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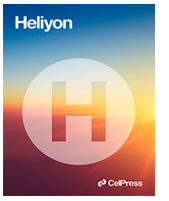

Research article

Glycyrrhetinic acid reduces lung inflammation caused by pneumococcal infection by reducing the toxicity of pneumolysin

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ABSTRACT

Objective: In this study, to provide new methods for the treatment of *Streptococcus pneumoniae* infection, we aimed to describe the anti-inflammatory and antibacterial value of glycyrrhetinic acid on the basis of its inhibitory effect on bacterial growth (without killing the bacteria) and its reduction of the toxicity of *S. pneumoniae*.

Methods: A mouse model was established via intranasal administration of *Streptococcus pneumoniae* D39, and glycyrrhetinic acid was subcutaneously injected for treatment. The wet–dry ratio, bacterial flora content and inflammatory factor levels in the mouse lungs were determined. Cell experiments were used to evaluate glycyrrhetinic acid-mediated inhibition of PLY hemolysis and A549 cell death, and WB was used to measure glycyrrhetinic acid-mediated inhibition of PLY oligomerization.

Results: Glycyrrhetinic acid reduced the levels of inflammatory factors, the dry–wet ratio, the abundance of *S. pneumoniae* in the lungs of infected mice, pneumolysin-mediated A549 cell death, erythrocyte hemolysis and PLY oligoplasia.

Conclusion: Glycyrrhetinic acid can reduce the virulence of *S. pneumoniae* by preventing the oligomerization of PLY.

Infectious diseases are caused by pathogenic microorganisms such as bacteria, viruses, fungi, Mycoplasma, Chlamydia, Rickettsia, and parasites [1,2]. To date, infection is still one of the main causes of death and disability in humans and has become a major problem facing the medical community [3–6].

At present, research on drugs for treating infectious diseases has focused mainly on antibacterial and antiviral effects. However, owing to the emergence of bacterial resistance and the rapid mutation of viruses, research on anti-infective drugs is challenging. Studies have shown that more than 45 % of lung inflammation in China is caused by bacteria. *Streptococcus pneumoniae* and Mycoplasma are the main sources of infection [1]. In addition, probiotics, bacteriophages, antimicrobial peptides, etc., can all be used as effective means to fight *S. pneumoniae*, but previous studies have reported high R&D costs and long cycles, and the problem of immature large-scale mass production still needs to be solved [7–13]. The use of vaccines will also accelerate the mutation of noninvasive serotypes to invasive serotypes. In addition, a series of drugs have exerted considerable evolutionary pressure on bacteria.

As a major branch of traditional medicine, Chinese medicine plays a special role in preventing and treating infectious diseases. In

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particular, when drug-resistant strains and mutant strains emerge, new antibacterial drugs that do not produce evolutionary pressure from marketed Chinese medicinal materials can be found quickly. Studies have shown that *Scutellaria baicalensis*, *Taraxacum officinale*, and Tussilago have antibacterial effects against *Staphylococcus aureus* [14]. *Polygonum cuspidatum* has inhibitory effects against *Staphylococcus aureus* and other bacterial biofilms [15]. A review on combating antibiotic resistance revealed that 50 Chinese medicinal materials, including *Pulsatilla striata*, *Terminalia chebula*, *Astragalus membranaceus*, *Aster tataricus*, cinnamon bark, *Coptis chinensis*, *Dendrobium officinale*, and *Ephedra sinica*, have direct antibacterial effects or synergistic or additive effects with antibiotics [16]. Considering the diversity of Chinese medicinal materials and the complexity of the underlying mechanisms, we performed antibacterial testing on available ingredients from these materials. This approach was chosen because analysis of the specific ingredients can more directly explain the antibacterial mechanism.

Glycyrrhetinic acid is one of the main active ingredients of the traditional Chinese medicine liquorice, and it is a pentacyclic triterpenoid. Glycyrrhetinic acid provides good protection against liver damage [17]. Moreover, glycyrrhetinic acid also has many effects, such as antiviral effects [18], autophagy regulation [19], intestinal permeability changes [20], and cancer prevention [21,22]. However, few studies have investigated the antibacterial activity of GA, and previous studies have described only the minimum inhibitory concentration [23] and have not clarified the specific mechanism involved. In this study, we describe the anti-inflammatory and antibacterial value of glycyrrhetinic acid from the perspective of inhibiting the growth of bacteria without killing bacteria and reducing the toxicity of *Streptococcus pneumoniae* to provide new methods for the treatment of *Streptococcus pneumoniae* infection.

1. Materials and methods

1.1. Cells and reagents

A549 alveolar epithelial cells were purchased from ATCC (Manassas, VA, USA) and cultured in DMEM (Gibco Life Technologies, Inc., Grand Island, NY, USA) supplemented with 10 % fetal bovine serum (FBS; Biological Industries, Israel). Glycyrrhetinic acid (Fig. 1, CAS No. 471-53-4, purity >98 %) was purchased from Chengdu Reifenside Biotechnology Co., Ltd.; Todd-Hewitt Broth (THB), dimethyl sulfoxide (DMSO), skim milk powder, trypsin, imidazole, tetramethylethylenediamine (TEMED) and β-mercaptoethanol (β-ME) were purchased from Sigma–Aldrich; a BCA protein quantification kit and ECL luminescent liquid were purchased from Thermo Fisher; horseradish peroxidase (HRP)-labeled goat anti-mouse secondary antibodies were purchased from Protech; polyvinylidene fluoride (PVDF) membranes were purchased from Roche; a live/dead cell activity detection kit was purchased from Invitrogen; fetal bovine serum albumin (FBS) was purchased from Biological Industries; and 30 % acrylamide, ammonium persulfate, sodium dodecyl sulfate (SDS) and disodium ethylenediaminetetraacetic acid (EDTA) were purchased from Dingguo Changsheng Biotechnology Co., Ltd.; and pneumolysin (ab240861) was purchased from Abcam. The preparation method for the glycyrrhetinic acid stock solution was as follows: 64 mg of glycyrrhetinic acid was weighed out, dissolved in 1000 ml of PBS, filtered with a 0.22 μM filter membrane, and stored at 4 °C.

1.2. Bacteria

Streptococcus pneumoniae D39 serotype 2 (NCTC7466) (a gift from Professor Huang Jian of Zunyi Medical University) was cultured in THY media at 37 °C and 5 % CO2 in an incubator.

1.3. Animals

BALB/c mice (female, 6–8 weeks old, 20–22 g) were purchased from Liaoning Changsheng Biotechnology Co., Ltd. (Laboratory Animal Production License No. SCXK (Liao) 2020–0001) and housed at a temperature of 24 ± 3 °C and a relative humidity of 40 ± 5 %, with alternating light and dark cycles, noise <55 Db, free access to water and food, and litter changed twice a week. The animal experiments were approved by the Experimental Animal Welfare Ethics Committee of Henan University of Chinese Medicine (IACUC-

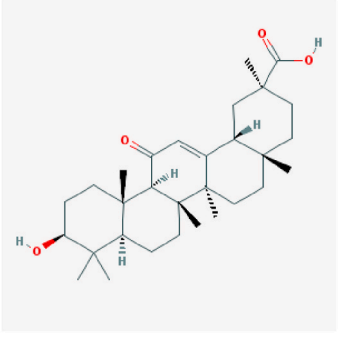

Fig. 1. Molecular structure of glycyrrhetinic acid.

202404012).

1.4. PLY and hemolysis test

PLY was purchased from Fitzgerald Company (80R-4390) in the United States. Different concentrations of glycyrrhetinic acid (0, 4, 8, 16, 32, and 64 μg/ml) were mixed with PLY and incubated in PBS at 37 °C for 30 min. Then, 25 μl of defibrinated sheep red blood cells was added to the mixture and incubated at 37 °C for 10 min. Finally, the mixture was centrifuged at 3000×*g* for 5 min, and the supernatant was collected to measure the hemolytic activity at an OD of 543 nm via a microplate reader.

1.5. Antibacterial activity and cytotoxicity assay

The inhibition curve was prepared via the microbroth method. Glycyrrhetinic acid at different concentrations (0, 4, 8, 16, 32, and 64 μg/ml) was incubated with *Streptococcus pneumoniae* in THB, and the growth of *Streptococcus pneumoniae* was monitored at 600 nm every 60 min via a UV spectrophotometer. A total of 2×10^4 A549 cells were added to each well of a 96-well plate and incubated overnight, after which 3.0 μl of PLY pretreated with glycyrrhetinic acid was added, and the plates were placed in an incubator at 37 °C for 5 h. Cells were treated with a live/dead (green/red) staining kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions, and cell viability was observed via a confocal laser scanning microscope (Olympus, Tokyo, Japan).

1.6. Western blot analysis

Streptococcus pneumoniae D39 was cultured at 37 °C in THY, and then different concentrations of glycyrrhetinic acid were added. After centrifugation (3000 rpm, 10 min), the 5 × SDS–PAGE supernatant was incubated at 100 °C for 10 min, separated by 12 % SDS–PAGE and transferred to a PVDF membrane, which was blocked with 5 % skim milk powder at room temperature for 2 h. The membrane was incubated with a monoclonal antibody against pneumococcal hemolysin (1:1000; Abcam, Cambridge, UK) at 4 °C overnight, and the secondary antibody (1:2000; Proteintech, Chicago, IL, USA) was added after washing with PBST and incubated at 37 °C for 1 h. After washing with PBST, the membrane was developed via a Tanon-4200 imager (Tanon, Shanghai, China) and enhanced chemiluminescence (ECL) reagent (Thermo Scientific, Rockford, IL, USA).

The detection of oligomers and monomers was performed as described above: first, glycyrrhetinic acid and PLY were incubated at 37 °C for 1 h and then boiled at 50 °C for 10 min (with 5 × SDS–PAGE loading buffer without β-mercaptoethanol). The oligomers and monomers of PLY were subsequently analyzed.

1.7. Mouse model of pneumococcal infection

D39 cells were cultured in THY at 37 °C until the mid-log phase (OD600 nm = 0.4), washed three times by centrifugation with PBS and resuspended again in PBS. Mice were anesthetized with light ether, and 1.5×10^8 colony forming units (CFUs) were inoculated into the left nostril for lung infection. Two hours after infection, the mice (n = 10) were subcutaneously injected with glycyrrhetinic acid or PBS every 8 h. The mice (n = 10) were killed 48 h after infection. The dry weight and wet weight of the lung tissue were measured, and the wet weight/dry weight ratio was calculated.

Bronchoalveolar lavage fluid was collected from the mice, and the levels of cytokines (IL-1β, IL-6, and TNF-α) were determined after centrifugation via an ELISA kit (eBioscience, San Diego, CA, USA). The left lung of each mouse was removed to observe the overall changes in the lung, and pictures were taken to collect images. Then, the sections were fixed with 10 % formalin solution for 24 h, dehydrated with gradient concentrations of ethanol (70 %, 80 %, 90 %, 95 %, and 100 %), cleared with xylene, embedded in paraffin, sliced, dewaxed with xylene and ethanol, stained with HE, and mounted. Finally, histopathological changes were observed under an optical microscope, and images were collected. The surface moisture of the lungs was removed with filter paper, and the samples were weighed and placed in a 4 ml sterile centrifuge tube. The lung tissue was fully ground with an electric tissue grinder, 900 ml of sterile PBS was added to resuspend the tissue cells, 100 μl of 1 % Triton X-100 was added, and the mixture was shaken for 5 min to fully lyse the cells. After the sample was diluted 10 times in a gradient, 10 μl was spread on a blood agar plate and cultured at 37 °C and 5 % CO for 24 h, after which the number of colonies was counted.

1.8. Statistical analysis

The data are expressed as the mean ± standard deviation (S.D.) (n = 10) and were analyzed via GraphPad Prism 6.0 (GraphPad Software) using two-tailed independent-sample t tests and one-way ANOVA followed by Tukey's *post hoc* multiple comparison test. *, $p < 0.05$ and **, $p < 0.01$.

2. Results

2.1. Glycyrrhetinic acid can reduce the inflammatory response in the lungs of mice infected with Streptococcus pneumoniae

After model establishment, the lungs of the mice were observed with the naked eye, and after D39 nasal drops were applied, the lungs became dark brown, and inflammation significantly increased. However, inflammatory infiltration in the lungs was significantly

reduced after glycyrrhetinic acid treatment, and HE staining of pathological sections revealed the same results, as shown in Fig. 2A–B. These results indicate that glycyrrhetinic acid controls the inflammatory response after pneumococcal infection.

2.2. Glycyrrhetinic acid can reduce the levels of inflammatory factors, the dry–wet ratio and the content of Streptococcus pneumoniae in the lungs of mice infected with Streptococcus pneumoniae

By continuing to explore the regulatory effect of glycyrrhetinic acid on lung inflammatory factors, we found that after D39 treatment, the levels of IL-1β, IL-6, and TNF-α in the mouse lungs were significantly reduced (Fig. 3C–E), and the lung wet–dry ratio also decreased after glycyrrhetinic acid treatment (Fig. 3A). These results demonstrate the regulatory effect of glycyrrhetinic acid on lung inflammation. To explain the regulatory effect of glycyrrhetinic acid on *Streptococcus pneumoniae*, we measured the content of *Streptococcus pneumoniae* in the lungs (Fig. 3B) and found that *Streptococcus pneumoniae* can actually reduce the abundance of the lung flora. This result suggests that glycyrrhetinic acid does not directly reduce lung inflammation but rather reduces the abundance of the flora, thereby reducing the local source of infection. Because the amount of bacteria responsible for infection is reduced, lungs treated with glycyrrhetinic acid are as clean as those that have never been invaded by bacteria.

2.3. Glycyrrhetinic acid can reduce the death of A549 cells mediated by pneumococcal hemolysin

We first evaluated the effect of glycyrrhetinic acid on the growth of *Streptococcus pneumoniae*. As shown in Fig. 5A, glycyrrhetinic acid did not inhibit or promote *Streptococcus pneumoniae* growth at concentrations of 4, 8, 16, 32, or 64 μg/ml. The overshoot of *Streptococcus pneumoniae* infection depends on the release of virulence factors, among which PLY is the main virulence factor of *Streptococcus pneumoniae* and is involved throughout the transmission, colonization and invasion of *Streptococcus pneumoniae*. Therefore, we explored whether glycyrrhetinic acid has a certain effect on the toxicity of PLY. As shown in Fig. 4A–D, after the addition of PLY, the number of dead A549 cells increased, but after treatment with 64 μg/ml glycyrrhetinic acid, the number of dead A549 cells decreased significantly, as shown in Fig. 4D. This result indicates that the therapeutic effect of glycyrrhetinic acid may be achieved by targeting PLY.

2.4. Glycyrrhetinic acid reduces the hemolysis of erythrocytes mediated by PLY, and its mechanism is related to the oligomerization of PLY

The pore-forming structure of PLY oligomers is the main way that pneumococcal virulence is expressed. Inhibiting the pore-forming activity of PLY can directly reduce pneumococcal virulence. To further explore whether glycyrrhetinic acid affects the oligomerization of PLY, we evaluated the hemolytic activity of PLY. As shown in Fig. 5B–C, glycyrrhetinic acid still reduced the hemolytic activity mediated by PLY. On the other hand, since oligomerization is the main mode of PLY virulence, we focused our attention on the direction of PLY oligomerization mediated by glycyrrhetinic acid. As shown in Fig. 5D, glycyrrhetinic acid directly reduced the process of PLY polymerization into polymers. Moreover, glycyrrhetinic acid at a concentration of 64 μg/ml or less had no inhibitory effect on the production of PLY, as shown in Fig. 5E.

In summary, these results illustrate the ability of glycyrrhetinic acid to inhibit pneumococcal virulence and reduce lung infection by targeting PLY oligomerization.

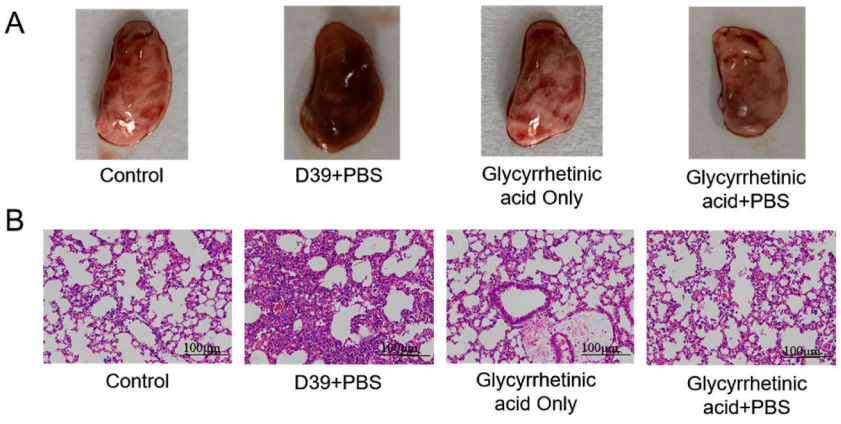

Fig. 2. Pulmonary inflammatory response after pneumococcal infection. A shows the macroscopic view of the lungs, and B shows the HE pathological result.

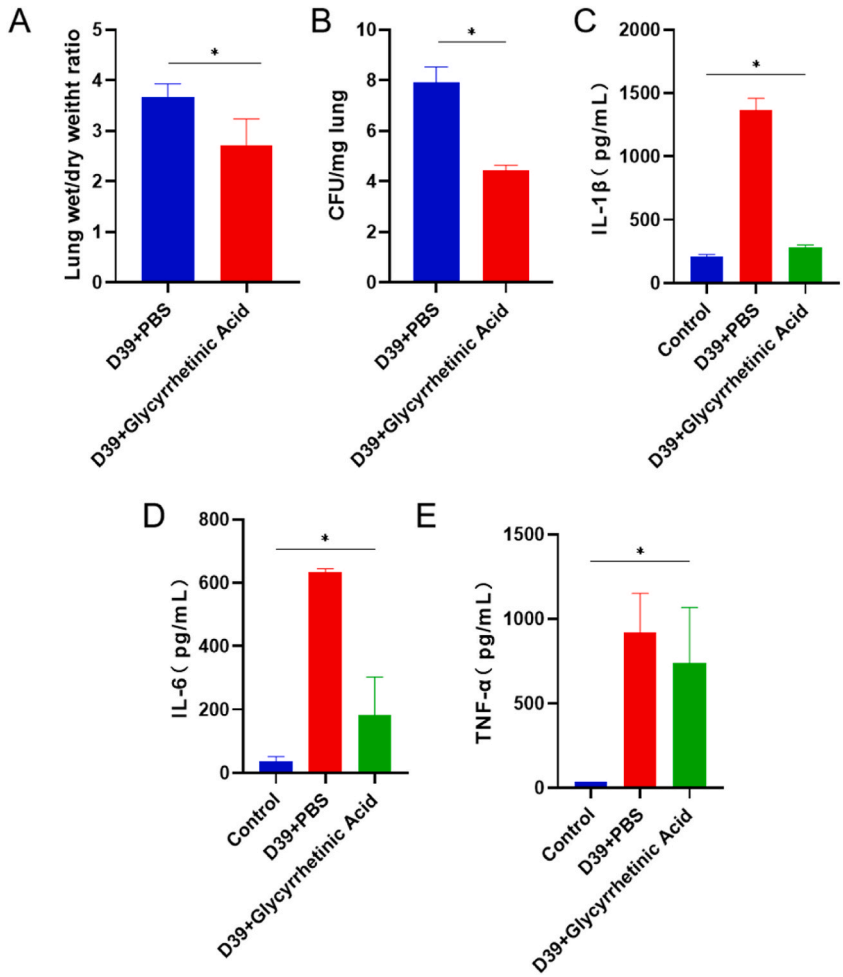

Fig. 3. Glycyrrhetinic acid affects the lung dry–wet ratio, the abundance of bacterial flora and inflammatory factors. The data are presented as the means ± S.D.s and the Significance was analyzed via a *t*-test. *, $p < 0.01$.

3. Discussion

3.1. Novel antibacterial potential of glycyrrhetinic acid based on targeting PLY

Licorice is a very sweet, moist, and soothing herb with anti-inflammatory and expectorant properties that can control coughs and have hormonal effects [24]. It also has detoxifying and liver-protective properties. Medicinally, it is used internally to treat Addison's disease, asthma, bronchitis, coughs, peptic ulcers, and arthritis [25,26]. In fact, the ethanol, ethyl acetate, acetone and chloroform extracts of licorice exhibit antibacterial activity (7–11 mm/20 μl) [27]. However, few studies have investigated the underlying cause of the antibacterial properties of licorice and identified the components involved. Glycyrrhizic acid is one of the main components. It has been shown to inhibit the cell cycle [28], induce tumor cell differentiation and apoptosis, and inhibit tumor cell invasion [29]. Glycyrrhetinic acid also has anti-inflammatory [30], antioxidant [31], antiviral [32], antiulcer [33], and antiallergic [34] activities and plays a role in the treatment of toxic hepatitis [32] and AIDS [35]. Furthermore, glycyrrhetinic acid can downregulate the expression of the inflammatory factors HMGB1, TLR4, IL-1β, TNF-α and TGF-β1 in LPS-treated RAW264.7 cells in a dose-dependent manner [30] and inhibit ANIT-induced activation of the activated NF-κB inflammatory pathway and increase the serum TNF-α concentration [36]. β-Glycyrrhetinic acid can inhibit bacterial growth and biofilm formation through supragingival plaque symbionts [37]. Eighteenβ-Glycyrrhetinic acid derivatives exhibited good inhibitory effects on gram-positive bacteria at a concentration of 2.5 μM [23]. Other studies have shown that glycyrrhetinic acid and its derivatives have strong antibacterial activity against several strains of *Staphylococcus aureus*, including MRSA [38]. However, there is still no detailed explanation for how glycyrrhizic acid inhibits bacteria, and the underlying mechanism remains unknown. In this study, *Streptococcus pneumoniae*, the main cause of pneumonia, was selected for experiments to explore the abovementioned aspects. Furthermore, PLY, an important virulence factor, is involved throughout the process of transmission, colonization, and invasion of *Streptococcus pneumoniae* [39]. The expression of PLY can increase the survival rate of *Streptococcus pneumoniae* in vitro [40]. After *Streptococcus pneumoniae* invades the human body, the release of

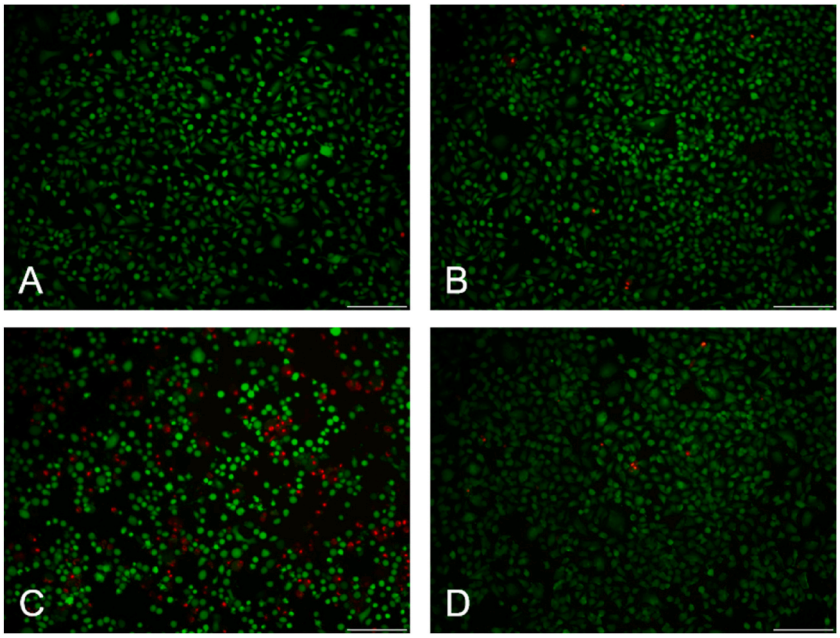

Fig. 4. Glycyrrhetinic acid alleviates PLY-mediated cell death in A549 cells. A is untreated, B is the 64 μg/ml glycyrrhetinic acid treatment, C is the PLY treatment, and D is the 64 μg/ml licorice phenol and PLY treatment. Red indicates dead cells, and green indicates live cells.

PLY can reduce the cilial beating of respiratory epithelial cells, thereby reducing the amount of *Streptococcus pneumoniae* cleared by cilia and facilitating the endocytosis of *Streptococcus pneumoniae* invading epithelial cells. Moreover, PLY also directly destroys epithelial cells. The rough method not only destroys the epithelial barrier but also helps *Streptococcus pneumoniae* effectively avoid phagocytosis by phagocytes when *Streptococcus pneumoniae* breaks through the endothelium and enters the blood [39]. PLY is composed of four domains that form a complex spatial structure. Many PLY monomers can form helical oligomers at room temperature. These oligomers present a curved protein conformation similar to the pore state [41]. This pore conformation can be directly inserted into the cell membrane, causing the osmotic pressure inside and outside the cell to change and rupture. PLY is the main virulence factor of *Streptococcus pneumoniae*. In this study, we revealed the special mechanism by which glycyrrhetinic acid inhibits the oligomerization of the four domains of PLY into a pore-forming structure (Fig. 6), thereby blocking the pore-forming activity of PLY and reducing cell death. As this mechanism does not directly inhibit the replication of *Streptococcus pneumoniae* and does not affect the survival mechanism of *Streptococcus pneumoniae*, it is essentially impossible to induce drug resistance. In fact, the antibacterial potential of this mechanism of glycyrrhetinic acid is still substantial and deserves further in-depth study.

3.2. Antibacterial potential and the development of natural compounds

At present, 700,000 people die from antibiotic resistance every year [42], and as the situation continues to worsen, it is estimated that 10 million people will die from antibiotic resistance in 2050, resulting in an economic loss of 100 trillion US dollars [42,43]. Faced with this serious situation, humans are eager to find ways to replace antibiotics in the treatment of infectious diseases to provide effective antibacterial effects in the postantibiotic era. Probiotics, bacteriophages, antimicrobial peptides, etc., can all be used as effective means to combat *Streptococcus pneumoniae* infections. Probiotics mainly stimulate the human body to respond to foreign bacteria by enhancing immunity, and their specific mechanisms and controllability need to be clarified [7–9]. Bacteriophages can effectively kill bacteria and are harmless to the human body, but each bacterium and even bacterial subspecies has unique specificity, and many resources need to be invested in studying the types of bacteriophages of each subspecies; therefore, mass production is difficult. Antimicrobial peptides also have the disadvantages of high synthesis costs, long cycles, and immature large-scale mass production [10]. Vaccination is a relatively well developed and recognized method with the greatest potential for preventing pneumococcal diseases. For example, there are currently two types of commercially available *Streptococcus pneumoniae* vaccines: pneumococcal polysaccharide (PPV) vaccines and pneumococcal conjugate (PCV) vaccines. Both are designed and developed on the basis of the capsular polysaccharide of *Streptococcus pneumoniae* and cover the most common serotypes that cause pneumococcal diseases. However, the current problem is that among the more than 90 pneumococcal capsular serotypes that have been discovered, the vaccine only covers a small number of serotypes, although the covered serotypes account for 80 % of invasive pneumococcal diseases [12,44]. *Streptococcus pneumoniae* can undergo rapid evolution by capturing homologous DNA from other pneumococci or closely related streptococcal species. In this way, noninvasive serotypes can mutate into invasive serotypes [45]. In addition, there is increasing clinical evidence that different serotypes of *Streptococcus pneumoniae* can mutate over time. An increase in pulmonary necrosis and empyema was also detected in the early population treated with the PCV7 vaccine [46–52]. Because of the use of pneumococcal

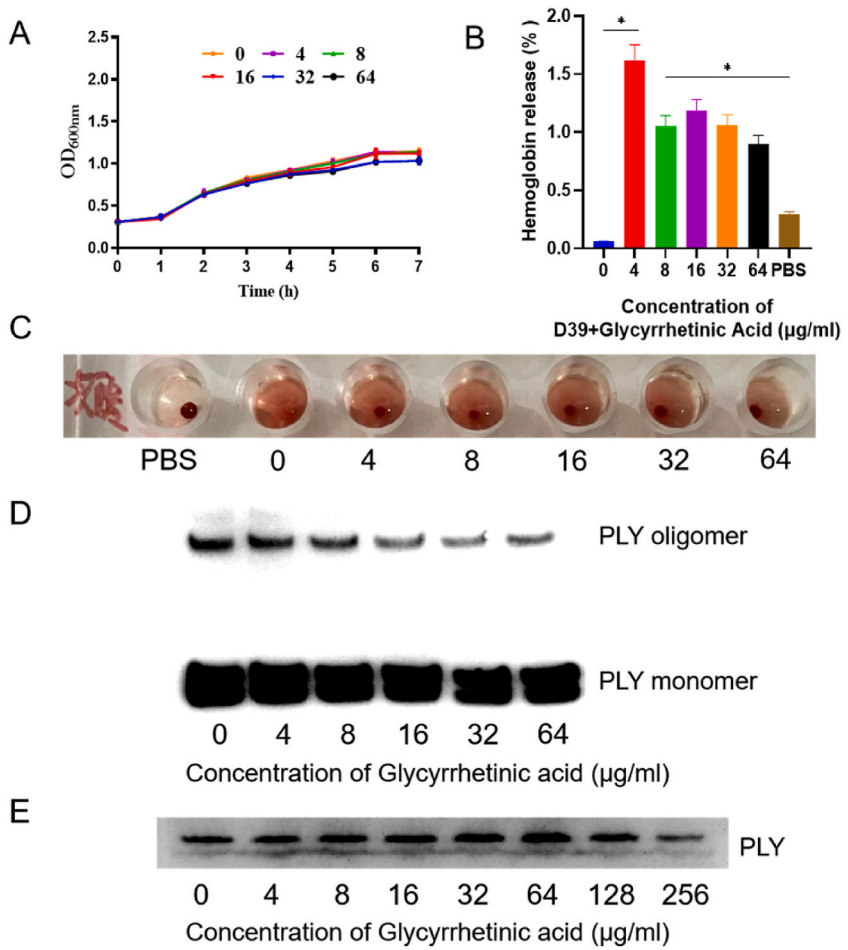

Fig. 5. Glycyrrhetinic acid can alleviate PLY-mediated erythrocyte hemolysis and reduce PLY oligomerization. A shows the effect of glycyrrhetinic acid on the growth of *Streptococcus pneumoniae,* B and C show the hemolytic activity of PLY, and D shows the PLY oligomerization mediated by glycyrrhetinic acid. E shows PLY production in the culture supernatants of D39 cocultured with various concentrations of glycyrrhetinic acid. The original materials for Fig. 5D and E are in Supplementary Materials 1 and 2. The data are expressed as the means ± S.D.s. The significance of the differences was analyzed via multiple comparison tests. *, $p < 0.01$.

vaccines, the pathogenicity of *Staphylococcus aureus* has also increased [53–55]. Therefore, new antibacterial methods are still needed. Unlike antibiotics (whose bactericidal effects include destroying bacterial membrane structure, inhibiting protein synthesis, and inhibiting the production of folic acid coenzymes, nucleic acids, and peptidoglycans), natural compounds extracted from plants can inhibit the fixation of *Streptococcus pneumoniae* and the activity of virulence factors. Notably, natural compounds do not necessarily directly inhibit the growth of *Streptococcus pneumoniae*. Without affecting the production of virulence factors by *Streptococcus pneumoniae*, reducing the activity of virulence factors, reducing the permeability of bacterial membranes, preventing the production of extracellular and intracellular microbial enzymes, interrupting bacterial metabolic pathways or destroying plaque and biofilm formation are all unique means by which natural compounds can effectively inhibit the growth of bacteria. In this study, glycyrrhetinic acid inhibited *Streptococcus pneumoniae* growth by inhibiting the activity of PLY, the main virulence factor of *Streptococcus pneumoniae* [56]. In addition, many natural compounds have effects similar to those of antibiotics. Their combined use can restore the sensitivity of bacteria to antibiotics and reduce the effective dose of antibiotics, thereby minimizing their side effects, prolonging the antibacterial spectrum of their effects, and reducing the cost of anti-infection treatment [57]. Therefore, natural compounds are reasonable and applicable candidate inhibitors of *Streptococcus pneumoniae*. Of course, identifying and isolating active compounds from plants is still a challenge in most countries with rich plant resources, but with the advancement of science and technology, more effective natural compounds can be identified and applied to medical and food fields, which will inevitably become a solution to the global problem of drug-resistant microorganisms.

4. Conclusion

Traditional herbal medicines are an enormous resource for screening drugs with specific functions and an indispensable part of

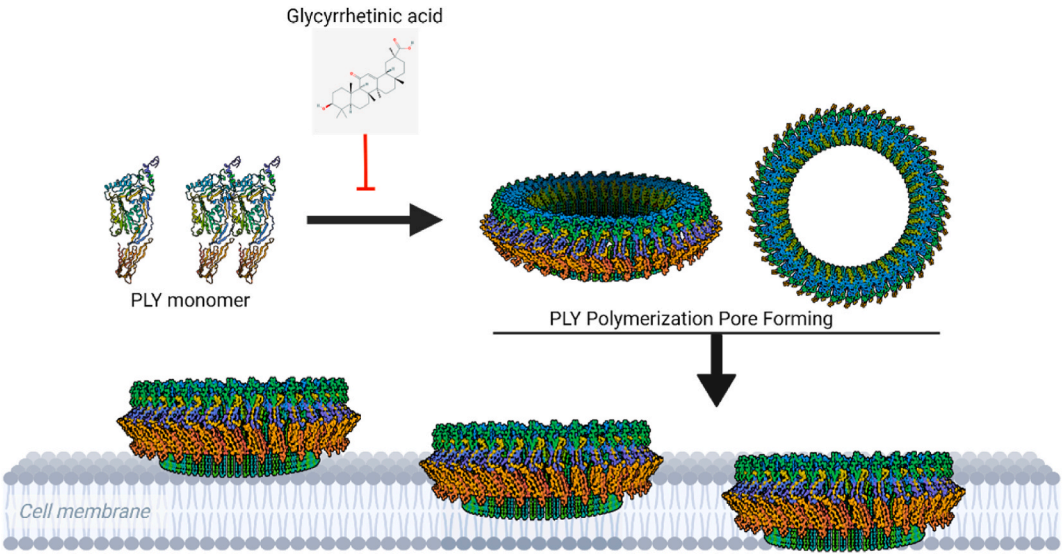

Fig. 6. Glycyrrhetinic acid reduces PLY oligomerization to form a pore-like structure.

drug development. In this study, glycyrrhizic acid, a component of the traditional Chinese medicinal material licorice, was examined, and that the results revealed the following: 1. Glycyrrhizic acid can reduce lung inflammation mediated by *Streptococcus pneumoniae* in mice. 2. Glycyrrhizic acid can reduce A549 cell death and erythrocyte hemolysis by targeting PLY, the main virulence factor of *Streptococcus pneumoniae*. 3. Glycyrrhizic acid can inhibit the process of PLY oligomerization into pore-shaped structures without inhibiting the growth of *Streptococcus pneumoniae* or the production of PLY, thereby reducing the toxicity of *Streptococcus pneumoniae* and playing a role in combating *Streptococcus pneumoniae* infection.

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Data and code availability statement

Data included in article/supplementary material is referenced in the article.

CRediT authorship contribution statement

Yan Xu: Data curation, Conceptualization. **Ying Ding:** Writing – review & editing, Supervision. **Hongji Wu:** Formal analysis, Data curation. **Donglin Li:** Software, Resources. **Yudi Li:** Writing – original draft, Visualization, Validation. **Yibo Hu:** Writing – review & editing, Writing – original draft, Visualization, Conceptualization. **Haoji Meng:** Project administration, Methodology, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e38611.

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