Infection and Drug Resistance

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ORIGINAL RESEARCH

18β-Glycyrrhetinic Acid Alleviates *P. multocida*-Induced Vascular Endothelial Inflammation by PARP1-Mediated NF-κB and HMGB1 Signalling Suppression in PIEC Cells

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Background: At present, the treatment and prevention of *Pasteurella multocida* infections in pigs mainly rely on antibiotics and vaccines, but inflammatory injury cannot be eliminated. The compound 18β-glycyrrhetinic acid (GA), a pentacyclic triterpenoid extracted from *Glycyrrhiza glabra L*. root (liquorice) and with a chemical structure similar to that of steroidal hormones, has become a research focus because of its anti-inflammatory, antiulcer, antimicrobial, antioxidant, immunomodulatory, hepatoprotective and neuroprotective effects, but its potential for the treatment of vascular endothelial inflammatory injury by *P. multocida* infections has not been evaluated. This study aimed to investigate the effects and mechanisms of GA intervention in the treatment of vascular endothelial inflammatory injury by *P. multocida* infections.

Materials and Methods: Putative targets of GA intervention in the treatment of vascular endothelial inflammatory injury by *P. multocida* infections were identified using network pharmacological screening and molecular docking simulation. The cell viability of PIEC cells was investigated via the CCK-8 assay. The mechanism of GA intervention in the treatment of vascular endothelial inflammatory injury by *P. multocida* infections were investigated using cell transfection and western blot.

Results: Through network pharmacological screening and molecular docking simulation, this study found that PARP1 may be a core target for GA to exert anti-inflammatory effects. Mechanistically, GA alleviates *P. multocida*-induced vascular endothelial inflammation by PARP1-mediated NF-κB and HMGB1 signalling suppression.

Conclusion: These findings, for the first time, demonstrate the potential therapeutic relationship among GA, PARP1 and inflammatory injury, providing a candidate drug, therapeutic targets and explanation for treating vascular endothelial inflammatory injury caused by *P. multocida* infection.

Keywords: network pharmacology, GA, PARP1, inflammatory injury, *P. multocida* infection

Introduction

Pasteurella multocida is a zoonotic pathogen with the ability to infect a wide range of hosts including humans as well as domestic, wild and companion animals.[1,2] Infection with *P. multocida* is responsible for significant morbidity and mortality in humans and animals.[1,3] The pathogen is one of the culprits causing major economic losses in the pig industry worldwide because of its persistently high prevalence: 8% prevalence with pneumonia or progressive atrophic rhinitis (PAR) between 2003 and 2007 in China,[4] 3.4% prevalence of 44,175 samples between 2013 and 2017 in China,[5] 15.6% prevalence of 33,813 isolated swine respiratory cases between 2003 and 2010 in the United States,[6] 15.8% prevalence between 2009 and 2010 in South Korea,[7] 16.8%

Received: 12 April 2023
Accepted: 21 June 2023
Published: 29 June 2023

prevalence between 2008 and 2016 in Korea.[8] In clinically diseased pigs, most *P. multocida* strains are characterised as capsular type A and D, causing respiratory disorder in pigs, such as PAR, pneumonia, pleurisy and septicaemia.[9–11] The haemorrhagic pneumonia caused by *P. multocida* infection suggests severe injury to the vascular endothelial cells at the site of pathogenesis and is accompanied by increased vascular endothelial permeability. At present, clinical prevention and treatment of *P. multocida* infection are mainly based on the application of antimicrobials and vaccines,[9,12] which do not eliminate the vascular endothelial inflammation injury caused by *P. multocida* infection but also cause the development of drug resistance. In addition, antimicrobials, glucocorticoids or non-steroidal anti-inflammatory drugs often cause adverse effects such as bacterial endotoxin release, immune suppression or gastric ulcers. Therefore, elucidating the molecular mechanisms of vascular endothelial inflammation injury caused by *P. multocida* infection and developing adjuvant therapies or effective drugs to control inflammation *P. multocida* infection are of great scientific interest and application to reduce the harm of *P. multocida* infection.

Poly (ADP-ribose) polymerases-1 (PARP1) is a direct predictor of inflammatory diseases, and its central role in inflammation is the regulation of nuclear factor-kappa B (NF-κB) activation and high mobility group box 1 (HMGB1) secretion.[13,14] The PARP1 itself promotes the transcription of NF-κB-dependent genes of inflammatory cytokines, such as interleukins 6 and 1β (IL-6, IL-1β) and tumour necrosis factor α (TNF-α).[15] Moreover, in in vivo models, PARP1 is involved in the regulation of inflammation in bacterial infections. In a model of *S. typhimurium*-induced murine colitis, loss of PARP1 reduced NF-κB-mediated proinflammatory gene expression, contributing to the alleviation of intestinal inflammation.[16] The PARP1 mediates bacterial endotoxin-induced HMGB1 release from macrophages, and PARP1-dependent translocation of HMGB1 from the nucleus to the cytoplasm can increase the activation of cellular inflammatory pathways.[14] However, the effect of *P. multocida* infection on PARP1 expression and whether PARP1 alleviates *P. multocida*-induced vascular endothelial inflammatory injury by regulating NF-κB and HMGB1 remain unclear.

According to traditional medicine, *Glycyrrhiza glabra L*. root (liquorice) is one of the oldest and most widely used herbs in the world and applied to moisten the lung and relieve cough and toxicity, dispel phlegm, promote meridians and harmonise the nature of other herbal medicines.[17] The compound 18β-glycyrrhetinic acid (GA), a pentacyclic triterpenoid extracted from liquorice and with a chemical structure similar to that of steroidal hormones, has become a research focus because of its anti-inflammatory, antiulcer, antimicrobial, antioxidant, immunomodulatory, hepatoprotective and neuroprotective effects.[18] In a previous study, GA inhibited *Staphylococcus aureus*-induced RAW264.7 cell inflammation by inhibiting the activation of NF-κB and the expression of HMGB1;[19] in addition, it has a protective effect on *Helicobacter pylori*-infected gastric mucosa through alleviating the expression levels of inflammation-related cytokines (IL-1β, TNF-α).[20] However, the protective role of GA against *P. multocida*-induced vascular endothelial inflammatory injury via PARP1 is still largely unclear.

In this study, drug target prediction and experimental validation were used to explore the mechanism by which GA alleviates vascular endothelial inflammatory injury caused by *P. multocida* infection through regulating the PARP1-NF-κB/HMGB1 pathway, with the aims to prove that GA has potential to serve as a novel antibiotic alternative and to provide a basis for clinically alleviating vascular endothelial inflammatory injury in *P. multocida*-infected piglets.

Materials and Method

Reagent and Chemicals

The GA (CAS NO. 471-53-4; Catalog # HY-N0180) was purchased from MedChemExpress, USA.

Network Pharmacological Analysis

The potential protein target of GA was identified by the drug target database, such as PharmMapper,[21] SwissTargetPrediction[22] and TCMSP.[23] The targets of vascular endothelial injury were obtained from the PubMed database (https://pubmed.ncbi.nlm.nih.gov) and the GeneCards database (https://www.genecards.org). The Veen software (www.bioinformatics.com.cn) was employed to select the common target of the potential protein target of GA and the targets of vascular endothelial injury in pigs. The core target was selected by the protein-protein interaction (PPI) network of the String database (https://cn.string-db.org), using the Cytoscape 3.7.2 software.[24] Subsequently, GO enrichment analysis and the Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis were

used to analyse the functions and the involved signalling pathways of core genes in the database for annotation, visualisation and integrated discovery (DAVID) (https://david.ncifcrf.gov), with the species limited to "*Sus scrofa*". Finally, the results of GO and KEGG analysis were visualized using a bioinformatics tool (www.bioinformatics.com.cn).

Molecular Docking

The SWISS-MODEL (https://swissmodel.expasy.org) was employed to construct the protein structure homology-modelling of PARP1.[25] Subsequently, molecular docking between PARP1 and GA was conducted using the software SYBYL-X 2.0. After molecular docking, the results were visualised by the PyMOL software.[26]

Bacterial Strains and Cell Culture

Pasteurella multocida strain HB03 (serogroups/genotypes A) was kindly provided by Professor Bin Wu and Dr. Zhong Peng (Huazhong Agricultural University, Wuhan). Strain HB03 was cultured in trypticase soy broth medium (Hopebio, China) supplemented with 5% bovine serum (NEWZERUM Ltd, New Zealand, Catalog # NCS-500) at 37°C for 8–12 h.

Porcine vascular endothelial cell line (PIEC) cells were kindly provided by Professor Hongkui Wei and Professor Menghong Dai at Huazhong Agricultural University (Wuhan, China). For culturing PIEC cells, RPMI medium modified (Cytiva, USA, Catalog # SH30809.01) with 1% penicillin-streptomycin solution (Gibco, USA, Catalog # 15070063) and 10% foetal bovine serum (PAN, Germany, Catalog # ST30-3302) was used. The cultures were cultured in a humidified incubator set at 37°C and 5% CO_2. After inoculating the PIEC cells in the cell culture plate for approximately 12 h, the cultures were preincubated with GA for 2 h and then co-treated with strain HB03 (MOI = 50) for another 10 h. All experiments were performed at least in triplicate on three separate occasions.

Cell Viability Assay

The cell viability of PIEC cells was investigated via the CCK-8 assay. For this, PIEC monolayer cells in each well of a 96-well plate were incubated with a series of GA (5–160 μg/mL) for 24 h, followed by the addition of 10 μL CCK-8 solution (Vazyme, China, Catalog # A311-02) into each well of the plate for 2 h. The absorbance of formazan was measured at 450 nm using a microplate reader (Spectra MIX i3x, Molecular Devices, China).

Cell Transfection

The PIEC cells were transiently transfected using the lipo8000™ transfection reagent (Beyotime, China, Catalog # C0533). For the PARP1 overexpression plasmids, PIEC monolayer cells in each well of a 6-well plate were transfected with 125 μL Opti-MEM (Gibco, USA, Catalog # 31985070) diluted in 3.75 μg of DNA (pcDNA3.0-PARP1 or pcDNA3.0) and 3.75 μL of lipo8000™ transfection reagent. For the RNAi assay, PIEC monolayer cells in each well of a 6-well plate were transfected with 125 μL Opti-MEM diluted with 7.5 μL of siRNA (siRNA of PARP1 or negative control siRNA (GenePharma, China) and 5 μL of lipo8000™ transfection reagent.

Western Blot

Challenged PIEC cells were lysed in RIPA buffer (Beyotime, China, Catalog # P0013B) with a protease and phosphatase inhibitor cocktail (Beyotime, China, Catalog # P1045), sonicated and centrifuged at 10,000 × g for 10 min at 4°C. The protein concentration of the supernatant was measured by the enhanced BCA protein assay kit (Beyotime, China, Catalog # P1010). Briefly, 20 μg of protein from each sample was separated via 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (Sangon Biotech, China, Catalog # C671102-0125) and then transferred to polyvinylidene difluoride membranes (Millipore, USA, Catalog # SE4M039I09). After being blocked with 5% BSA in tris-buffered saline with Tween 20 for 2 h at room temperature, the blots were incubated overnight with the primary antibodies-anti-PARP1 polyclonal antibody (Proteintech Group, China, Catalog # 13371-1-AP, 1:1000 dilution) anti-β-actin polyclonal antibody (Proteintech Group, China, Catalog # 20536-1-AP, 1:1000 dilution), anti-phospho-NF-kappa-B transcription factor p65 monoclonal antibody (p-NF-κB p65) (Thermo Fisher Scientific, USA, Catalog # MA5-15160, 1:1000 dilution), anti-IL-1β polyclonal antibody (Abclonal, China, Catalog # A1112, 1:1000 dilution), anti-IL-18 polyclonal antibody (Abclonal, China, Catalog # A16737, 1:1000 dilution), anti-HMGB1 polyclonal antibody (Abclonal, China, Catalog # A2553,

1:1000 dilution) and anti-NF-κB p65 polyclonal antibody (Abclonal, China, Catalog # A11204, 1:1000 dilution). After washing, the blots were incubated with secondary anti-IgG antibody (Abclonal, China, Catalog # A11204, 1:5000 dilution) and then visualised with enhanced chemiluminescence reagents (Abclonal, China, Catalog # RM00021) by the FluorChem E (ProteinSimple, China). All western blots were densitometrically quantified using the ImageJ software (ImageJ, NIH).

Statistical Analysis

Statistical analysis was performed using SPSS 18.0 for Windows. All results were presented as mean ± standard deviation. Results were statistically analysed by one-way ANOVA followed by Duncan's post hoc analysis. Statistical significance was set at $p < 0.05$, and a value of $p < 0.01$ was considered to be strongly significant.

Results

Identification of the Core Targets from Network Pharmacological Analysis

To explore the potential core targets of GA for attenuating *P. multocida*-mediated vascular endothelial inflammatory injury, we explored the core targets from the target database. Target database analysis yielded 216 and 749 targets for GA and vascular endothelial injury, respectively (Figure 1A and B, Supplementary Table 1). The 69 common targets of GA

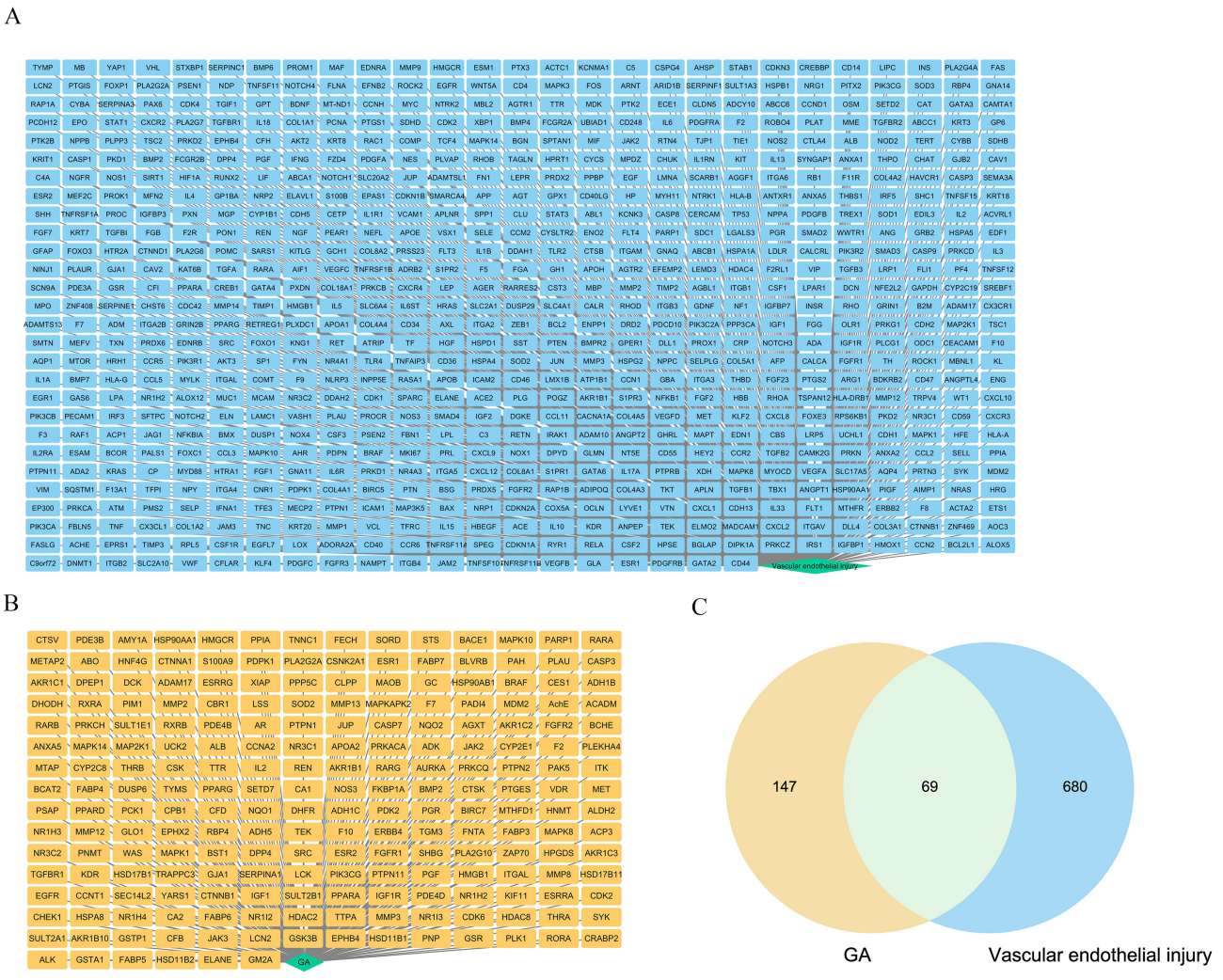

Figure 1 Target databases were applied to summarise the targets of 18β-glycyrrhetinic acid (GA) and vascular endothelial injury. (**A**) Summary of vascular endothelial injury targets using PubMed database and GeneCards databases; (**B**) Summary of GA using the drug target database; (**C**) The common vascular endothelial injury targets and GA targets were summarised by the Veen software.

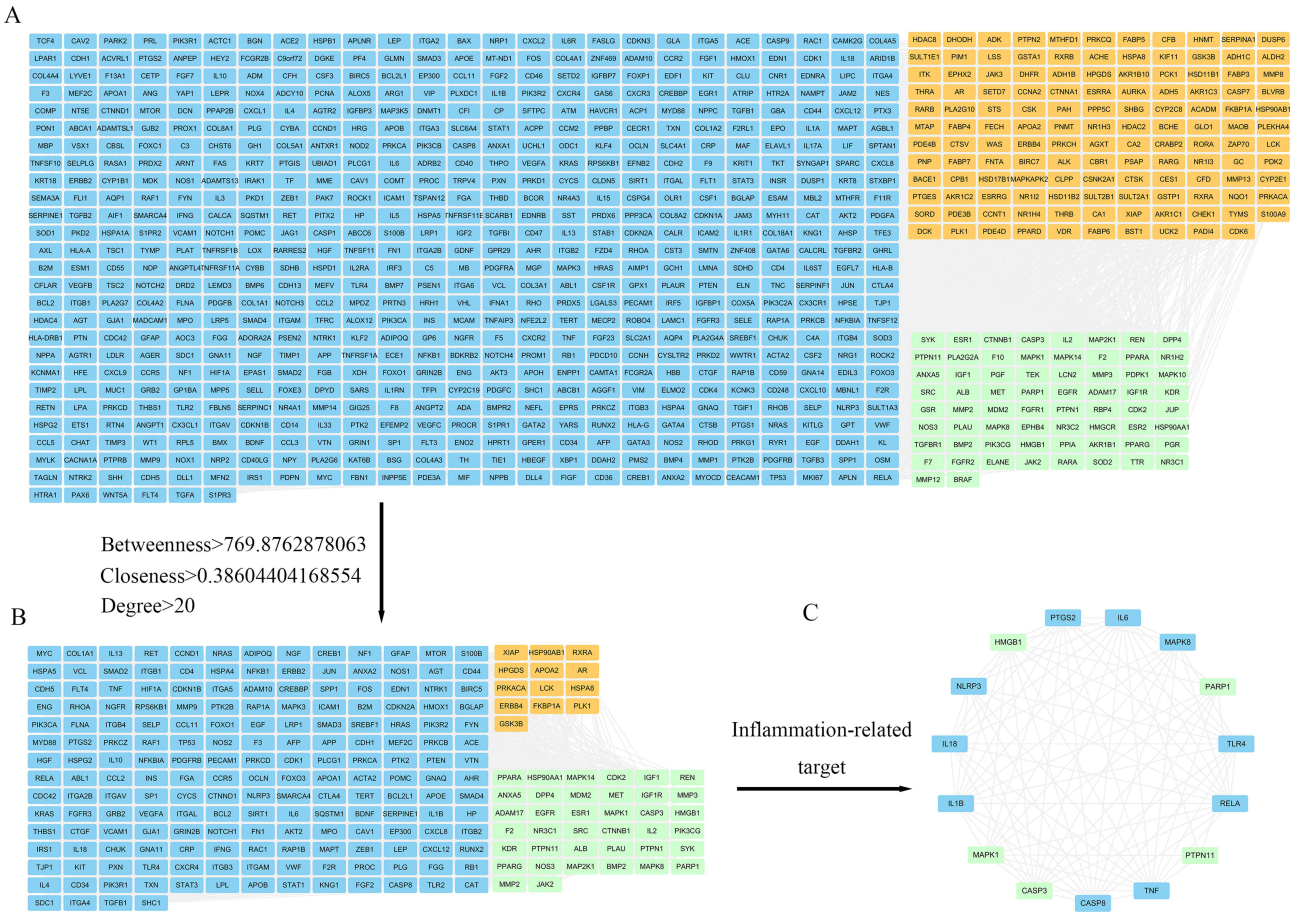

Figure 2 The protein-protein interaction (PPI) network and Cytoscape analyses were applied to screen the core targets of 18β-glycyrrhetinic acid (GA) in alleviating vascular endothelial injury. (**A**) PPI network diagram after String database screening; (**B**) Diagram of core targets for further screening based on the Cytoscape database. Orange nodes represent GA targets, blue nodes represent vascular endothelial injury targets, and green nodes represent common targets of vascular endothelial injury and GA. (**C**) Screening of core targets related to inflammation.

and vascular endothelial injury targets were analysed by the Venn diagram (Figure 1C, Supplementary Table 1). To further reveal the core targets of GA and vascular endothelial injury, all targets were mapped into the STRING database and conducted PPI network. As shown in Figure 2A and B, blue nodes represent vascular endothelial injury targets, orange nodes represent GA targets, and green nodes represent common targets. The core targets of GA and vascular endothelial injury were further selected, including PARP1, HMGB1, IL-1β, IL-18, RelA (NF-κB p65), PTPN11 and MAPK1 (Figure 2C). In addition, this study also compared the GA docking scores with niraparib (FDA-approved PARP1 inhibitor) docking scores, the docking scores of niraparib and GA with PARP1 are 5.9030 and 5.4304, respectively, indicating that both niraparib and GA have the potential to bind with PARP1 (Figure 3 and Table 1). Collectively, these data suggest that GA alleviated *P. multocida*-induced vascular endothelial inflammatory injury, possibly by altering the expression of core targets such as PARP1, HMGB1, IL-1β, IL-18 and NF-κB p65.

Core Targets Participate in Inflammatory Disorders

To understand the putative functions of the core targets, they were mapped to the GO and KEGG databases for analyses. Among the enriched biological processes, some processes related to the regulation of inflammatory injury were mainly enriched, such as positive regulation of interleukin-8 production, cellular response to lipopolysaccharide (LPS), positive regulation of IκBα kinase/NF-κB signalling and inflammatory response (Figure 4A). According to the KEGG analysis, core targets involved in the top 40 signalling pathways were mainly involved in the PI3K-AKT signalling pathway, the MAPK signalling pathway, coronavirus disease-COVID-19 and the JAK-STAT signalling pathway (Figure 4B). Based on

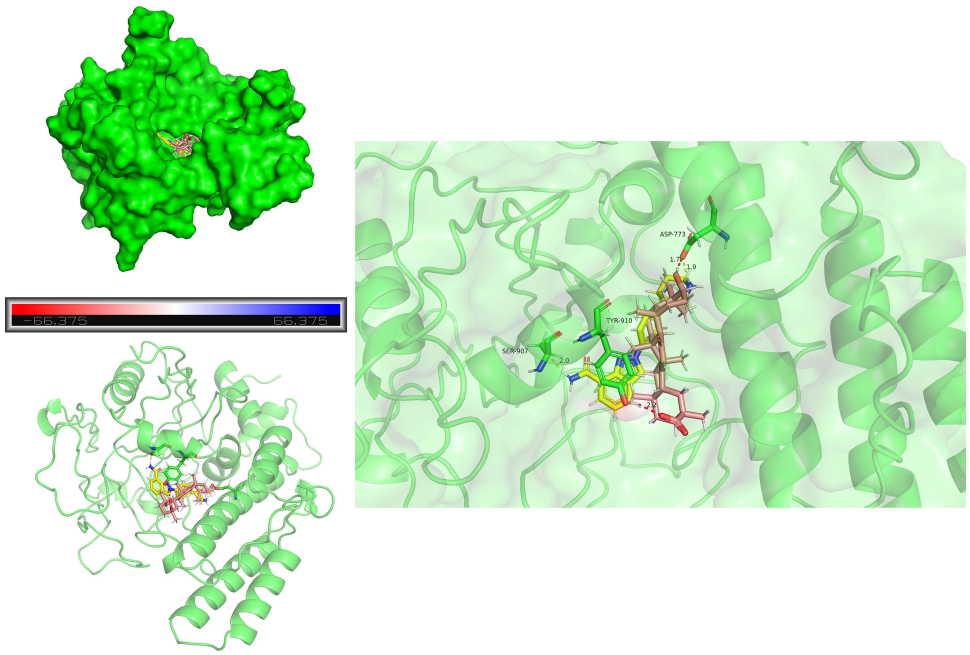

Figure 3 Molecular docking was applied to model the potential of 18β-glycyrrhetinic acid (GA) and niraparib to bind PARP1. Brown stick molecules represent GA, yellow stick molecules represent niraparib and green stick molecules represent amino acid residues of PARP1.

the results of the comprehensive GO and KEGG analysis, core targets of GA and vascular endothelial injury may participate in the inflammatory disorders of vascular endothelial cells.

Dose-Effect of GA on the Cytotoxicity of PIEC Cells

The CCK-8 assay was used to evaluate the dose-effect of GA on the cytotoxicity of PIEC cells. As presented in Figure 5A, below 40 μg/mL, GA was almost non-cytotoxic, with a cell viability > 80%. However, at concentrations of 80–160 μg/mL, GA exhibited significant cytotoxicity to PIEC cells ($p<0.01$). Consequently, 10, 20 and 40 40 μg/mL were used as the working concentrations of GA for the whole experiment.

GA Attenuates *P. multocida*-Induced Vascular Endothelial Inflammatory Injury

To evaluate whether GA can attenuate endothelial inflammatory injury, this study modelled vascular endothelial inflammatory injury using PIEC cells induced by *P. multocida*. The minimum inhibitory concentration (MIC) of GA against *P. multocida* was 1024 μg/mL. In addition, the western blot results showed that the expression levels of PARP1, p-NF-κB p65, HMGB1, IL-1β and IL-18 were upregulated after *P. multocida* infection ($p<0.01$), with a significant dose-dependent reversal of *P. multocida*-induced inflammatory protein (PARP1, p-NF-κB p65, HMGB1 and IL-1β) expression by 10–40 μg/mL of GA ($p<0.01$) (Figure 5B and C); 10 μg/mL ($p=0.950$) and 20 μg/mL ($p=0.869$) concentrations of GA did not produce a significant effect on *P. multocida* -induced expression of IL-18. However, the concentration of 40 μg/mL of GA significantly reduced the expression of IL-18. These findings indicate that *P. multocida* infection can cause an abnormal increase in inflammatory markers of vascular endothelial cells, whereas GA can effectively alleviate *P. multocida* infection-induced vascular endothelial inflammatory injury.

Table 1 The Docking Value of 18β-Glycyrrhetinic Acid (GA) and Niraparib with PARP1

Chemicals	Docking Score	Binding Site
GA	5.4304	ASP-773, TYR-910
Niraparib	5.9030	ASP-773, SER-907

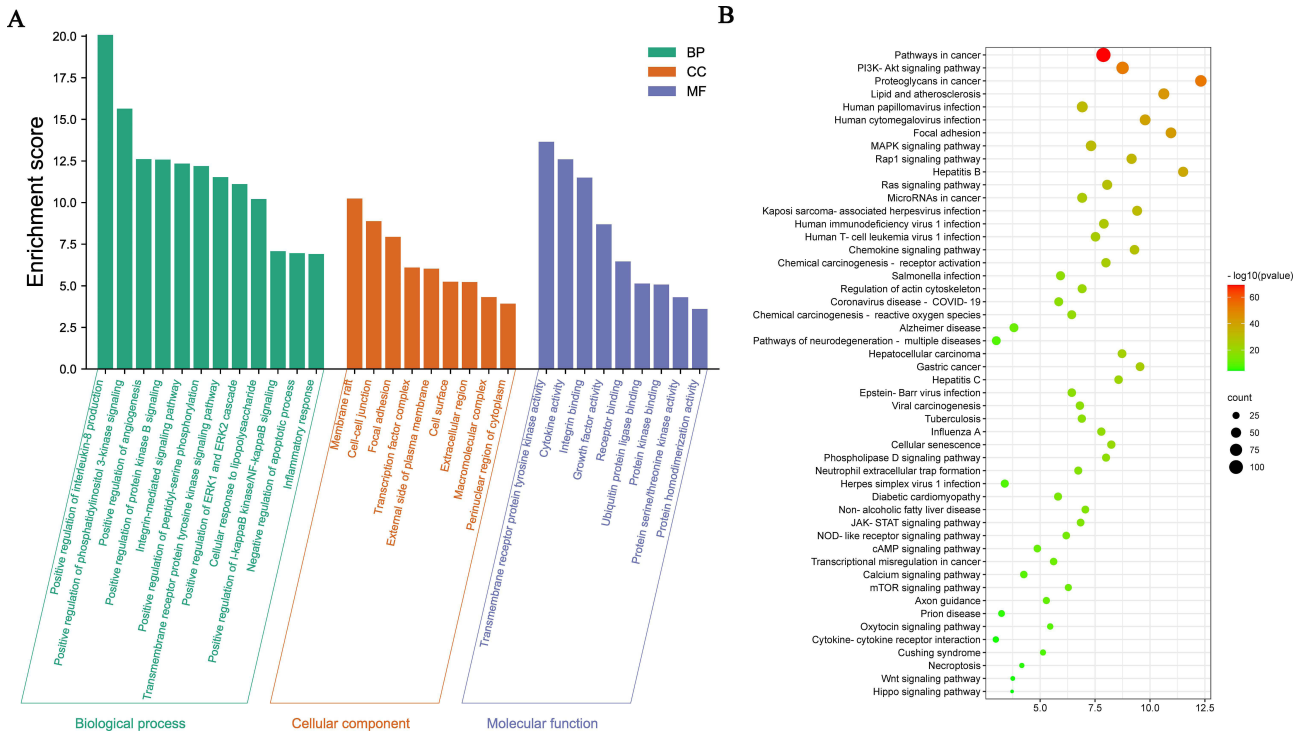

Figure 4 GO enrichment analysis and the Kyoto encyclopedia of genes and genomes (KEGG) analysis of the core targets of 18β-glycyrrhetinic acid (GA) in alleviating vascular endothelial inflammatory injury using the database for annotation, visualisation and integrated discovery (DAVID). (**A**) GO analysis of core targets; (**B**) KEGG analysis of core targets.

GA Attenuates *P. multocida*-Induced Vascular Endothelial Inflammatory Injury by Inhibiting the PARP1 Pathway

To gain insight into how GA alleviates the cellular inflammatory damage induced by *P. multocida* infection, a comprehensive analysis was performed in combination with PARP1 overexpression and interference assays, bacterial infection and GA treatment. As shown in Figure 6A and B, overexpressed PARP1 significantly promoted the expression of PARP1, p-NF-κB p65, HMGB1, IL-1β and IL-18 ($p<0.01$). However, GA was able to alleviate inflammatory protein elevation induced by PARP1 overexpression in a concentration-dependent manner. Knockdown of PARP1 also down-regulated the expression of inflammatory proteins such as PARP1, p-NF-κB p65, HMGB1, IL-1β and IL-18 ($p<0.01$) and inhibited the increase in inflammatory proteins induced by *P. multocida* (Figure 7A and B). The results showed that GA alleviates *P. multocida*-induced vascular endothelial inflammatory injury through the PARP1-HMGB1 or the PARP1- NF-κB p65 signalling pathway.

Discussion

As one of the main pathogenic bacteria causing respiratory diseases in humans and animals, the study of the interaction between *P. multocida* and vascular endothelial cells plays an important role in exploring the pathogenesis of hemorrhagic pneumonia. The clinical treatment of *P. multocida* infections mainly occurs via the application of antimicrobials. However, antimicrobials do not solve the problem of inflammatory injury of vascular endothelial cells caused after *P. multocida* infection, and the massive and illegal use of antimicrobials is often accompanied by the occurrence of antimicrobial resistance.[27] Therefore, it is of great significance to explore the mechanism of vascular endothelial inflammatory injury induced by *P. multocida* infection and to search for new, effective drugs for adjuvant treatment or the control of inflammation in the disease to reduce the harm of *P. multocida* infection. In the present study, we used PIEC cells as a model and found that GA alleviates *P. multocida*-induced vascular endothelial inflammation by PARP1-mediated NF-κB and HMGB1 signalling suppression (Figure 8), suggesting that GA and PARP1 are potential alternatives to antibiotics and candidate therapeutic targets for the adjuvant treatment of *P. multocida* infection, respectively.

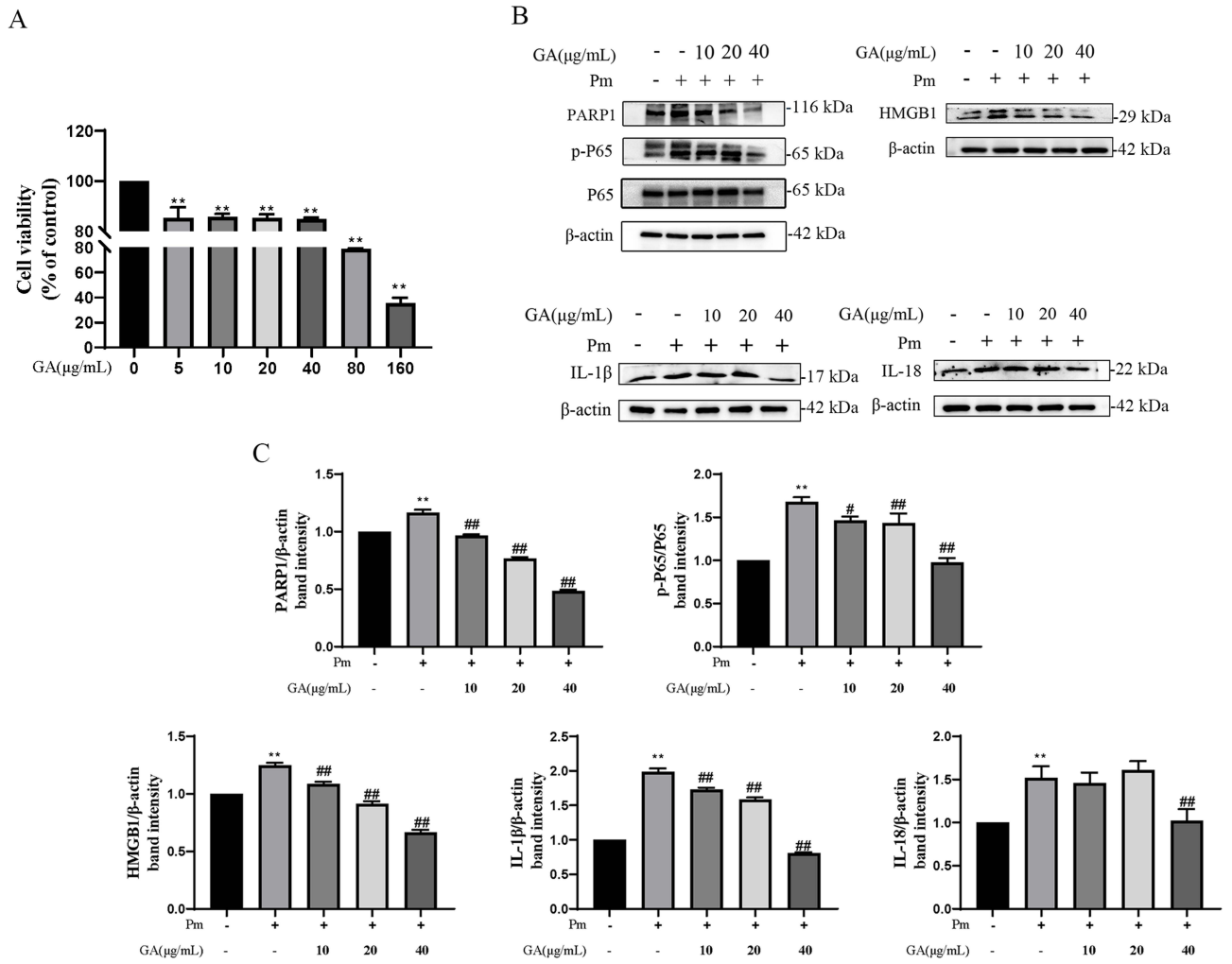

Figure 5 18β-glycyrrhetinic acid (GA) attenuates vascular endothelial inflammatory injury induced by *P. multocida* infection in PIEC cells. (**A**) Cytotoxicity of GA on PIEC cells using the CCK-8 assay; (**B**) GA reduced the expression of inflammatory proteins induced by *P. multocida* infection in PIEC cells; (**C**) Protein density quantified using the ImageJ software. **represents the control group vs *P. multocida* infection group, $p < 0.01$; #And ##Represent other groups vs *P. multocida* infection group, $p < 0.05$ and $p < 0.01$, respectively.

Network pharmacology is a new discipline to study the mechanism of drug-target-disease interactions in the context of larger biological networks.[28] To explore the possible target of GA in host cells that are responsible for the vascular endothelial inflammatory injury induced by *P. multocida* infection, network pharmacology analysis was conducted. Some inflammation-related core targets of GA and vascular endothelial injury were enriched by PPI network and Cytoscape analysis,[29,30] such as PARP1, HMGB1, IL-1β, IL-18, NF-κB p65, indicating that inflammatory pathways play an important role in GA alleviating *P. multocida*-induced vascular endothelial inflammatory injury. Moreover, GO analysis showed that core targets are mainly enriched in biological functions related to the regulation of inflammatory injury, such as positive regulation of interleukin-8 production, cellular response to LPS, positive regulation of IκBα kinase/NF-κB signalling and inflammatory response. Based on the results of the KEGG enrichment analysis, GA involves multiple signalling pathways in the treatment of *P. multocida* infection, mainly including the PI3K-AKT signalling pathway, the MAPK signalling pathway, coronavirus disease-COVID-19 and the JAK-STAT signalling pathway. In addition, studies have shown that the NF-κB/MAPK signalling pathway is involved in the regulation of LPS-induced inflammatory responses in IPEC-J2 cells.[31] According to another study, HMGB1 can enhance the inflammatory response by activating the p38 MAPK signalling pathway.[32,33] Meanwhile, PARP1 could serve as a regulator of inflammatory diseases through promoting NF-κB activation and HMGB1 secretion.[13,14] The network pharmacology results shown herein revealed that the activation of PARP1 might contribute to the activation of NF-κB and HMGB1, which may finally be beneficial for vascular endothelial inflammatory injury after *P. multocida* infection.

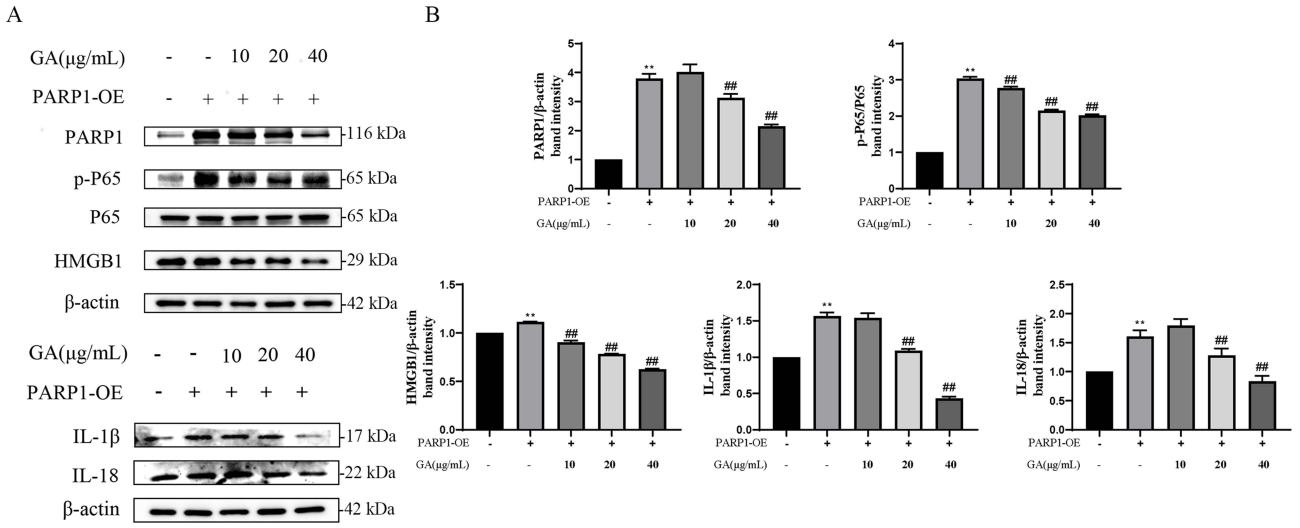

Figure 6 18β-glycyrrhetinic acid (GA) alleviates overexpressed PARP1-induced vascular endothelial inflammatory injury in PIEC cells. (**A**) Pre-treatment with GA could alleviate the overexpressed PARP1-induced expression of inflammation-related protein, such as PARP1, HMGB1, p-P65, IL-18 and IL-1β; (**B**) Protein density quantified using the ImageJ software. PARP1-OE represents overexpressed PARP1. **Represents the control group vs the PARP1-OE group, $p < 0.01$; ##Represents other groups vs PARP1-OE group, $p < 0.01$.

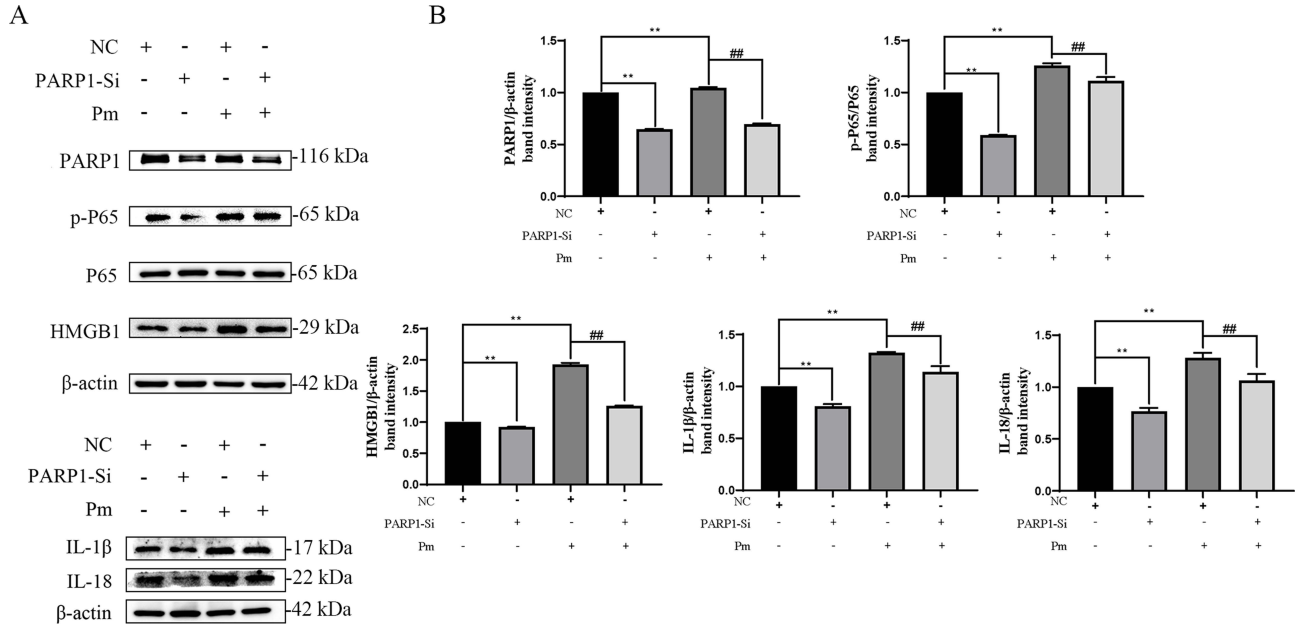

Figure 7 Knockdown of PARP1 decreased vascular endothelial inflammatory injury induced by *P. multocida* infection in PIEC cells. (**A**) Knockdown of PARP1 decreased *P. multocida* infection-induced expression of inflammation-related protein, such as PARP1, HMGB1, p-P65, IL-18 and IL-1β; (**B**) Protein density quantified using the ImageJ software. PARP1-Si represents Knockdown of PARP1. NC represents negative control siRNA. ##Represents NC group+ *P. multocida* infection group, $p < 0.01$; **Represents other groups vs NC group, $p < 0.01$.

The GA can be an anti-inflammatory drug candidate for the treatment of inflammatory responses evoked by various external stimuli. In a previous study, it inhibited *Staphylococcus aureus*-induced RAW264.7 cell inflammation by inhibiting the activation of NF-κB and the expression of HMGB1[19] and showed a protective effect on *Helicobacter pylori*-infected gastric mucosa through alleviating the expression levels of inflammation-related cytokines (IL-1β, TNF-α).[20] Further, GA suppressed allergic airway inflammation through NF-κB and Nrf2/HO-1 signalling pathways in an ovalbumin-induced asthma mouse model.[34] In LPS- or TNF-α-induced inflammatory cell models and collagen-induced arthritis animal models, GA exerts its anti-inflammatory activity through the MAPK/NF-κB signalling pathway.[35] The above studies demonstrate

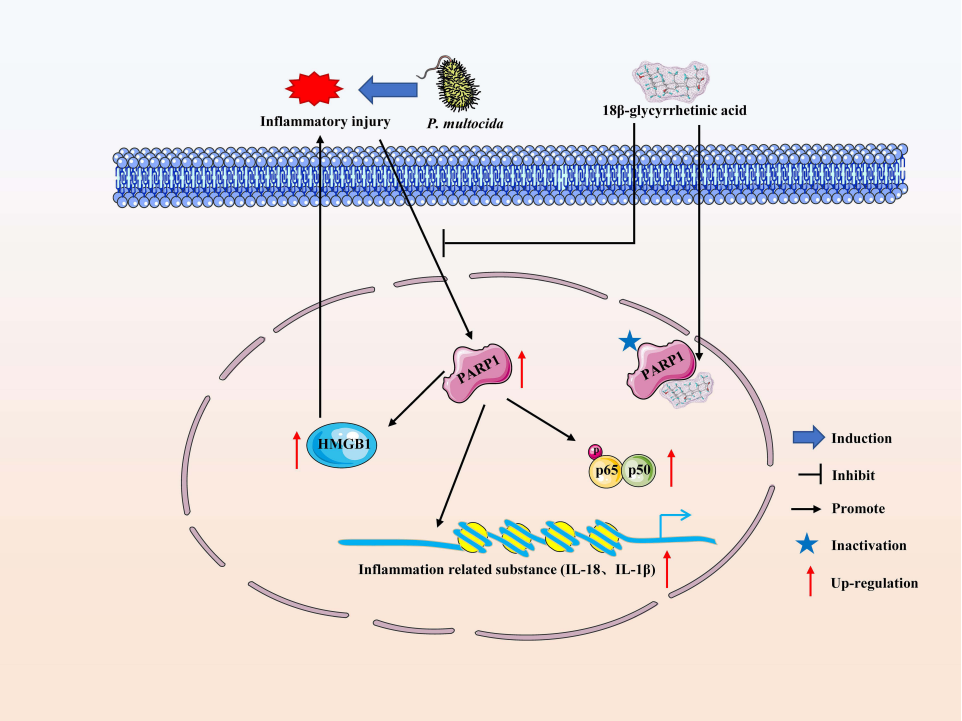

Figure 8 18β-glycyrrhetinic acid (GA) alleviates *P. multocida*-induced vascular endothelial inflammation by PARP1-mediated NF-κB and HMGB1 signalling suppression in PIEC cells. *P. multocida*-infected PIEC cells could significantly active the PARP1-mediated NF-κB and HMGB1 signalling pathways and induce the PARP1-mediated expression of inflammatory-related substances, such as IL-1β and IL-18. On the one hand, GA may inhibit the activity of PARP1 by targeting it, on the other hand, it may inhibit the NF-κB and HMGB1 signalling pathways by reducing the expression of PARP1, thus inhibiting *P. multocida* infection-induced vascular endothelial inflammatory injury in PIEC cells.

that GA presents great anti-inflammatory activity in vivo or in vitro by inhibiting pathways such as NF-κB and HMGB1. This study found that GA could inhibit the NF-κB and HMGB1 pathways to alleviate the vascular endothelial inflammatory injury induced by *P. multocida* infection. In addition, in this study, GA potentially targeted PARP1 by network pharmacology and molecular docking technology, which in turn exerted the inhibition of NF-κB and HMGB1 pathway activation.

The PARP1 is involved in the regulation of inflammation in bacterial infections. In LPS-induced mouse septic shock and *S. typhimurium*-induced mouse colitis models, the absence of PARP1 decreased NF-κB-mediated inflammatory disorders.[16,36] Moreover, PARP1 mediates LPS-induced HMGB1 secretion from macrophages, and PARP1 activity-dependent HMGB1 translocation from the nucleus to the cytoplasm can promote the activation of cellular inflammatory pathways.[14] However, loss of PARP1 activity impedes the cytosolic translocation of HMGB1, which can prevent the HMGB1-mediated TLR4- NF-κB signalling pathway to attenuate LPS-induced myocardial inflammation.[37] In the present study, either PARP1 overexpression or PARP1 deletion demonstrated the ability of PARP1 to promote the expression of NF-κB and HMGB1, which in turn were involved in the progression of vascular inflammatory injury induced by *P. multocida* infection. In addition, the molecular docking score is greater than 4,[38] implying that PARP1 might be a key target of GA. And GA at lower antimicrobial concentrations (10 ~ 40 μg/mL) on the inflammatory response of endothelial cells induced by *P. multocida* infection. Thus, we speculate that GA may play a role in alleviating vascular endothelial inflammatory injury induced by *P. multocida* infection by targeting PARP1 and inhibiting PARP1 activity. The above studies demonstrate that GA and PARP1 could serve as a novel antibiotic alternative and therapeutic target for the prevention and treatment of vascular endothelial inflammatory injury induced by *P. multocida* infection, respectively.

This study has, however, some limitations. First, some unconfirmed and unrecorded targets of the target database may not be enriched in the present study, implying that the target database of network pharmacology needs to be further improved. Second, the molecular docking results showed that PARP1 may have the potential to bind with GA, but its specific binding situation awaits further investigation. Third, the results of the present study showed that PARP1 could regulate the protein expression of NF-κB and HMGB1, but whether GA inhibits inflammation by targeting PARP1 to regulate the nuclear

translocation of NF-κB and HMGB1 during *P. multocida* infection awaits further exploration. Fourth, pharmacodynamic and animal therapeutic trials are needed to further verify the potential of GA as an alternative to antibiotics in the treatment of vascular endothelial inflammatory injury caused by *P. multocida* infection.

Conclusion

Collectively, through the mining of network pharmacology studies and the verification of in vitro experiments, this study proves the powerful potential of network pharmacology in disease and drug target screening and that PARP1 is a potential target for GA for the treatment of vascular endothelial inflammatory injury caused by *P. multocida* infection. Further, PARP1-mediated NF-κB and HMGB1 signalling suppression may be a critical signalling pathway for treating vascular endothelial inflammatory injury caused by *P. multocida* infection.

Ethics Statement

Part of the target data for this manuscript was obtained from a public database, such as GeneCards. The ethics committee of Wuhan Polytechnic University has reviewed the data in this manuscript, which does not violate relevant ethical issues and agrees to publish it.

Acknowledgments

We sincerely acknowledge Professor Hongkui Wei and Professor Menghong Dai at Huazhong Agricultural University (Wuhan, China) for the gift of PIEC cells. We also thank the Professor Bin Wu and Dr. Zhong Peng at Huazhong Agricultural University (Wuhan, China) for the gift of *P. multocida* strain HB03.

Funding

This work was supported by National Natural Science Foundation of China (NSFC) (32202867) and Key Research and Development Plan of Hubei Province, China (2022BBA0055).

Disclosure

The authors declare that they have no conflicts of interest in this work.

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