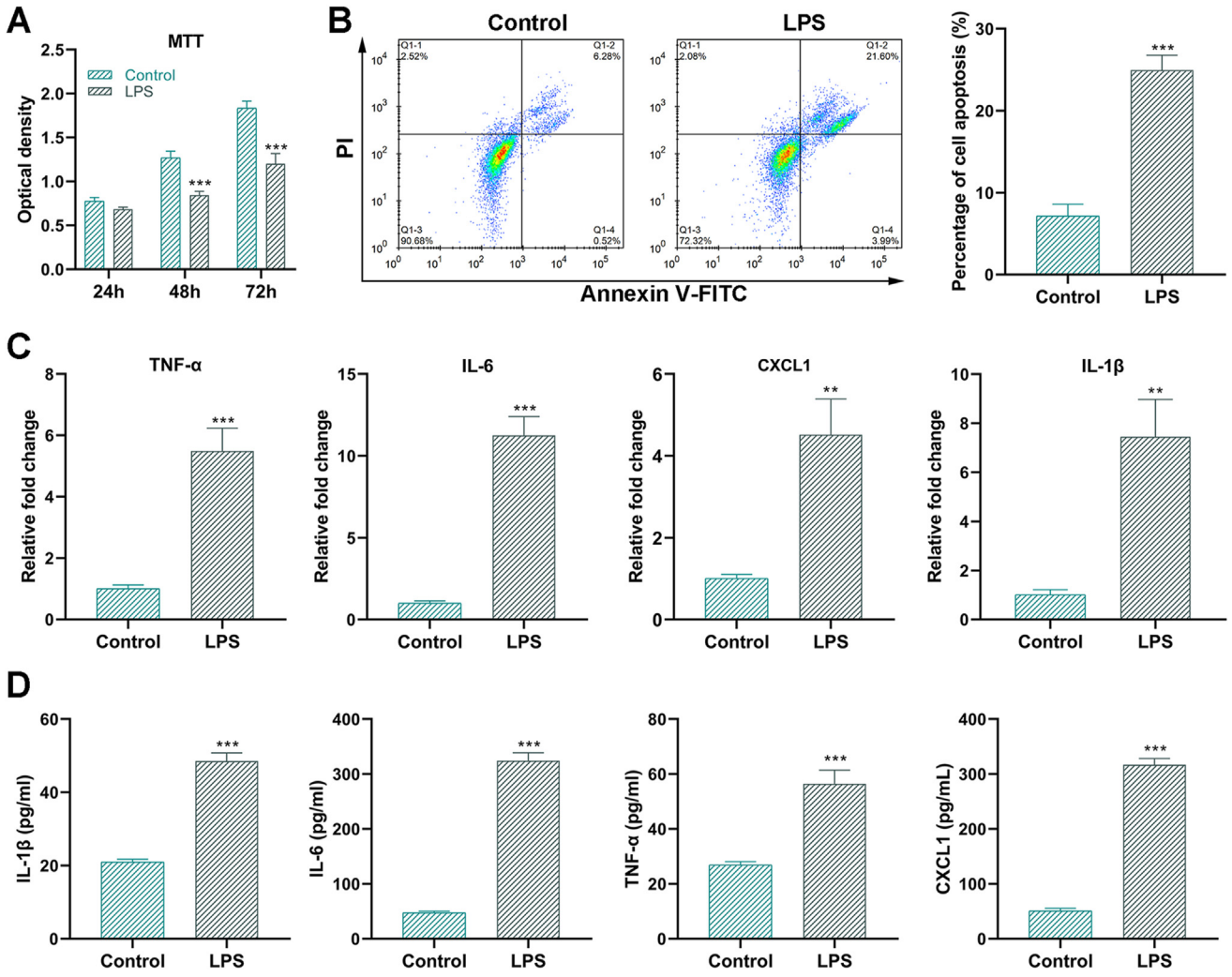

Figure 2 LPS stimulation caused an impaired cell viability, apoptosis, and inflammation in rDPSCs. (A) MTT assay detected the cell viability of rDPSCs cultured under LPS for 24, 48, and 72 h. (B) Flow cytometry detected cell apoptosis based on Annexin V-FITC/PI double staining. (C) RT-PCR analyzed the transcriptional levels of pro-inflammatory factors (TNF-α, IL-6, CXCL1 and IL-1β) in rDPSCs. (D) Detection of pro-inflammatory factors (TNF-α, IL-6, CXCL1 and IL-1β) in the supernatant of rDPSCs by ELISA. $^{**}P < 0.01$ and $^{***}P < 0.001$ versus the control group.

HRP-labeled secondary antibody (A0208, Beyotime). Finally, by using ECL Substrate Kit (Abcam) and Image J software (Bethesda, MD, USA), protein bands were visualized and quantified, respectively.

Enzyme-linked immunosorbent assay (ELISA)

The supernatant of rDPSCs from each group was collected. The levels of TNF-α (ER1393, Wuhan Fine Biotech, Wuhan, China), IL-6 (MBS269892, MyBioSource, San Diego, CA, USA), IL-1β (MBS2023030, Mybiosource), and CXCL1 (ER1014, Wuhan Fine Biotech) were measured by ELISA, and the experimental procedures were strictly performed according to the kit instructions.

Immunofluorescence staining

The treated rDPSCs were washed twice with PBS when reaching approximately 60% confluence. After 4% PFA of fixation for 10 min and 0.1% Triton-X 100 of permeabilization for 15 min at room temperature, the rDPSCs were rinsed for subsequent blocking with BSA for 30 min. Afterward, rDPSCs were cultivated with the primary antibody against NLRP3 (PA5-79740, ThermoFisher) overnight at 4 °C,

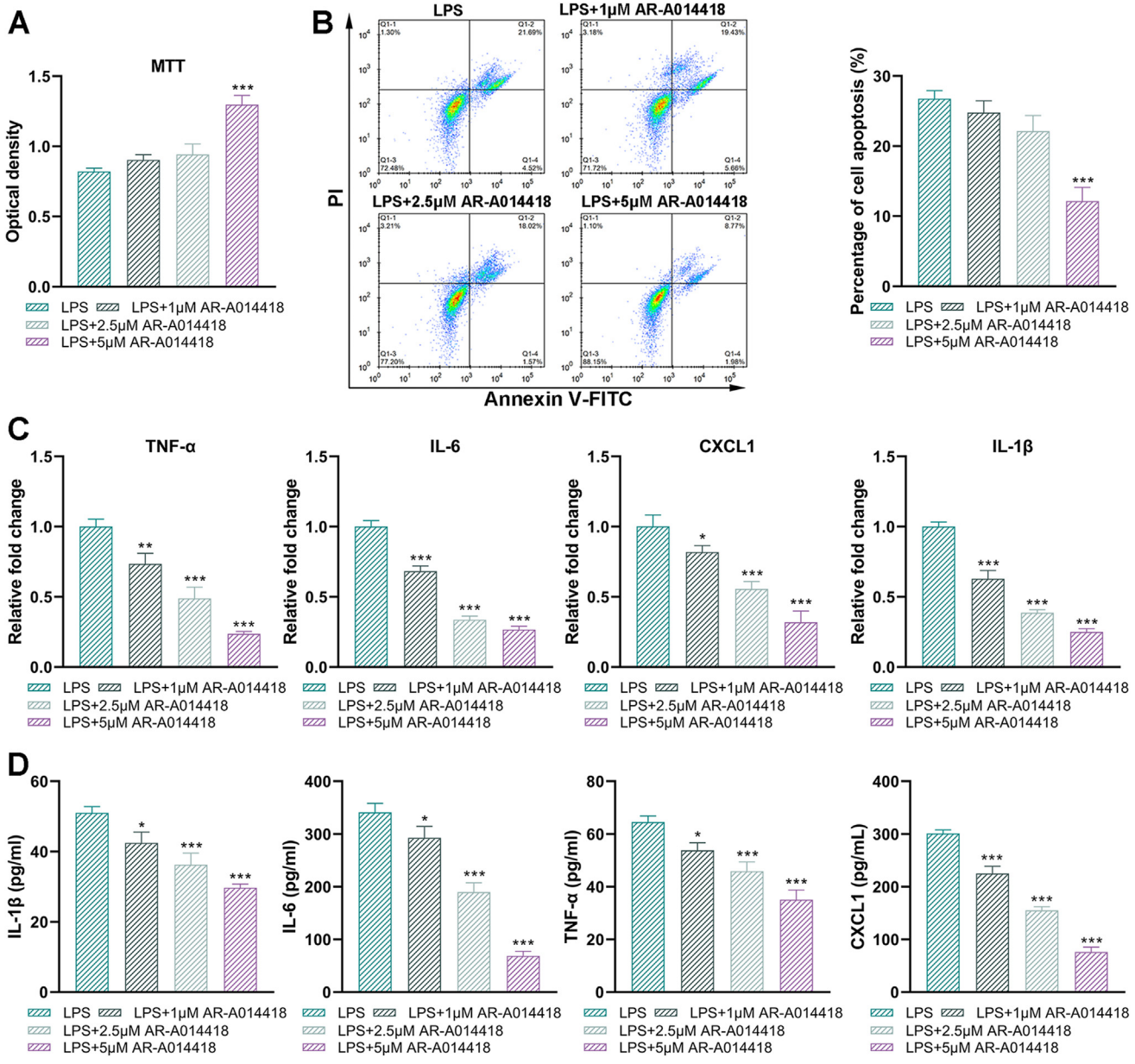

Figure 3 AR-A014418 attenuated LPS-induced inflammation in rDPSCs. rDPSCs were treated with LPS for 48 h, and given a series of concentrations (0, 1, 2.5, and 5 μM) of AR-A014418. (A) MTT assay detected the cell viability of rDPSCs. (B) Flow cytometry detected cell apoptosis based on Annexin V-FITC/PI double staining. (C) RT-PCR analyzed the transcriptional levels of pro-inflammatory factors (TNF-α, IL-6, CXCL1 and IL-1β) in rDPSCs. (D) Detection of pro-inflammatory factors (TNF-α, IL-6, CXCL1 and IL-1β) in the supernatant of rDPSCs by ELISA. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ versus the LPS group.

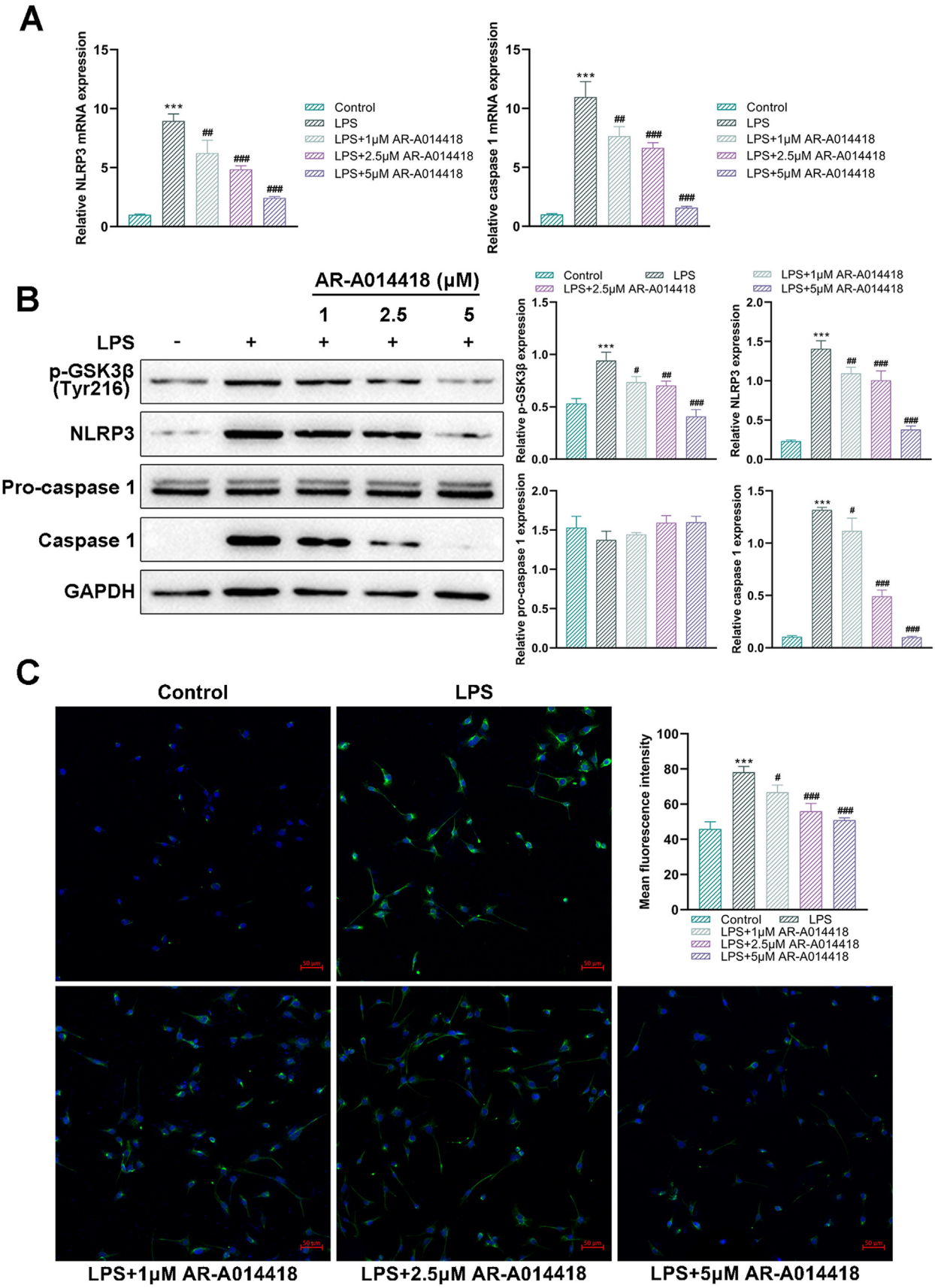

Figure 4 AR-A014418 suppressed NLRP3 inflammasome activation in LPS-induced rDPSCs. rDPSCs were treated with LPS for 48 h, and given a series of concentrations (0, 1, 2.5, and 5 μM) of AR-A014418; rDPSCs cultured for 48 h without LPS and AR-A014418

followed by incubation with Dylight 488-labeled secondary antibody (35,552, ThermoFisher) for 1 h. Finally, the stained cells were observed using confocal fluorescence microscopy (Leica microsystems, IL, USA) after counterstaining nuclei with DAPI (Beyotime) for 10 min.

Statistical analysis

All data were expressed as mean ± standard deviation (SD). The Student's t-test was used for comparison between the two groups. For multiple-group conditions, one-way ANOVA was performed with Bonferroni's method. $P < 0.05$ were considered statistically significant.

Results

Successful isolation of rDPSCs

The rDPSCs extracted from the incisors of rats were observed under a microscope, of which morphology was shown as Fig. 1A. ARS staining and Oil red O staining respectively verified the abilities of rDPSCs in osteogenic and adipogenic differentiations (Fig. 1B and C). Moreover, surface antigens of rDPSCs were characterized using FCM. (Fig. 1D). FCM results displayed that a high percentage of rDPSCs expressed and CD 90 (96.94%) and CD29 (97.14%), while only a few rDPSCs expressed CD34 (1.54%) and CD45 (1.75%) antigens. Colletively, the rDPSCs were successfully isolated.

LPS stimulation caused an impaired cell viability, apoptosis, and inflammation in rDPSCs

To verify the effects of LPS on the cell viability, apoptosis, and inflammation in rDPSCs, MTT, Annexin V-FITC/PI staining, PCR, as well as ELISA were carried out after exposing the rDPSCs to 10 μg/mL of LPS. The results showed that the cell viability of rDPSCs was profoundly suppressed after exposing LPS 48 and 72 h (Fig. 2A). 48 h was selected as the treatment time for subsequent experiments. The increase in apoptosis was also observed after LPS induction (Fig. 2B). The transcription levels of TNF-α, IL-6, IL-1β, and CXCL1 were significantly elevated by LPS exposure (Fig. 2C). Next, whether the rDPSCs released such pro-inflammatory cytokines in response to LPS was investigated using ELISA. The results showed that the secretion of TNF-α, IL-6, IL-1β, and CXCL1 was markedly increased after exposure to LPS (Fig. 2D).

AR-A014418 attenuated LPS-induced inflammation in rDPSCs

Next, LPS-treated rDPSCs were exposed to a series of (0, 1, 2.5, and 5 μM) AR-A014418. MTT and FCM results showed that 1 and 2.5 μM of AR-A014418 did not affect cell viability and apoptosis (Fig. 3A and B), while 5 μM of AR-A014418 significantly attenuated LPS-induced injury in rDPSCs. For inflammation, incubating AR-A014418 with LPS-induced rDPSCs significantly and dose-dependently reduced the expression of pro-inflammatory cytokines, including TNF-α, IL-6, CXCL1, and IL-1β, at both transcription and secretion levels (Fig. 3C and D). As a whole, these data implied that AR-A014418 could attenuate LPS-induced inflammation in rDPSCs, and block LPS-induced injury when the concentration reached 5 μM.

AR-A014418 suppressed NLRP3 inflammasome activation in LPS-induced rDPSCs

In order to uncover the mechanism behind the anti-inflammatory property of AR-A014418, we investigated the activation of NLRP3 inflammasome in LPS-induced rDPSCs. As illustrated in Fig. 4A, the LPS stimulation remarkedly elevated the transcription of NLRP3 and Caspase-1. In the meantime, protein levels of NLRP3 and Cleaved caspase-1 were also induced by LPS (Fig. 4B). As expected, the administration of AR-A014418 evidently decreased the phosphorylation of GSK3β at Tyr216, which is in a dose-dependent manner. In the meantime, the elevation of NLRP3 and Caspase-1 mRNA and the increased protein levels of NLRP3 and Cleaved caspase-1 induced by LPS in rDPSCs were significantly reversed by AR-A014418 treatment, while the concentration of 5 μM exhibited a potent effect that totally blocked the role of LPS in NLRP3 inflammasome activation of rDPSCs (Fig. 4A and B). Moreover, this finding was corroborated by the result of immunofluorescence staining for NLRP3 (Fig. 4C). Collectively, AR-A014418 suppressed the inflammatory response in LPS-induced rDPSCs via the GSK3β/NLRP3 inflammasome pathway.

Discussion

To maintain oral health and even prevent systemic diseases, it's of significance to search for effective periodontitis intervention. As an endotoxin stimulus, LPS is usually utilized to construct both the *in vivo* and *in vitro* periodontitis models.[22,23] It's believable that LPS can damage the periodontal ligament which is critical for the connection of teeth and gingiva.[24] More importantly, LPS not only leads to direct periodontal tissue injury by inducing the secretion of matrix metalloproteinases and excessive inflammation,[25] but also causes worsening gingival tissue destruction and even alveolar bone resorption.[26] The inflammation in periodontal tissue stimulates gingival fibroblasts via the activation of TNF-α and IL-1β and facilitates bone resumption. In our study, we found that LPS also induced the inflammation of rDPSCs, as revealed by the significant increase in the secretion of pro-inflammatory cytokines, such as IL-1β and TNF-α. A previous publication

considered as the control. (A) RT-PCR analyzed the transcriptional levels of NLRP3 and Caspase 1. (B) Western blot was performed to assess the expression levels of *p*-GSK3β, NLRP3, Pro caspase 1, and Caspase 1. (C) Immunofluorescence staining for NLRP3 in rDPSCs (Scale bar:100 μm). $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ versus the control group; $\#P < 0.05$ and $\#\#\#P < 0.001$ versus the LPS group.

reported that TNF-α can not only activate immune cells but also enhance osteoclast differentiation.[27] In addition, IL-1β at high concentration results in periodontal histopathology lesions as well as the increase of alveolar bone loss.[28]

In response to periodontal infection, inflammation was induced. Subsequently, it caused alveolar bone loss, which has considered to be one of the critical points of clinical management for patients with periodontal disease. Simultaneous anti-inflammatory and osteogenic interventions have long been essential to delay the progression of periodontitis.[29] Over the last few decades, increasing evidence supports GSK3 as an important player during the regulation of innate and adaptive immune cells for secretion of pro- and anti-inflammatory cytokines.[18] As one of the downstream effectors of the PI3K pathway, GSK3 is involved in the production of pro-inflammatory cytokines, such as including IL-6, TNF-α, and IL-1β, known to stimulate alveolar bone loss.[30] In addition, GSK3 also acts as a cytosolic Wnt signaling inhibitor, which is capable of inducing β-catenin degradation, rendering to the repression of osteoblast differentiation.[31] Multiple previous studies revealed that GSK3 is stimulated by LPS in diverse disease models, such as acute lung injury, cardiac dysfunction, as well as periodontitis.[32–34] Therefore, we supposed that GSK3 could be a promising potential therapeutic target for the treatment of periodontal disease.

In the past decades, a number of small molecule GSK3 inhibitors have been identified; however, more than half of the published inhibitors not only target GSK3, but also affect cyclin-dependent kinase 2 (cdk2) or cdk5.[35,36] The 6-bromoindirubin-3′-oxime, a pharmacological inhibitor of GSK3, has been reported to attenuate diverse inflammatory diseases via the positive regulation of inflammatory factors. AR-A014418, an amino thiazole, was identified in a high-throughput biochemical screening by Bhat et al., which can inhibit GSK3β activity in an ATP-competitive way, with high specificity for GSK3β as it does not affect the activity of other 26 protein kinases, especially cdk2 and cdk5 that are inhibited by the majority of GSK3 inhibitors.[37] Besides, Several studies reported that AR had potent activities against diverse types of cancer with no adverse effects in rodents.[38,39] More importantly, we previously reported that AR-A014418 can promote osteogenic differentiation and migration of rDPSCs by regulating the β-catenin/PI3K/Akt signaling.[21] In this study, our results indicated that AR-A014418 could attenuate LPS-induced inflammation in rDPSCs by decreasing the levels of TNF-α and IL-1β.

As for the perspective of cell viability, it was investigated that the viability was obviously impaired in LPS-treated rDPSCs at 48 h and 72 h, in comparison to non-treated cells. Apoptosis is a distinctive mechanism of active cellular death and plays a key role in the pathological development of various types of disorders. Our data showed that the apoptosis of rDPSCs was significantly increased after LPS stimulation, which confirmed that the constant stimulation of LPS could cause rDPSC injury. In addition, LPS stimulation also elevated the transcription and secretion of cytokines (TNF-α, IL-6, CXCL1, and IL-1β) of rDPSCs. Of the cytokines mentioned, TNF-α is capable of directly inducing cellular apoptosis through a well-characterized signaling pathway.[40] More recently, several studies have shown that elevated proinflammatory cytokine expression and their synergistic activities are involved in pathogenesis in several clinical and inflammatory disorders.[41,42] In this study, we found that AR-A014418 reversed the LPS-induced up-regulation of cytokines in rDPSCs with a dose-dependent way. However, only the concentration reached 5 μM, AR-A014418 exerted an obvious protective effect against injury induced by LPS on rDPSCs.

Many times, multiple stimuli, such as damage-associated and microbial-associated molecular patterns, can activate inflammasomes to enhance the maturity and release of IL-18 and IL-1β, thereby stimulating inflammatory response. The NLRP3 inflammasome has been established as a pivotal player in maintaining chronic inflammation.[43] As known, the activation of NLRP3 inflammasome involves two steps process, including priming and activation signaling. Priming is a security component related to transcriptional and posttranslational priming mechanisms, which guarantees an appropriate and timely inflammatory reaction.[44] NLRP3 oligomerizes and interacts with ASC to form the inflammasome which consequently offers a platform for caspase-1 activation, thereby cleaving pro-IL-1β and pro-IL-18 into IL-1β and IL-18.[45] LPS stimulated TLR4 is the most common stimulus controlling productions of pro-IL-1β and pro-IL-18.[46] Through activating NF-κB, an essential inflammation transcription factor, TLR4 and inflammatory cytokine may significantly enhance NLRP3 expression.[47] Both IL-1β and IL-18 have been observed in the gingival crevicular fluid collected from patients with periodontitis; moreover, the up-regulation of NLRP3 in gingival tissue was also investigated.[48] It has been reported that porphyromonas gingivalis caused the cleavage of caspase-1 to exaggerate periodontitis.[49] In this study, LPS stimulation not only caused the increase in IL-1β production but also the activation of caspase-1 in rDPSCs, suggesting the NLRP3 inflammasome was activated in LPS-induced rDPSCs. A previous study showed that blocking the NLRP3 inflammasome in the experimental periodontitis model induced by porphyromonas gingivalis led to the suppression of pro-inflammatory cytokine as well as alveolar bone resorption.[50] Considering that GSK3 has been considered an important molecule in host inflammatory response, it's reasonable that GSK3 inhibition has the capacity of suppressing NLRP3 inflammasome activation. As expected, our data showed that AR-A014418 could reduce the transcription and secretion of IL-18, TNF-α, L-1β, and IL-6, as well as the cleavage of caspase-1 by inhibiting the expression of NLRP3 *in vitro*.

In conclusion, the current study demonstrated that AR-A014418 attenuated LPS-induced inflammation in rDPSCs via the NLRP3 inflammasome pathway. Further research is warranted before clinical application.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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