Anti-Inflammatory Effects and Mechanisms of Pudilan Antiphlogistic Oral Liquid

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1. INTRODUCTION

Inflammation is clinically manifested as a reddened, swollen, hot, and usually painful condition. In order to eliminate stimuli and promote tissue repair and healing, innate and adaptive immunological cells are activated and recruited, contributing to local or systemic inflammation in the body. It is a fundamental protective response of the body. Still, excessive inflammation would cause damage to the body, leading to disorders like type 2 diabetes, asthma, Alzheimer's disease, rheumatoid arthritis, colitis, cancer, and so forth. The most commonly used anti-inflammatory drugs are chemical synthetics, including steroidal anti-inflammatory drugs (SAIDs) and non-SAIDs. However, these potent therapeutics are often accompanied by irritating adverse effects such as obesity, hormonal disorders, aggravated infections, gastrointestinal irritation, liver damage, and so forth. Because anti-inflammatory drugs are a significant class in clinical treatment, second only to anti-infectious agents, developing anti-inflammatory drugs with potent efficacy and negligible side effects is an urgent issue for medical workers to deal with.

Complementary and alternative medicine, especially traditional Chinese medicine (TCM), is widely used for anti-inflammatory purposes. Inflammation is recognized as the "fire evil" in the theory of TCM, which requires herbs of cold and calm nature to tackle. Pudilan antiphlogistic oral liquid (PDL), collected in Chinese Pharmacopoeia, was a well-known TCM formula prepared from four herbs of cold and calm nature to tackle. Pudilan antiphlogistic oral liquid (PDL), Scutellaria baicalensis Georgi, Isatis tinctoria L., Corydalis bungeana Turcz., and Taraxacum mongolicum Hand.-Mazz. PDL has been clinically used for inflammatory purposes. In 2011, PDL was recognized to have anti-inflammatory effects such as pharyngitis, tonsillitis, and other respiratory infections. Previous reports showed that PDL had a therapeutic effect on pneumonia in mice infected with Streptococcus pneumoniae and had the effect of enhancing immunity of immunocompromised mice.

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Nevertheless, investigation on the underlying mechanisms of the potential protective effects of PDL against inflammation has been quite limited. Considering the complex composition and multitarget interactions of TCM, it is extremely difficult to conduct a systematic and in-depth study on the effects and mode of action of PDL. Systemic pharmacology is built to study the interaction between drugs and the body from a systemic level, which helps to study the discipline and mechanisms of the action of the drugs, especially some ancient but potent formulae. Studies have found that the construction of complex biochemical networks of living organisms such as cells presented a large-scale period from transients (such as metabolic reactions) to long-term regulation (such as cell development), from different localities within cells to tissues and organs. Large-scale leaps will also present multiple dimensions of the three-dimensional network from small chemical molecules, genes to proteins. Therefore, systemic pharmacology can solve a series of problems brought about by the complexity of TCM, which provides a new approach to predict the active ingredients and candidate targets through a holistic process of active

![Image](https://doi.org/10.1021/acsomega.1c04797)
compound screening, target “fishing”, component−target−disease (C−T−D) network construction, and target enrichment analysis.\textsuperscript{16–19}

Therefore, in this study, an innovative and systematic approach was used to predict the anti-inflammation targets of PDL comprehensively. We investigated the effect of PDL on several inflammation models and attempted to explore the underlying mechanism of PDL on inflammation \textit{via} systems pharmacology analysis, and then subsequent experimental validation was presented \textit{in vivo} and \textit{in vitro}.

\begin{figure}[h]
\centering
\includegraphics[width=\linewidth]{image}
\caption{Effect of PDL on LPS-induced ALI in mice. (A) Experimental design. (B) Wet/dry ratio of mice lung tissue. (C) MPO activity of mice lung tissue. (D) IL-6 in BALF. (E) IL-1β in BALF. (F) TNF-α in BALF. (G) Total number of cells in BALF. (H) Neutrophils count in BALF. (I) HE staining of the lung tissue, red arrowheads indicate the thickened bronchial wall (\(n = 9–12\), scale bar = 100 \(\mu\)m; magnification: \(\times 200\)). (J) ALI score. Data are the mean \pm SD, \(n = 8–12\), \#\(P < 0.01\) vs control, *\(P < 0.05\) vs model, and **\(P < 0.01\) vs model.}
\end{figure}
2. RESULTS AND DISCUSSION

2.1. PDL Markedly Alleviated the β-Hemolytic Streptococcus-Induced Inflammatory Response in Acute Pharyngitis Rats. An acute pharyngitis rat model was established as shown in Figure 1A. As shown in Figure 1B, the expression of IL-6 and IL-1β in serum significantly increased after β-hemolytic streptococcus infection, whereas PDL administration could significantly downregulate these pro-inflammatory factors. Furthermore, pathological staining showed that the pharyngeal mucosa of control rats was covered with multiple layers of squamous epithelium, with thin layers of connective tissue under the epithelium and no degeneration or necrosis of the epithelial cells after the pharyngeal infection with β-hemolytic streptococcus. In contrast, the hypopharyngeal wall tissue of the pharyngeal mucosa of model rats was infiltrated by mononuclear macrophages and neutrophils, accompanied with local hyperemia and edema. More importantly, PDL administration could relieve the infiltration of inflammatory cells in the pharyngeal tissue (Figure 1C,D). Collectively, PDL dramatically alleviated inflammatory responses in the acute pharyngitis rat model, in consistence with the clinical efficacy of PDL.

2.2. Effect of PDL on LPS-Induced Acute Lung Injury (ALI) in Mice. To confirm the effects of PDL (1.3, 3.9, and 11.7 g/kg) on inflammation of the lower respiratory tract, we

Figure 3. Effects of PDL on xylene-induced ear swelling in mice. (A) Experimental design. (B) Ear swelling of mice. (C) HE staining of mice ear tissues, red arrowheads indicate the thickened ear (n = 10, scale bar = 100 μm; magnification: ×100). (D) Statistics of inflammatory cells. Data are the mean ± SD, n = 10, ###P < 0.01 vs control, *P < 0.05 vs model, and **P < 0.01 vs model.
examined the effects of PDL on LPS-induced ALI in mice (Figure 2A). The results showed that PDL (11.7 g/kg) reduced the wet/dry ratio of mouse lung tissue (Figure 2B). Meanwhile, PDL significantly restrained the activity of myeloperoxidase (MPO) in mice (Figure 2C) and reduced the level of IL-6, IL-1β, (P < 0.01, P < 0.01, and P < 0.01), and TNF-α in bronchoalveolar lavage fluid (BALF) (Figure 2D-F). PDL could significantly reduce the number of neutrophils in BALF (Figure 2G,H). After hematoxylin and eosin (HE) staining of the lung tissue and then pathological scoring was performed, the result showed that 11.7 g/kg PDL significantly alleviated the inflammatory infiltration of the lung tissue and reduced blood exudation (Figure 2I,J).

2.3. Effect of PDL on Xylene-Induced Ear Swelling in Mice. Mice were given PDL or aspirin (ASA, 0.2 g/kg) for 7 days by intragastric administration (Figure 3A). Except for the control group, the other groups were stimulated with 30 μL of xylene to establish the acute ear swelling model (Figure 3A). The ear thickness was measured using a thickness gauge, and the ear tissue was microscopically examined after HE staining. It showed that PDL significantly decreased the ear swelling of mice (Figure 3B). The results of the lymphocyte count showed that PDL significantly reduced the number of inflammatory cells infiltrating the ear tissue (Figure 3C). HE staining showed that 3.9 and 11.7 g/kg PDL could substantially improve the inflammatory infiltration and ear swelling (Figure 3D).

2.4. Effect of PDL on Carrageenan-Induced Paw Edema in Rats. Rats were given PDL or ASA (0.2 g/kg) for 7 days by intragastric administration. Except for the control group, the rats replicated the acute paw edema model by the subcutaneous injection of carrageenan (Figure 4A). The paw volume of the rats was measured every hour, and the change curve of edema rate was drawn in Figure 4B. The results showed that 2.7 and 8.1 g/kg PDL significantly inhibited the paw edema rate of rats 2 h after administration. During the period of 3–7 h, each dose of PDL significantly inhibited the paw edema rate of rats (Figure 4C).

2.5. Effect of PDL on Acetic Acid-Induced Evans Blue Leakage. Mice were given PDL and ASA (0.2 g/kg) for 7 days by intragastric administration. All mice were injected with 2% Evans blue physiological saline solution intravenously. Mice were subsequently injected intraperitoneally with 0.6% acetic acid saline solution to replicate the capillary permeability-increasing model (Figure 5A), and mice in the control group were injected with saline. The results showed that PDL significantly inhibited the increase in the capillary permeability of mice induced by acetic acid (Figure 5B).

2.6. Predicted Active Components and Targets of PDL against Inflammation. To elucidate the mechanism of PDL against inflammation, we first utilized a systems pharmacology approach to predict the active components in PDL and their potential targets. As a result, 370 chemical ingredients of the four herbal medicines in PDL were retrieved from the traditional Chinese medicine systems pharmacology (TCMSP) database and related literatures, including 143 in S. baicalensis Georgi, 169 in L. tinctoria L., 29 in C. bungeana Turcz, and 29 in T. mongolicum Hand.-Mazz. As listed in Table S1, 45 ingredients were screened out as the active virtual candidates in PDL following the criteria **P < 0.01 vs model.** As a result, 370 chemical ingredients of the four herbal medicines in PDL were retrieved from the traditional Chinese medicine systems pharmacology (TCMSP) database and related literatures, including 143 in S. baicalensis Georgi, 169 in L. tinctoria L., 29 in C. bungeana Turcz, and 29 in T. mongolicum Hand.-Mazz. As listed in Table S1, 45 ingredients were screened out as the active virtual candidates in PDL following the criteria **P < 0.01 vs model.**

According to the target prediction system and several databases, we retrieved 185 potential targets out of the predicted 45 ingredients in PDL. Besides, 471 targets were screened out based on the keyword “inflammation” (Figure S1), and there were a total of 38 intersections between PDL targets and inflammation-related targets (Figure S2).
Network analysis provides an efficient tool for strengthening our understanding of the action mechanisms of multicomponent and multitarget TCM regulatory networks. As a result, an herb—component—target—disease (H—C—T—D) network of PDL was composed of 88 nodes and 290 edges. The top 10 ingredients of the degree value were quercetin, wogonin, luteolin, baicalein, corynoline, indirubin, acacetin, moslososoflavone, cotispine, and corycine. Also, the top 10 targets were PTGS2, NOS2, PIK3CG, F2, NOS3, PPARG, F7, ATP7B, C5, and BCL2 (Figure 6A).

Afterward, KEGG pathway analysis was carried out on the predictive target genes of PDL, through which we selected the pathways according to the P-value for further study. As shown in Figure 6B, the top 18 pathways were listed. PDL might regulate inflammation mainly through hypoxia inducible factor (HIF-1), tumor necrosis factor (TNF), nuclear factor kappa-B (NF-κB), and NOD-like receptor (NLR) pathways, and so forth.

According to the DAVID system, PI3K and p38 play pivotal roles in HIF-1 and TNF signaling, the top two pathways in our enrichment analysis (Figure S3).

2.7. Effect of PDL on Inflammation was through PI3K

We next verified the efficacy of PDL regulating these two targets on a protein level in the β-hemolytic streptococcus-induced acute pharyngitis model. As shown in Figure 7, PDL had negligible effects on total PI3K and P38, whereas their corresponding phosphorylated protein were both downregulated by PDL. It indicated that PDL might exert its anti-inflammatory influence by restraining the activation of PI3K and its downstream protein P38, in accordance with the prediction by systems pharmacology.

2.8. Effects of Wogonin and Corynoline on the NLRP3 Inflammasome Priming Phase. An NLR pathway is also one of the potential pathways for PDL to exert anti-inflammatory effects. The role of the NLRP3 inflammasome in various diseases has become the focus of attention recently. The NLRP3 inflammasome can identify pathogen-associated molecular patterns or danger-associated molecular patterns and, in turn, mediates host cells’ immune response to pathogens and cell damage. Activation of NLRP3 inflammasomes requires two steps. Transcriptional levels of NLRP3, pro-IL-1β, and procaspase-1 can be elevated by infection, stress, or injury inducers as the first signal through the NF-κB pathway.23 ATP, protozoa parasite, crystal or other substances (uric acid salt, calcium oxalate, silica, asbestos, etc.), and metabolites in the body can be used as the second signal,23 makes NLRP3 protein oligomers, ASC, and caspase 1 form the NLRP3 inflammasome. The NLRP3 inflammasome can cut pro-IL-1β into mature IL-1β, which released into extracellular would induce inflammation.24 The NLRP3 inflammasome plays a key role in inflammation and has gradually become one of the therapeutic targets. Systems pharmacology predicted that quercetin, wogonin, luteolin, baicalein, corynoline, indirubin, and acacetin in PDL were important potential active components. According to the composition analysis, quercetin, wogonin, luteolin, baicalein, and corynoline were the active components with the high content in PDL (Table S1). The effects of baicalein, quercetin, and luteolin on the activation of the NLRP3 inflammasome have been reported,25,26 but wogonin and corynoline have been rarely reported. We wondered whether these two components could inhibit the activation of the NLRP3 inflammasome to achieve the inhibitory effect of IL-1β.

3-(4,5-Dimethyl-2-thiazoyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) results showed that wogonin had no significant effect on the cell activity of primary peritoneal macrophages at 0.1—100 μM, and corynoline had no significant impact on the cell activity of peritoneal macrophages at 0.1—10 μM, while inhibited cell proliferation at 100 μM (P<0.01) (Figure 8A,B). Before stimulating peritoneal macrophages with LPS and ATP, we pretreated wogonin (10 μM) and corynoline (1 μM), which dissolved in DMSO, for 1 to 24 h. The level of IL-1β was drastically reduced by wogonin (1, 10, and 100 μM) and corynoline (0.1, 1, and 10 μM), respectively. Then, we tested the onset time of wogonin and corynoline. The level of IL-1β was significantly inhibited by 10 μM wogonin and 1 μM corynoline after incubation for 1—24 h (Figure 8C,D).

There are two signaling stages in the activation of the NLRP3 inflammasome, which are stimulated by LPS and ATP.27 The first signal is initiated by different TLR ligands, including the expression of NF-κB transcription and downstream NLRP3 and pro-IL-1β protein, induced by the LPS danger signal.28 The second signal is stimulated by ATP, which contributes to the formation of NLRP3 inflammatory complex, the activated caspase-1 shears pro-IL-1β into activated IL-1β, which leads to inflammation in the body.29 To investigate the action stage of wogonin and corynoline, we administrated wogonin and corynoline after the stimulation of LPS, then stimulated by ATP. The results showed no significant change in the release level of IL-1β (Figure 8E), indicating that the inhibition of IL-1β release by wogonin and corynoline was due to the affection of the first signal in the activation of the NLRP3 inflammasome.

On the other hand, cells were treated with 10 μM wogonin and 1 μM corynoline, respectively, for 1 h and then stimulated with LPS for a further 4 h. WB showed that wogonin and corynoline could inhibit the phosphorylation of NF-κB p65, the expression of NLRP3 and pro-IL-1β protein, which led to the reduction of downstream pro-inflammatory factors IL-1β release (Figure 8F).

2.9. DISCUSSION

As a commercial anti-inflammatory oral prescription of TCM, PDL has been widely applied in treating anti-inflammatory diseases, especially upper respiratory infection diseases.30 It has...
the functions of reducing fever and detoxifying, anti-inflammatory, and antiswelling. In order to better provide the guidance on clinical medication, we evaluated the anti-inflammatory effects of PDL on several classic inflammatory models. The results showed that PDL has a significant inhibitory effect on various types of inflammation. For instance, in an acute pharyngitis model, represents an upper respiratory infection, PDL decreased the levels of pro-inflammatory cytokines IL-6 and IL-1β in the peripheral blood and reduced inflammatory infiltration of pharyngeal tissues. In ALI model, PDL could reduce the degree of oedematous lung tissues, decrease the number of neutrophils, and inhibit the release of typical inflammatory cytokines IL-6, IL-1β, and TNF-α, which suggested PDL could inhibit the lower respiratory tract inflammation. To further investigate whether PDL can also resist nonspecific inflammation, we established three classic animal models, including xylene-induced ear swelling, carrageenan-induced acute paw edema, and capillary permeability-increasing models. Our results showed that PDL could significantly alleviate the ear swelling and reduce the inflammatory infiltration in mice ear tissues, inhibit the paw edema rate of rats, and decrease the capillary permeability in mice. These data demonstrated that PDL has a potent anti-inflammatory efficacy on both respiratory tract inflammation and nonspecific inflammation.

The potent anti-inflammatory efficacy of PDL has been verified in both clinical application and in vitro experiments. However, a systemic dissection of its underlying mechanisms is still stagnant because of the complex composition and multiple targets of PDL. Because of the holistic conception and complex composing principle of TCM, the existing methods widely used in studying western medicines are not scientifically eligible to

Figure 6. Network pharmacology analysis of PDL against inflammation (A) H−C-T-D network interaction of PDL. The H−C-T-D network of PDL was established using Cytoscape 3.7.1, which helped us identify the corresponding targets of each ingredient in PDL and get an overview on the potential modes of action of PDL on inflammation. (Red triangle: herb; purple diamond: component; orange square: target; and green circle: disease). (B) KEGG pathway analysis of the PDL-targeting genes. The DAVID Bionformatics Resources 6.8 System was utilized to perform the enrichment analysis of the potential target genes of PDL on inflammation, and the pathways with p-values ≤ 0.05 were considered for better prediction and were selected for further verification of the mechanisms of PDL against inflammation.
explore the pharmacological mechanisms of herbal formulae.\textsuperscript{31} Under these circumstances, the development of systems pharmacology (also known as network pharmacology) provides us with a novel and efficient approach to predict the active ingredients and their corresponding molecular targets. It is of significance to facilitate the modernization of TCM.

In this study, we utilized computational systems pharmacology methods for bioactive ingredients screening, target fishing, network establishment, and enrichment analysis to predict the mechanisms of PDL against inflammation. Subsequently, we verified the predictive findings with an acute pharyngitis model \textit{in vivo}. As a result, 370 chemical ingredients were retrieved based on the four herbal medicines in PDL from the TCMSp database, among which 45 components were predicted to possess bioactive efficacy. Taken baicalin and norwogonin in \textit{S. baicalensis Georgi},\textsuperscript{32,33} eupatorine and liquiritigenin in \textit{I. tinctoria} L.,\textsuperscript{34,35} dihydrosanguinarine and stylepine in \textit{C. bungeana Turcz.},\textsuperscript{36,37} and quercetin and luteolin in \textit{T. mongolicum Hand.-Mazz.}\textsuperscript{38,39} as examples, several chemical ingredients in these 45 compounds have been reported to exhibit potent anti-inflammation activities. These data, at least, partly demonstrated the rationality and scientificity of the methodology of a network pharmacology approach.

Furthermore, we identified 38 inflammation-associated genes targeted by PDL \textit{via} overlapping the 185 potential target genes into 471 inflammation-associated genes retrieved from three databases, as mentioned above. To decipher the underlying molecular mechanisms of PDL on anti-inflammation, we established an H–C–T–D network with 88 nodes and 290 edges using Cytoscape 3.7.1 software. Moreover, we performed enrichment analysis on the potential target genes to better understand the main biological processes that PDL exerted its anti-inflammatory efficacy. According to the \textit{p}-value analyzed by the DAVID Bioinformatics Resources 6.8 System, of all the pathways involved, HIF-1 and TNF pathways were ranked the top two signaling pathways.

HIF-1 is a pivotal regulator of the transcriptional response to hypoxia, a notable feature of disorders where tissues suffer from chronic inflammation.\textsuperscript{40} HIF-1 signaling is involved in multiple diseases, including arthritis, inflammatory bowel disease (IBD), asthma, cancer, and so forth.\textsuperscript{41,42} TNF is a well-recognized cytokine with crucial functions in maintaining homeostasis and mediating diseases like rheumatoid arthritis, IBD, ankylosing spondylitis, as it plays a crucial role in the process of inflammation, apoptosis, and necroptosis.\textsuperscript{33–45} Interestingly, P38 and p38 are the mutual and critical genes involved in both HIF-1- and TNF-mediated signaling pathways. As a family of lipid kinases sharing a core motif, P38ks promote the production of pro-inflammatory cytokines like IL-6 by activating NK-\textit{xB},\textsuperscript{46,47} as we found in the acute pharyngitis rat model, and prominently the phosphorylation of P38 and p38 in \textit{β}-hemolytic streptococcus-induced acute pharyngitis. The serum levels of IL-6 and IL-1β significantly increased in accordance with the enhanced p-Pi3K and p-p38 levels. Therefore, PDL possessed remarkable efficacy in ameliorating the inflammatory responses of acute pharyngitis, which might be highly correlated with deactivating P38 and p38 signaling instead of inhibiting the total protein expression and further decrease the production of pro-inflammatory cytokines in the peripheral blood. Taken together, our data confirmed the therapeutic targets of PDL predicted by computational prediction were indeed involved in anti-inflammatory mechanisms \textit{in vivo}.

Moreover, the systems pharmacology study showed that quercetin, wogonin, luteolin, baicalein, corynoline were the active components with the high content in PDL. Previous studies have demonstrated that baicalin\textsuperscript{38,49} or wogonoside,\textsuperscript{50} which have high contents in PDL, affect anti-inflammation by modulating the NLRP3 inflammasome signaling. The contents of wogonin and corynoline are also high in PDL, which rank closely followed by baicalin and wogonoside.\textsuperscript{20} Few of the studies that reported wogonin and corynoline, might play an essential role in the anti-inflammatory process. Therefore, we hypothesized that similar to baicalin and wogonoside, wogonin and corynoline might alleviate inflammation also by suppressing NLRP3 inflammasome activation. This study found that wogonin and corynoline could not suppress the release of IL-1β after activation of the first stimulation signal. However, adding compounds before LPS can effectively inhibit IL-1β release. It suggested that wogonin and corynoline inhibit the first stimulation signal of inflammasome activation against inflammation. The WB assay showed that wogonin and corynoline reduced the activation of the NF-\textit{xB}, inhibited the expression of the downstream protein involved in NLRP3 inflammasome activation, including NLRP3 and pro-IL-1β. It indicated that wogonin and corynoline inhibited the first signal of NLRP3 inflammasome activation to reduce the release of IL-1β. Actually, studies\textsuperscript{51} have shown that baicalin, baicalein, and wogonin,\textsuperscript{52} the main components of PDL, possessed anti-inflammatory effects previously. The anti-inflammatory activity is mainly due to their abilities to scavenge the reactive oxygen species and the improvement of antioxidant status by attenuating the activity of NF-\textit{xB} and suppressing the expression of several inflammatory cytokines and chemokines, including monocyte chemotactic protein-1 (MCP-1), cyclooxygenases, lipoygenases, cellular adhesion molecules, TNF, and interleukins.\textsuperscript{53} Nevertheless, the pharmacological mechanisms of PDL on anti-inflammation still needs to be further studied, and more therapeutic targets are expected to be explored.

Collectively, PDL has a potent anti-inflammatory efficacy in both respiratory tract inflammation and nonspecific inflammation animal models \textit{in vivo}. The approach of systems pharmacology successfully helped us screen out the bioactive components and predicted the potential therapeutic targets of PDL, HIF-1- and TNF-mediated signaling, which provided valid evidence for us to verify the underlying molecular mechanisms of PDL. We found that PDL downregulated HIF-1-and TNF-
mediated signaling pathways through inhibiting PI3K and p38 phosphorylation in vitro. The bioactive components of PDL including wogonin and corynoline exerted anti-inflammatory potency via inhibiting NLRP3 inflammasome activation.

3. CONCLUSIONS

The combination of systems pharmacology and further verification experiments preliminarily provided the evidence that several bioactive ingredients in PDL synergistically exerted anti-inflammation efficacy by regulating HIF-1 and TNF pathways and the NLRP3 inflammasome. Besides, our study further confirmed that systems pharmacology could significantly contribute to the comprehensive understanding of the material basis and mechanisms of actions of TCM.

4. MATERIALS AND METHODS

4.1. Reagents. PDL was provided by Jichuan Pharmaceutical Co., Ltd (1807012; Jiangsu, China). Dexamethasone (DEX) was from Shanghai ShangYao Xinyi Pharmaceutical Co., Ltd (015180207, Shanghai, China). LPS was purchased from Sigma
(35H4086, MO, USA). ASA was from Bayer Health Care Manufacturing S.r.l (B41873, Leverkusen, Germany). Corynoline (CAS: 18797-79-0, ID: AQSY-0DVP) and wogonin (CAS: 632-85-9, ID: HAS8-PMVN) were purchased from the China National Institutes for Food and Drug Control (Beijing, China). Amoxicillin (AMX) was purchased from Shanxi Tongda Pharmaceutical Co. (181202, Shanxi, China).

4.2. Animals. ICR mice (18–22 g) were purchased from the Nanjing Qinglongshan animal breeding farm (Jiangsu, China, SCXK(Su) 2017-0001) and Zhejiang Vital River Laboratory Animal Technology Co. Ltd. (Zhejiang, China, SCXK(Zhe) 2018-0001). SD rats (180–220 g) were purchased from Shanghai Jiesijie Laboratory Animal Technology Co. Ltd. (Shanghai, China, SCXK(Hu) 2018-0004) and Beijing Vital River Laboratory Animal Technology Co. Ltd., (Beijing, China). All animals were raised at Nanjing University of Chinese Medicine under specific pathogen-free conditions at 22–25 °C and 40–65% humidity. All procedures involving animals were approved by the Animal Care and Use Committee of Nanjing University of Chinese Medicine and were performed strictly according to the Guide for the Care and Use of Laboratory Animals.

4.3. Acute Pharyngitis Model Establishment and Administration. Sixty SD rats, half male and half female, were randomly allocated into six groups including control, model, AMX at 0.18 g/kg, PDL at 0.9 g/kg, PDL at 2.7 g/kg, and PDL at 8.1 g/kg groups. Administration was performed from day 1 to day 7, and mice in the control and model groups were given an equal volume of normal saline once a day. The acute pharyngitis rat model was established by injecting 4 × 10^7 CFU β-hemolytic streptococcus (identification number: 32210; obtained from the National Institutes for Food and Drug Control, NIFDC, Beijing, China) in 0.1 mL of normal saline into the pharyngeal mucosa on day 5 and day 6. On day 7, mice were euthanized, and subsequently, the pharyngeal mucosa was embedded in paraffin. Afterward, pharyngeal mucosa was dissected at a thickness of 5 µm and 6 µm. Histological analysis was performed with HE staining. The thickness of mouse ears was measured using a thickness gauge, and the ear tissue was microscopically examined after HE staining.

4.4. All Model Establishment and Administration. Mice were given PDL (1, 3, 9, and 11.7 g/kg) and DEX (5 mg/kg) for 7 days by intragastric administration. Except for the control group, each group was treated with LPS physiological saline solution (5 mg/kg) for mouse airway instillation to replicate the ALL model.

4.5. Acute Ear Swelling Model Establishment and Administration. Mice were given PDL (1, 3, 9, and 11.7 g/kg) and ASA (0.2 g/kg) for 7 days by intragastric administration. Except for the control group, the other groups used 30 µL of xylene to smear the mouse auricle to reproduce the acute ear swelling model. The thickness of mouse ears was measured using a thickness gauge, and the ear tissue was microscopically examined after HE staining.

4.6. Acute Paw Edema Model Establishment and Administration. Rats were given PDL (0.9, 2.7, and 8.1 g/kg) and ASA (0.2 g/kg) for 7 days by intragastric administration. Except for the control group, the rats in the other groups replicated the acute paw edema model by subcutaneous carrageenan injection. The paw volume of the rats was measured every hour. Swelling rate (%) = (foot volume after inflammatory−foot volume before inflammatory)/foot volume before inflammatory × 100%.

4.7. Capillary Permeability Model Establishment and Administration. Mice were given PDL (1, 3, 9, and 11.7 g/kg) and ASA (0.2 g/kg) for 7 days by intragastric administration. Mice were injected with 2% Evans blue physiological saline solution in the tail vein. Except for the control group, mice in each group were subsequently injected intraperitoneally with 0.6% acetic acid saline solution to replicate the capillary permeability-increasing model. The capillary permeability was determined according to the content of Evans blue in the abdominal fluid of mice.58

4.8. Cell Culture. Primary peritoneal macrophages cells were extracted from mice as described previously59 and seeded into 96-well plates at a density of 5 × 10^3 per well, cultured with 1640 medium containing 10% fetal bovine serum and 1% penicillin/streptomycin. Cells were pretreated with wogonin (0.1, 1, 10, and 100 µM), corynoline (0.1, 1, 10, and 100 µM), or medium with 0.05% DMSO as the vehicle (control) for 24 h, then test the viability using the MTT assay.

Peritoneal macrophages cells were cultured under the same condition as above. Cells were treated with wogonin or corynoline before or after adding LPS. ELISA detected the level of IL-1β, the expressions of NLRP3, NF-κB p65, p-P65, and pro-IL-1β were detected by WB.

4.9. Lung Dry/Wet (W/D) Determination. Mice were anesthetized by intraperitoneal injection of 50 mg/kg pentobarbital sodium, before the rats were euthanized using CO2 as previously described. Mice were sacrificed to obtain lung tissues, which were drained and measured for wet weight. Afterward, lung tissues were incubated at 80°C for 4 h, and then tissues were weighed to obtain measures of dry weight.

4.10. MPO. The measures of the activity of MPO as the instructions of the kit described. MPO activity (U/tissue wet weight) = (measured OD value − control OD value)/11.3 × sample volume (g).

4.11. Neutrophils Count. The total number of cells in BALF was counted, 0.02 mL was taken for smear and Regann’s staining, and cell classification and counting were performed under a microscope.

4.12. Determination of IL-6, IL-1β, and TNF-α Production. Production of IL-6 (4300713, ebioscience, CA, USA), IL-1β (B288648, Biolegend, CA, USA), and TNF-α (B288663, Biolegend, CA, USA) in BALF were measured using ELISAs according to the manufacturer’s instructions. Total protein levels in the homogenates were examined using a bicinchoninic acid (BCA) kit (041318180603, Beyotime, Shanghai, China). IL-6, IL-1β, and TNF-α protein levels were assessed with the formula: concentration of IL-6, IL-1β, and TNF-α in the homogenate/total protein (pg/mg).

4.13. Histological Analysis. Histological analysis was performed as described previously. Pharyngeal mucosa was inflated with 4% paraformaldehyde and fix in formalin. Afterward, pharyngeal mucosa was embedded in paraffin and dissected at a thickness of 5 µm for HE staining. Sections were then stained according to a standard protocol. Stained sections were analyzed by pathologist using single-blind method. The following observations were recorded: (1) the degeneration and necrosis of epithelial cells, and the inflammatory cells infiltration in or under the epithelium; (2) the exudate within the pharynx cavity; (3) the hyperemia and dropsy of the pharyngeal wall or inflammatory cell infiltration within the pharyngeal wall. The degree of inflammation was evaluated semiquantitatively using the scores of 0–3 to indicate no, mild, moderate, and severe inflammation.

4.14. Western Blotting. WBs were performed as described previously. Tissue homogenates and cells were prepared in lysis buffer (Beyotime, Shanghai, China), consisting of 1 nM...
phenylmethanesulfonyl fluoride (Beyotime, Shanghai, China) to extract protein, and the protein concentration was detected using an BCA protein detection kit. An equal amount of protein solubilization was added to the band and separated by 10% sodium dodecyl sulfate polyacrylamide gel. Then the isolated protein was transferred to a polyvinylidene fluoride membrane (Millipore Corporation, Darmstadt, GER). After incubation in 5% skimmed milk containing 0.1% TBST for 1 h, the membrane was incubated with primary antibodies against mouse P13K (A1520, Santa Cruz Biotechnology, CA, USA), phospho-P13K(D1718, Santa Cruz Biotechnology, CA, USA), p38-C0218, Santa Cruz Biotechnology, CA, USA), and phospho-p38 antibody (I1719, Santa Cruz Biotechnology, CA, USA), NLRP3(A27381510, Adipogen, Liestal, SUI), NF-κB p65-(#F2912, Santa Cruz Biotechnology, California, USA), p-NF-κB P65 (16, Cell Signaling Technology, Boston, MA, USA), and Pro-IL-1β (#12242, Cell Signaling Technology, Boston, MA, USA) or GAPDH (BC004109, Proteintech, PA, USA) in 1:1000 dilution overnight at 4 °C. Then, the secondary antibodies were added, and the membrane was incubated at room temperature for 1 h. In each sample, the target protein expression level was normalized to GAPDH.

### 4.15. Systems Pharmacology.

The active compounds of PDL were obtained from the TCMSP database (http://tcmsp. com/index.php), a unique system pharmacology platform designed for herbal medicines. Then, four in silico ADME models, including human oral bioavailability (OB), drug-likeness (DL), lipophilic prediction (A log P), and small intestinal epithelial cell permeability prediction (Caco-2) were employed to explore the candidate compounds in PDL. The threshold values for these screening models were set to BO ≥ 30%, DL ≥ 0.18, A log P ≥ 5, and Caco-2 ≥ −0.4, respectively. The compounds which satisfy all the criteria are listed as candidate molecules. We downloaded the 3D structures of the screened active ingredients in PDL on PubChem (https://pubchem.ncbi.nlm.nih.gov/) or on Sci-Finder (http://scifinder.nist.gov) and then uploaded them to PharmMapper (http://www.lilab-ecust.cn/pharmmapper/index.html), a pharmacophore matching and potential target identification platform. The active ingredient performs reverse docking in the server to fish the potential targets corresponding to the chemical components. Then, we collected inflammatory targets from three sources. One was the Therapeutic Target Database (TTD, http://db.idrblab.net/ ttd/), a database that provides information about the known and explored therapeutic protein and nucleic acid targets, the targeted disease, pathway information, and the corresponding drugs directed at each of these targets. Another resource was the DrugBank database (https://www.drugbank.ca, version 4.3), a unique bioinformatics and cheminformatics resource that combines detailed drug data with comprehensive drug target information. The other one is DisGeNET (https://www.disgenet.org), a discovery platform containing one of the largest publicly available collections of genes and variants associated with human diseases. We selected the keyword "inflammation" used for drugs approved by the Food and Drug Administration (FDA) to treat inflammation and human gene/protein targets. The targets corresponding to the potential ingredients in PDL and the targets corresponding to inflammation were taken as the intersections. The establishment of the C−T−D network using Cytoscape 3.7.1 software could help identify each compound’s protein targets and understand the mechanism of action of multicomponents and multitargets in TCM. The predicted targets of PDL were analyzed by the KEGG pathway using the DAVID Bioinformatics Resources 6.8 System (https://david. nciifcr.gov/), and p-value ≤ 0.05 was considered to have a significant enrichment effect.

### 4.16. Statistical Analysis.

Data are expressed as the mean ± SD. One-way ANOVA analysis was used for multiple group comparisons using GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA). Values of P < 0.05 indicated statistical significance.

### ASSOCIATED CONTENT

* Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c04797.

The known therapeutic targets in inflammation, the intersections between the potential targets of PDL and the inflammation-related therapeutic targets previously reported, the genes involved in HIF-1 and TNF signaling pathways, pharmacokinetic parameters of 45 predicted components in PDL (PDF)

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G.T. and M.H. designed the study and helped with data analysis; X.G. and K.B. performed data analysis and manuscript writing; X.Y. performed the experiments and systems pharmacology data analysis; and Y.Z., Y.X., and J.Z. were involved in data retrieval.

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