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ORIGINAL RESEARCH

Herbacetin Inhibits Asthma Development by Blocking the SGK1/NF-κB Signaling Pathway

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Background: Asthma severely interferes with people's lives through coughing, wheezing and inflammation of the lungs. Herbacetin is a class of natural compounds that inhibit the development of inflammation. However, whether Herbacetin inhibits asthma has not been definitively studied.

Methods: Lipopolysaccharides (LPS)-induced lung epithelial (BASE-2B) cells injury model was established, and then the relief of damaged BASE-2B cells with different concentrations of Herbacetin was examined. The cell counting kit (CCK8) was used to detect the effect of Herbacetin on the proliferation ability in ovalbumin (OVA)-induced asthma mice model, and Western Blot and flow cytometry were used to detect the effect of Herbacetin on the apoptosis in OVA-induced asthma mice model. Additionally, pulmonary pathology was detected by HE and Masson staining, and serum inflammatory factors were detected by alveolar lavage fluid.

Results: Herbacetin reduces BESA-2B cells induced by LPS level of inflammation, and reactive oxygen species (ROS) generation, inhibits cell apoptosis, promotes cell proliferation, OVA-induced mice lung histopathology test HE staining, serum inflammatory factors show the same results. Western Blot shows that Herbacetin regulates the expression of Caspase-3, Bax, and Bcl-2. SGK1 overexpression increased the rate of apoptosis, and Herbacetin reversed this phenomenon. By silencing the expression of SGK1, it was found that Herbacetin was an inhibitor of SGK1, which could inhibit the NF-κB/p-P65 pathway in asthmatic airway inflammation.

Conclusion: Herbacetin reduces pro-inflammatory cytokine levels by inhibiting the SGK1/NF-κB pathway. Our data suggest that Herbacetin has a significant anti-inflammatory effect on asthma and can be used as a potential therapeutic agent.

Keywords: asthma, herbacetin, SGK1, NF-κB, anti-inflammatory

Introduction

Asthma is a chronic inflammatory respiratory disease. Epithelial cells and endothelial cells participate in the inflammatory response by releasing inflammatory mediators and cytokines, which not only narrows and blocks the airway, but also contributes to airway hyperresponsiveness.[1] Asthma has the potential to induce lung cells to produce a significant number of deformed leukocytes in their nuclei, resulting in oxidative stress harm to the respiratory tract and damage to the peripheral vascular endothelium. This can ultimately lead to severe lung damage and worsen the symptoms of bronchial asthma. The pathogenesis of asthma is closely related to the release of inflammatory mediators caused by immune imbalance. Cytokines IL-4, IL-5, IL-9, and IL-13 secreted by Th2 lymphocytes can mediate allergic inflammation of the airways, pro-inflammatory cytokines TNF-α and IL-1β can aggravate the level of inflammation, and oxidative stress can amplify the inflammatory response.[2]

Serum/glucocorticoid-regulated kinase 1 (SGK1) has been found to be overexpressed in a variety of autoimmune diseases.[3] The SGK1 inhibits autoimmune responses and improves self-tolerance by fostering the formation of regulatory T cells and neutralizing Th17 cells. Consistent with the expression of the pro-inflammatory cytokine IL-6 in mouse CRS models, SGK1 is highly expressed in Th2 and Th9 cells and plays a key role in increasing inflammation and the occurrence of tissue fibrosis.[4,5] In asthmatic diseases, SGK1 can lead to increased inflammation and oxidative stress, which can induce apoptosis of airway epithelial cells and aggravate asthma disease.

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The Sedum plant is the main source of extraction of Herbacetin glycosides. The dry root and rhizome of Sedum possess beneficial properties of nourishing and strengthening, as well as clearing heat and moistening the lungs. Recent pharmaceutical research has shown that Herbacetin has the ability to enhance lung function in asthmatic rats, which could be attributed to its notable antioxidant and anti-inflammatory effects. Herbacetin structure contains five phenolic hydroxyl groups and has strong antioxidant activity in vitro. It can effectively remove free radicals, inhibit the effect of oxidative damage to proteins, and stabilize the REDOX state in cells.[6,7] Recent studies have shown that Herbacetin is an SGK1 inhibitor, Herbacetin is a promising lead compound as SGK1inhibitor.[8] Although clinical studies have been conducted on the treatment of various respiratory diseases, research specifically on the treatment of bronchial asthma has been lacking. However, further experimental demonstrations are needed to determine whether Herbacetin can alleviate asthma progression.

In this study, we used an OVA-induced asthma model to explore the potential therapeutic effects of Herbacetin, known for their strong anti-oxidant and anti-inflammatory activity. Our aim was to investigate the mechanism of action of Herbacetin by assessing its impact on oxidative stress and inflammatory factors in the lungs of asthma rats. The findings of this study hope to establish a foundation for the future clinical application of Herbacetin.

Methods

Chemicals and Solvents Purchasing

Herbacetin was purchased from Tongtian Biotechnology (Shanghai, China) and prepared as a 100 mM stock in dimethyl sulfoxide (DMSO). The DMSO made up about 0.05% of the volume of the culture medium. Fetal bovine serum (FBS) and cell culture medium were obtained from Gibco (Gaithersburg, MD, USA). Small interfering (si)-SGK1, Lentivirus (LV)-SGK1, were synthesized by JiKai Gene (Shanghai, China) transduced according to the manufacturer's instructions.

Cells and Animals

BASE-2B cells were obtained from the American Type Culture Collection (Manassas, VA, USA). These cells were maintained in RPMI 1640 medium (Sigma, St. Louis, MO, USA) supplemented with 100 U / mL streptomycin (Gibco, Carlsbad, CA, USA), 100 μg/mL penicillin (Gibco) and 10% fetal bovine serum (Gibco) in an atmosphere of 5% CO_2. The cell experiment was divided into five groups, in which four groups of BASE-2B cells were treated with 2ng/mL LPS for 24h, and then different concentrations of Herbacetin (5, 10, and 20 μm) were added to incubate the LPS treated BASE-2B cells for 24h. In the Control group, equal volume of normal saline was added.

Total 25 C57BL/6J mice were obtained from Shanghai Slac Lab-Oratory Animal Technology Co. China. All animals were fed a standard laboratory diet and housed in a 12 h light/dark cycle at 25 ° C with unrestricted access to food and water for the duration of the experiment. Twenty mice were randomly selected to induce an asthma model by intraperitoneal injection of OVA and $Al(OH)_3$ every 7 days for 28 consecutive days.[9] The other 5 mice were given the same amount of saline as the Control group. After 28 days, 15 asthma model mice were randomly selected to receive different doses of Herbacetin (25, 50, 100mg/kg), once every 7 days for 14 consecutive days, in which Herbacetin was dissolved in saline solution. The other 5 mice asthma models were given the same amount of saline. The animal models established according to the above methods were divided into Control group, OVA+saline group, OVA+HBN-25 group, OVA+HBN-50 group and OVA+HBN-100 group.

Cell Counting Kit (CCK8)

At 24 h of cell culture, 10 μL of 0.5 mg / mL CCK8 solution was added to each well for 1 hour each time at a temperature of 37 ° C, according to the kit instructions. The solution was placed on the special shaker of the culture plate for appropriate turns, and then the optical density value of each well at a wavelength of 490 nm was measured by a multifunctional microplate reader, and the inhibition rate of cell proliferation was calculated.

Colony Formation Assay

Total 5×10^3 cells were placed in 6-well plates and cultured with conditioned medium according to the grouping, and the medium was changed every two days. When the cells grew to no less than 50 cells per colony, the cells were fixed with 4% paraformaldehyde, stained with crystal violet, and photographed under a microscope for statistics.

Flow Cytometry

The Annexin V-FITC / PI double staining method was used for detection (TMR Red, Roche, Basel, Switzerland). Cells were seeded in 6-well plates at a density of 1×10^5 cells/well and cultured for 24 h, then conditioned medium was given to culture according to grouping, with three parallel cells in each group. After continuous culture for 24 h, floating cells and adherent cells were collected in the supernatant and the cells were washed three times with PBS. According to the apoptosis detection kit, 5μL of Annexin V-FITC and PI reagent were successively added to the cell suspension in the dark, and the collected cells were cultured at room temperature in the dark for 15 minutes. Flow cytometry (BD Biosciences, USA) was used to detect cell apoptosis, and Flowjo10 software (Tree Star, San Carlos, CA) was used to analyze the rate of apoptosis of each group.

Detection of Intracellular Levels of Reactive Oxygen

Dihydroergotamine (DHE, Key Gen Bio TECH, China) was used to measure total ROS levels according to the manufacturer's guidelines. In brief, 10 μM of DHR working solution was added to the wells containing KGN and incubated for 1 h in the dark. A NIKON Eclipse 80i fluorescence microscope was used to observe the intensity of the staining.

TdT-Mediated dUTP Nick End Labeling (TUNEL) Assay

Cells were divided into groups, cultured in conditioned medium for 24 hours to 80% confluence, fixed with 4% paraformaldehyde, incubated with permeable immunostaining solution, prepared with TUNEL staining working solution, added in turn for 60 minutes, added antiquench agent, observed and counted under a microscope (BX51, OLYMPUS, Tokyo, Japan).

Enzyme-Linked Immunosorbent Assay (ELISA)

The expression levels of the interleukin-6 (IL-6), interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) were quantitatively detected. After the conditional model was treated according to the group, samples of each group were collected, the suspension was prepared according to the requirements, the ELISA detection reagent was prepared and the ELISA detection was performed according to the requirements.

Hematoxylin/Eosin (HE) Staining

The tissues were fixed in 4% formalin buffer and embedded in paraffin. For histochemical analysis, paraffin blocks were sectioned into 5-μm-thick slices and subjected to HE staining.

Immunofluorescence (IF) Assay

The tissues were fixed with 4% formaldehyde for 30 min, permeabilized with 0.5% Triton X-100 for 10 min and blocked with goat serum for 30 min. The cells were then incubated with primary antibody at room temperature for 3 h and goat anti-rabbit secondary Alexa 555(Cell Signaling Technology) for 1 h. After the nuclei were counterstained with DAPI, the slides were viewed by immunofluorescent microscopy.

Immunohistochemistry (IHC) Staining

Typically, the antigen extraction process involves initial hydration of paraffin sections, followed by embedding, subsequent incubation with 3% hydrogen peroxide, and finally incubation with citrate buffer. After blocking with bovine serum albumin (BSA) at a concentration of 5%, tissues were treated with primary and secondary antibodies. Subsequently, the tissue samples were subjected to incubation with an HRP substrate solution, followed by a DAB substrate chromogen solution, in a sequential manner during the final stage.

Western Blot

Protein samples were prepared according to groups, and total proteins from cells were extracted. The BCA method was used for protein quantification, and the proteins were denatured at high temperature. Twenty micrograms of protein was resolved by SDS / PAGE and then transferred onto the PVDF membrane, hybridized with antibodies of Caspase-3 (1:1000), Bax (1:2000), Bcl-2 (1:1000), NF-κB p65 (1:1000), p-P65 (1:2000), SGK1 (1:500), phosphorylated(p) SGK1 (1:500), and GAPDH (1:1000) (all obtained from Abcam, Cambridge, MA) at 4 ° C overnight. Horseradish peroxidase conjugated secondary antibody (1:2000, Abcam, Cambridge, MA), Detection was performed using enhanced chemiluminescence reagent (Amersham ECL prime, Buckinghamshire, UK).

Statistical Analysis

Each and every statistical analysis was performed using GraphPad Prism 9 (CA, USA). The mean and standard deviation format is often used to present quantitative findings from studies utilizing biological replicates. A two-tailed Student's *t*-test was used to analyze the quantitative data, and p-values of less than 0.05 were considered statistically significant. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.

Results

Effect of Herbacetin in vitro

Figure 1A shows the structural formula of Herbacetin. Next, we investigated the repair effect of Herbacetin on the BESA-2B bronchial asthma cell model induced by LPS. The cytotoxic effect of Herbacetin was evaluated by CCK8 assay at 24 h after treatment with different doses of the Herbacetin. This analysis showed that Herbacetin did not affect BASE-2B cell viability in the lung epithelium at concentrations of 5, 10, and 20μm (Figure 1B). Herbacetin reduced ROS production in a dose-dependent manner compared to the control group (Figure 1C). The TUNEL demonstrated the same results (Figure 1D). To investigate the effect of Herbacetin on cell cloning, Herbacetin pretreatment significantly enhanced BASE-2B cell proliferation. The cells were stimulated with of LPS (2 ng/mL) for 24 h and then treated with different concentrations of Herbacetin (5, 10, and 20 μm) for 24 h (Figure 1E). Flow cytometry was used to detect the rate of apoptosis of BASE-2B cells in the 5 groups (Figure 1F). Compared to the saline group, the results showed that the protein expression level of Bcl-2 increased significantly in the Herbacetin intervention group. On the contrary, the expression level of the Caspase-3 and Bax proteins was significantly reduced (Figure 1G). p-SGK1 and p-65 showed the same trend (Figure 1H), indicating that the apoptosis rate of BASE-2B cells was reduced by Herbacetin.

Effect of Herbacetin in vivo

Herbacetin alleviates lung inflammation in OVA-induced bronchial asthma. The control group received normal saline and Herbacetin (25, 50, 100mg/kg) was administered intraperitoneally (Figure 2A). It improved the number of inflammatory cells in the bronchoalveolar lavage fluid (NALF) and the secretion of TNF-α, IL-1β and IL-6 in BALF (Figure 2B). HE staining showed that Herbacetin could improve the degree of lung injury in OVA-induced asthma mice, and the degree of airway inflammation was significantly alleviated (Figure 2C). Masson showed that the positive area around the trachea was significantly increased in OVA-induced asthma mice. The degree of collagen deposition in the saline group was significantly higher than in the Herbacetin intervention group (Figure 2D).

The results of IHC labeling showed that decreased expression of p-P65 was induced in the Herbacetin intervention group, thus inhibiting the inflammatory response (Figure 3A). DHE showed that the saline group was significantly higher than the Herbacetin intervention group. Herbacetin alleviated the increase in the level of oxidative stress induced by OVA and restored antioxidant capacity (Figure 3B). The results of the double immunolabeling showed that Herbacetin regulated the expression level of SGK1 and downregulated the expression level of p-p65 in a concentration-dependent manner (Figure 3C). The apoptotic pathway of Caspase-3 cells was regulated, the expression of Bax was up-regulated and the expression of Bcl-2 was down-regulated in Herbacetin intervention group (Figures 3D). Herbacetin reduced lung tissue injury in a dose-dependent manner (Figures 3E).

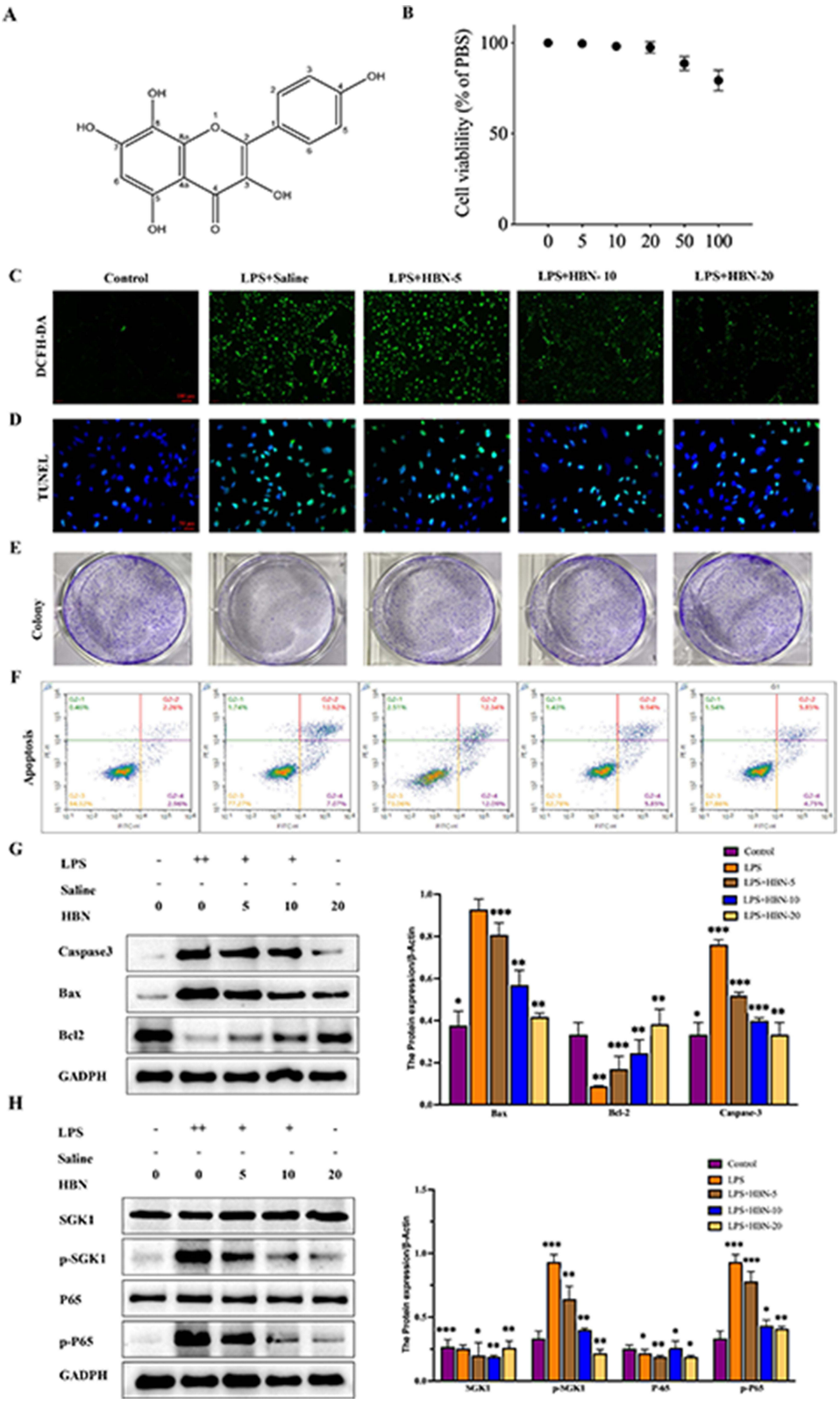

Figure 1 Protective of Herbacetin in BESA-2B cells. (**A**) Molecular structure of Herbacetin; (**B**) Cells were pretreated with Herbacetin for 2h, and then rinsed thrice with PBS. Subsequently, the pretreated cells then were incubated with LPS for another 24h, The cytotoxic of Herbacetin was examined after incubation 24h treatment using CCK8 assay; (**C**) Intracellular ROS levels were indicated by DCFH-DA fluorescence intensity and detected by high content screening; (**D**) Confocal analysis of cells by TUNEL (green) and DAPI (blue). (**E**) Cells were cultured until the number of cells in the vast majority of individual clones was greater than 50 and cell clones were examined. (**F**) Representative flow cytometry scatterplots of PI (Y-axis) vs Annexin V-FITC (x-axis), and cells were analyzed using Annexin-V-FITC apoptosis detection kit by flow cytometry. (**G**) Cell lysates were prepared and analyzed by western blotting with the apoptosis-related antibodies, n=4 for each group. (**H**) Cell lysates were prepared and analyzed by western blotting with the SGK, P-SGK, P65, P-P65 antibodies, n=4 for each group. All data were analyzed using one-way ANOVA and data were expressed as means ± SD, *p < 0.05, **p < 0.01, ***p < 0.001.

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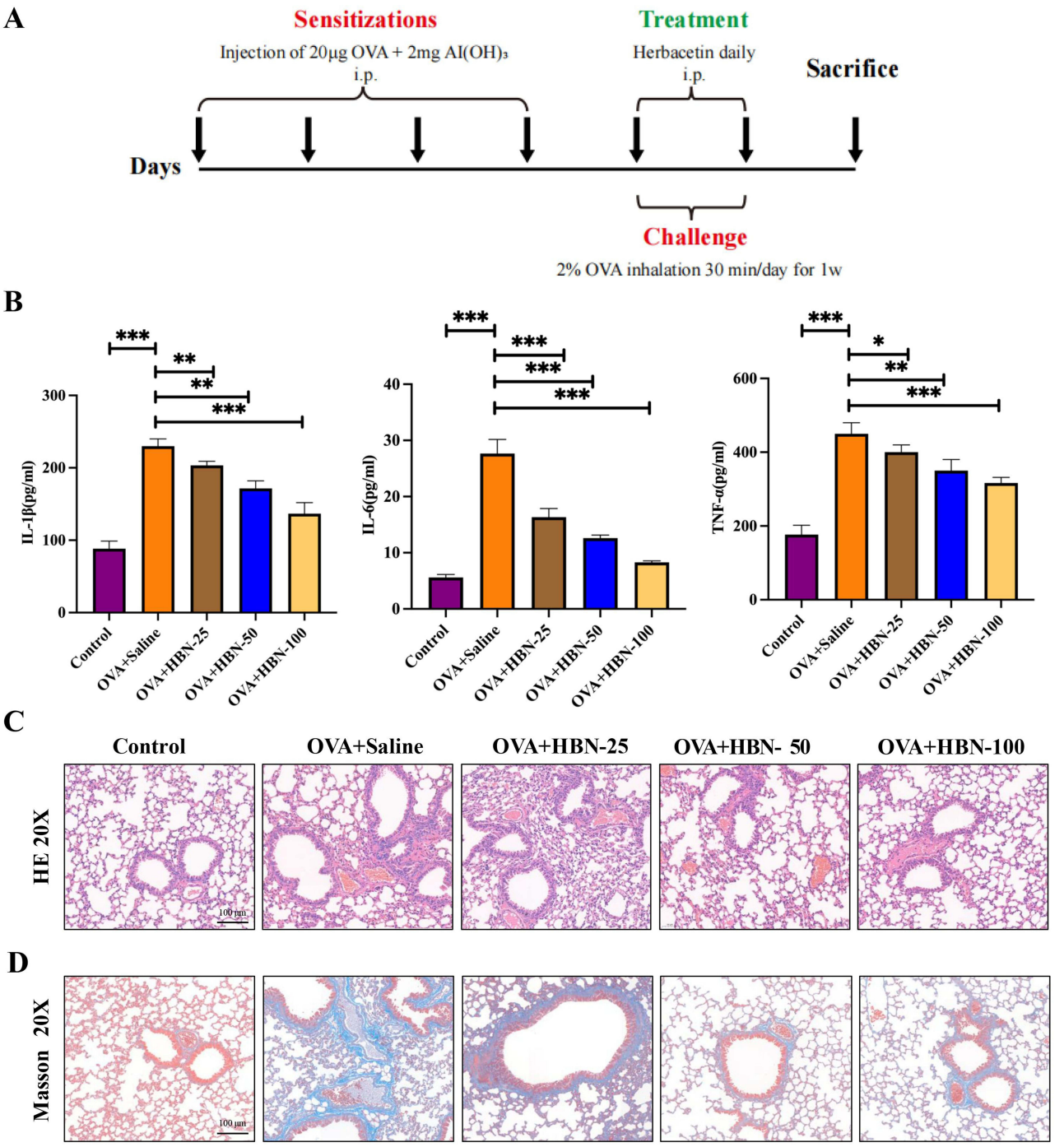

Figure 2 Protective of Herbacetin on OVA-induced asthma in mice. (**A**) Schedule of animal treatments. Sensitization: 20 μg OVA+2 mg Al $(OH)_3$ was given by intraperitoneal injection. Treatment: Herbacetin daily was given by intraperitoneal injection and 2% OVA inhalation 30min/day for one week. The mice were divided into 5 groups, n=5 for each group. (**B**) The expression of inflammatory factors in bronchoalveolar lavage fluid of mice was detected by ELISA. (**C** and **D**) Pulmonary pathological staining HE (20X) and Masson. *p < 0.05, **p < 0.01, ***p < 0.001.

Herbacetin Inhibited the Expression of SGK1 in vitro

Herbacetin exerts its effect by inhibiting SGK1 expression in lung epithelial cells. To clarify the mechanism of action of Herbacetin in the regulation of lung epithelial cells, the expression of SGK1 was first knocked down. ELISA was used to detect the expression levels of IL-6, TNF-α and IL1β in the cell supernatant. The results showed that IL-6, TNF-α and IL-β were significantly negatively regulated in the si-SGK1+Herbacetin group (Figure 4A). Compared to the blank control group, the oxidative stress levels of the Herbacetin intervention group and the silence group decreased after LPS induction,

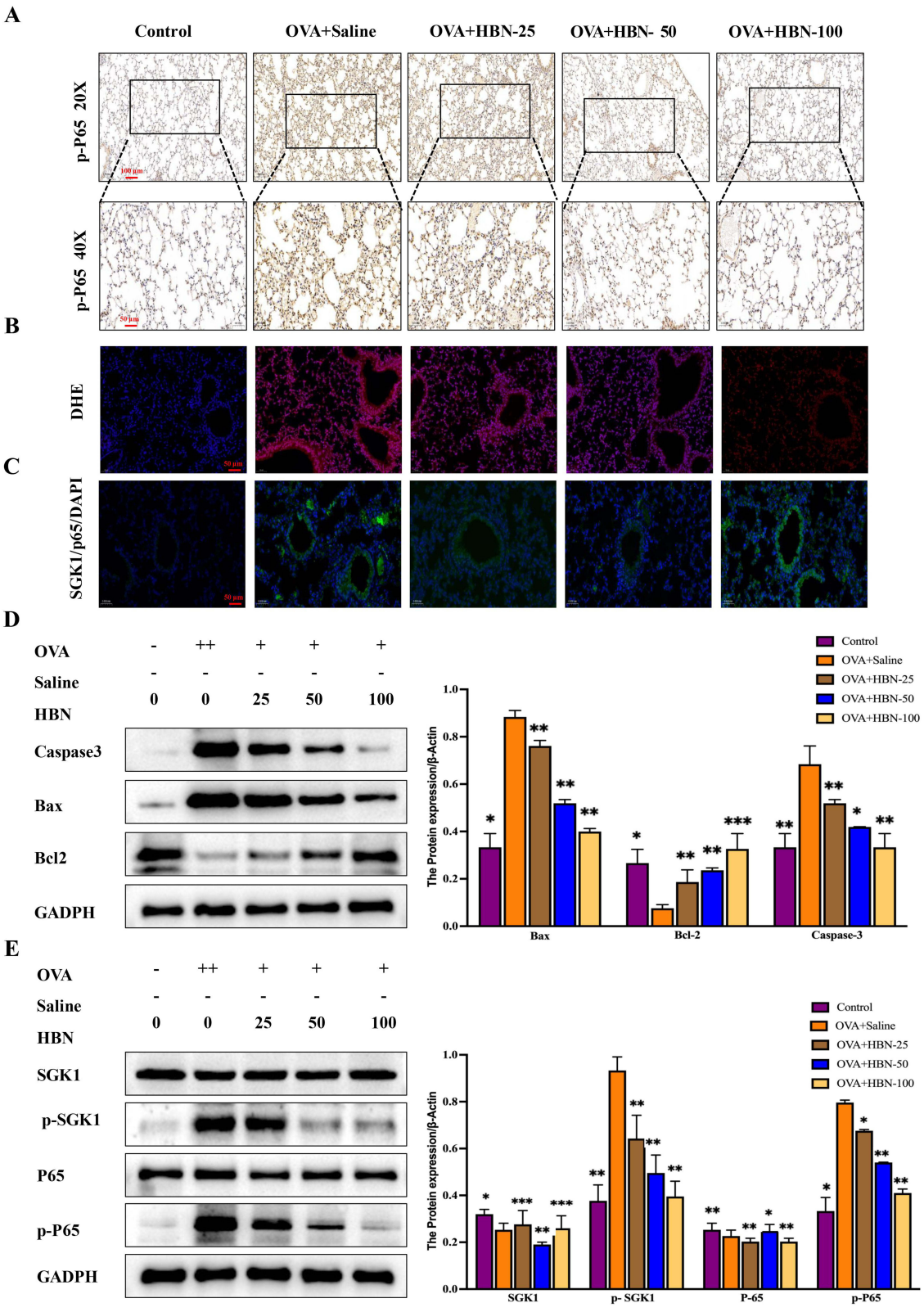

Figure 3 Herbacetin reduces inflammation in mice with OVA-induced asthma. (**A**) Immunohistochemical analysis of the expression of p-P65 in Pulmonary tissues from each group. (**B**) Intracellular ROS levels were indicated by DHE fluorescence intensity and detected by high content screening. (**C**) immunofluorescence analysis of the expression of SGK1 and p65 in Pulmonary tissues from each group. (**D** and **E**) Tissue lysates were prepared and analyzed by western blotting with the indicated antibodies, n=4 for each group. All data were analyzed using one-way ANOVA and data were expressed as means ± SD, *p < 0.05, **p < 0.01, ***p < 0.001.

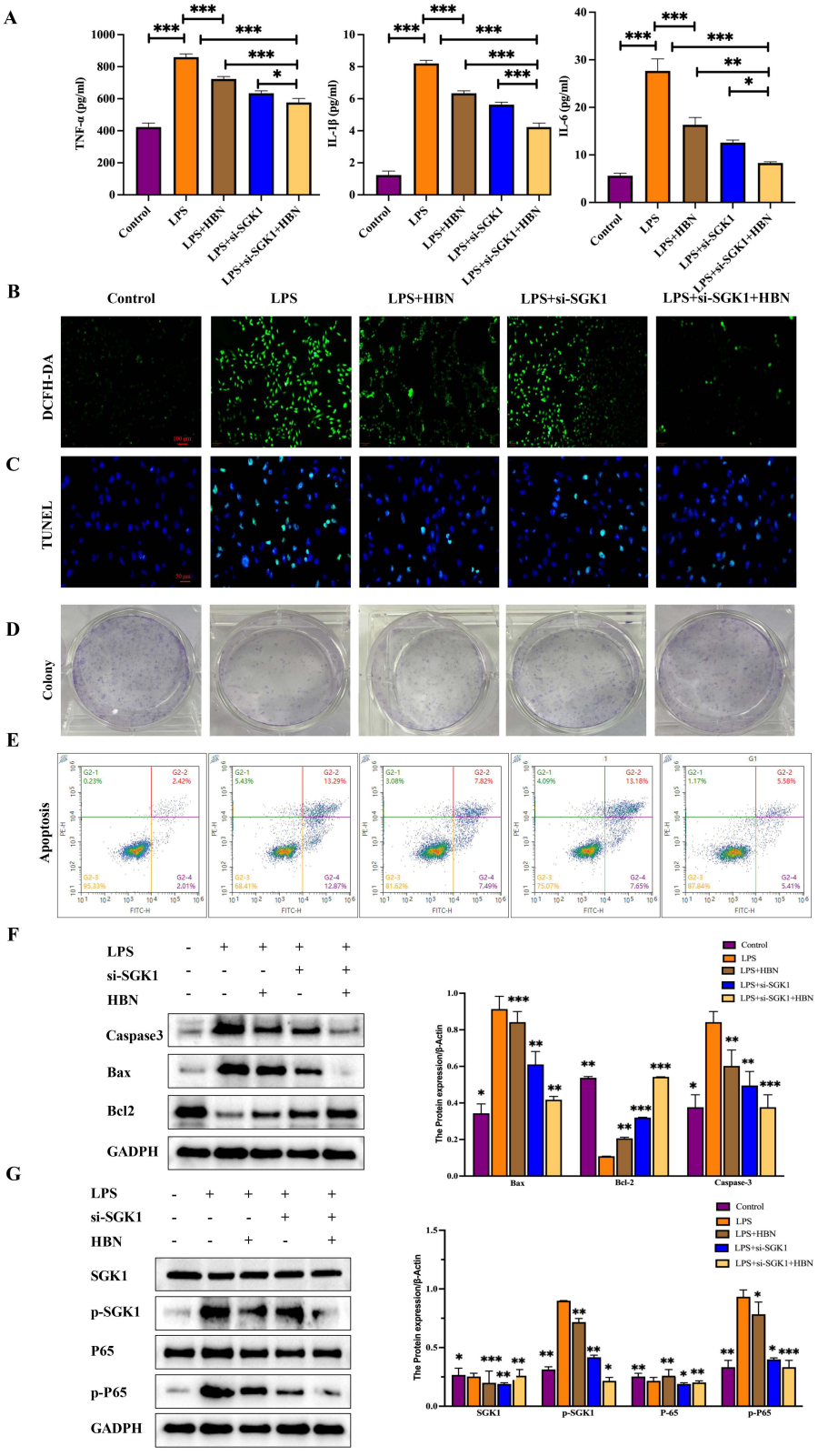

Figure 4 Herbacetin inhibited SGK1 in vitro. (**A**) The levels of IL-1β, IL-6, and TNF-α in the BASE-2B supernatant was detected by ELISA. (**B**) Intracellular ROS levels were indicated by DCFH-DA fluorescence intensity and detected by high content screening. (**C**) Confocal analysis of cells by TUNEL (green) and DAPI (blue). (**D**) Cells were cultured until the number of cells in the vast majority of individual clones was greater than 50 and cell clones were examined. (**E**) Representative flow cytometry scatterplots of PI (Y-axis) vs Annexin V-FITC (x-axis), and cells were analyzed using Annexin-V-FITC apoptosis detection kit by flow cytometry. (**F** and **G**) Cell lysates were prepared and analyzed by western blotting with the indicated antibodies, n=4 for each group. All data were analyzed using one-way ANOVA and data were expressed as means ± SD, *p < 0.05, **p < 0.01, ***p < 0.001.

and the ROS level was stable after Herbacetin pretreatment and reinduction of si-SGK1 (Figure 4B). The TUNEL results showed that despite LPS induction, si-SGK1 combined with Herbacetin showed a good antiapoptotic effect, and the positive rate of fluorescence was significantly lower than that of the si-SGK1 group and the Herbacetin intervention group (Figure 4C). The results of plate cloning showed that the number of cell clones in the Herbacetin + si-SGK1 intervention group was more than in the Herbacetin and si-SGK1 intervention groups and in the LPS group (Figure 4D). Flow cytometry showed the same trend, the apoptosis rate decreased in the si-SGK1 group and the Herbacetin intervention group, and further decreased in si-SGK1+Herbacetin. The results of the cell cloning assay indicated that the level of proliferation was also restored (Figure 4E).

Consistent with the previous results, LPS-induced expression of Caspase-3 and Bcl-2 was increased. However, the intervention of siSGK1+Herbacetin reversed this phenomenon and inhibited the expression of Caspase-3 and Bcl-2. Compared to the normal group and the si-SGK1 and Herbacetin intervention groups, the expression level of Bax also significantly increased (Figure 4F). Knockdown of SGK1 and Herbacetin intervention had a synergistic effect on the expression of p-SGK1 and down-regulate the expression of p-P65, indicating that Herbacetin exerts its effect by inhibiting the SGK1/NF-κB pathway in vitro (Figure 4G).

Effects of Herbacetin on SGK1-Induced Activation of NF-κB Signaling Pathways

Over-expression of si-SGK1 showed resistance to Herbacetin (Figure 5A). HE results showed that compared with the model group, the LV-SGK1 group showed more serious lung injury, and Herbacetin treatment alleviated this phenomenon. Compared to the model group, IHC results showed that Herbacetin regulated the expression of p-P65 levels in the LV-SGK1 model group (Figure 5B and C). DHE staining showed that oxidative stress in lung tissue was significantly enhanced after the intervention of the LV-SGK1 model group (Figure 5D). The results of the double immunolabeling showed that the suppression of Herbacetin in si-SGK group had a synergistic effect and the expression level of SGK1 was decreased, and the expression level of p-P65 was regulated (Figure 5E).

Discussion

Bronchial asthma (Asthma, Bronchial asthma) is caused by a variety of cells, including airway inflammatory cells, the structure of cells, and components involved in chronic inflammatory airway disease.[10] The clinical manifestations of asthma are recurrent wheezing, chest tightness, cough and dyspnea.[11] Although the degree of airway obstruction and airflow limitation is highly reversible, even if the standardized treatment of asthma patients is strictly implemented in clinical practice, it can eventually lead to irreversible airway obstruction and airway remodeling with the continuous course of the disease.[12,13] According to incomplete statistics, the prevalence of asthma in the world ranges from 1% to 18% and there are about 300 million asthmatics in the world.[14] Airway remodeling is defined as a series of morphological changes of the airway, including bronchioles, and this structural change affects the entire layer of the airway wall, including the epithelium, the basement membrane, smooth muscle and blood vessels.[15] The airway mucosa is made up of tracheal and bronchial epithelial cells and subepithelial ECM.[16] In the process of controlling asthma, internal and external factors, including biological, physical, chemical and allergy, stimulate the airway epithelium and the epithelial damage caused.[17]

Herbacetin and myricetin, which have been proven to be used in the treatment of liver cancer and breast cancer.[18,19] According to the chemical structure and that it belongs to the general nature of Herbacetin, it has certain anti-inflammatory effects.[20,21] The results of the present study demonstrated that Herbacetin effectively inhibited LPS-induced cell damage in BASE-2B lung epithelial cells in a dose-dependent manner, and apoptosis, also known as a mechanism of programmed cell death, was observed. Injured airway epithelial cells can express a variety of pro-inflammatory cytokines and chemokines, promote the infiltration and activation of inflammatory cells, release inflammatory mediators, inhibit apoptosis of inflammatory cells, and aggravate airway inflammation.[22,23] Induction of ROS and imbalance of the antioxidant defense system result in and ultimately lead to oxidative damage. As our results showed, glycyrrhizin had a potent inhibitory effect on LPS-induced ROS.

OVA-induced asthma is a common method for asthma modeling.[24] OVA has strong immunogenicity and is often used as an immune stimulator in the preparation of asthma models. It is injected together with antigen substances to affect the immune regulatory network and cause the body to produce immune responses.[25] In this study, OVA-induced C57BL/6 mice were used to construct a mice model of bronchial asthma, which is characterized by airway inflammation, increased

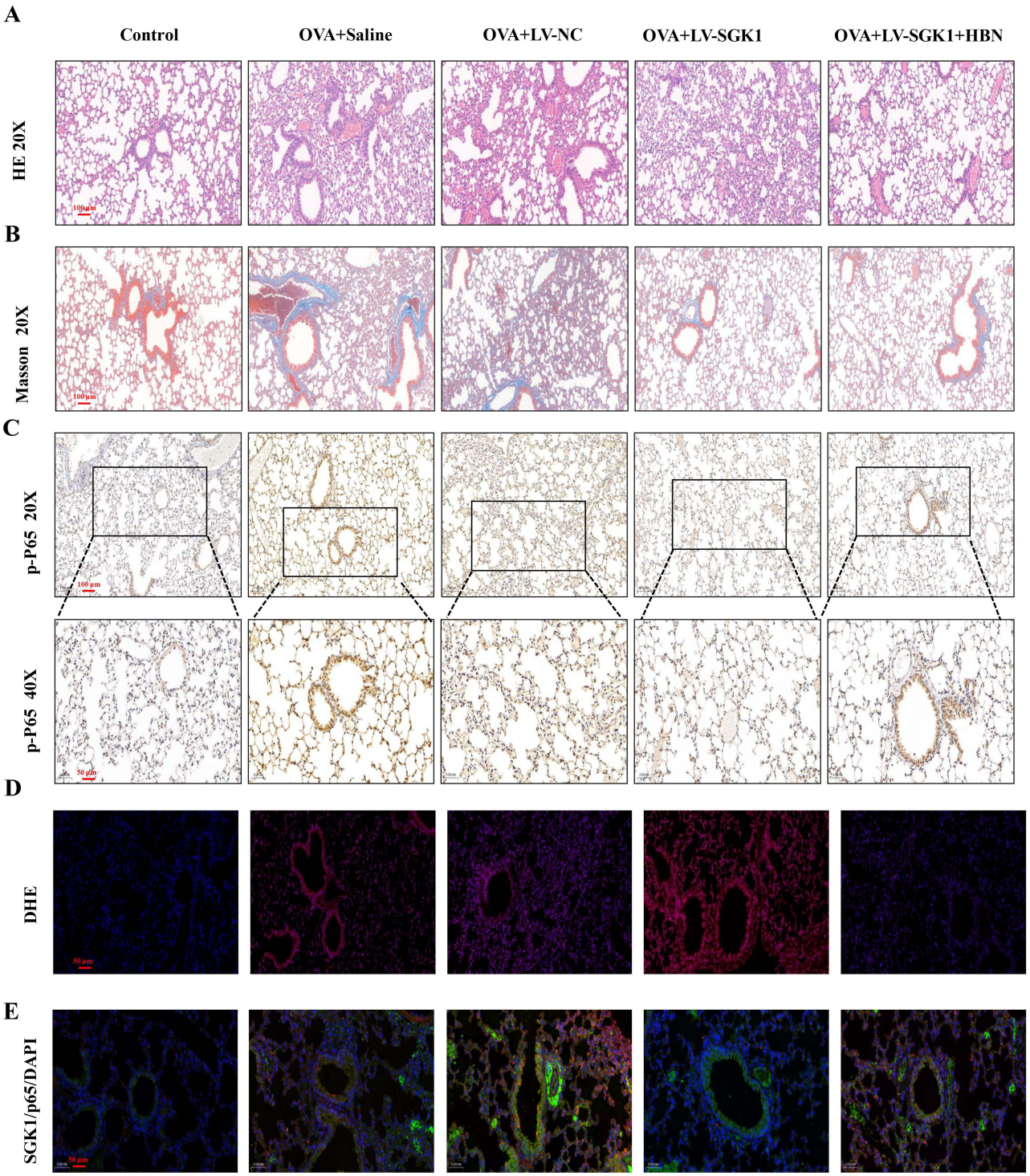

Figure 5 Herbacetin inhibits pulmonary inflammation in asthmatic mice by inhibiting SGK1. (**A** and **B**) Images show the Pulmonary tissues damage through H&E and Masson staining. (**C**) Immunohistochemical analysis of the expression of p-P65 in Pulmonary tissues from each group. (**D**) Intracellular ROS levels were indicated by DHE fluorescence intensity and detected by high content screening. (**E**) immunofluorescence analysis of the expression of SGK1 and p65 in Pulmonary tissues from each group. All data were analyzed using one-way ANOVA and data were expressed as means ± SD.

expression, increased number of eosinophils, lymphocyte infiltration, and mucus hypersecretion. Mice were sensitized by intraperitoneal injection at 0, 7, and 14 days. In the early stage of the challenge, nebulization was used.[26] HE staining of the lungs showed that the bronchial wall of the mice in the model group was thickened, with viscous fluid, exudate in the

lumen, and some inflammatory cells. Herbacetin intervention groups can effectively alleviate the pathological state of the lung. Masson shows the characteristics of stimulating concentration and affects the proliferation, differentiation, and collagen synthesis of fibroblasts. Consistent with in vitro results, the expression of cleaved Caspase-3 was significantly increased in airway epithelial cells from asthmatic rats with airway remodeling, indicating that airway epithelial apoptosis was significantly increased in the airway remodeling group. The expression of serum inflammatory factors in the bronchoalveolar lavage fluid of mice showed that Herbacetin could significantly negatively regulate the expression of inflammatory factors. Herbacetin was shown to significantly reverse the production of inflammatory mediators induced by inflammatory injury in NF-κB macrophages and inhibit NF-κB activation.

Pathogenic factors of asthma are still unclear, such as genetic immune mechanism, environmental exposure, respiratory virus infection, airway neuroregulation and neural signal transduction mechanism.[27] Currently, it is believed to be related to individual allergic constitution and environmental factors, and it has an obvious genetic tendency and aggregation of the family. Among them, the proportion of genetic factors can reach 48–79%, which also indicates that abnormal gene changes play an important role in the pathogenesis of asthma.[15] The literature shows that inflammatory cytokines can act on airway epithelial cells, smooth muscle cells, and lung fibroblasts and affect the occurrence of asthma airway hyperresponsiveness and airway remodeling by promoting or inhibiting the expression of SGK1 in these airway structural cells.[28] SGK1 is a kind of stress-induced survival factor, and in physiological and pathological conditions, such as growth factors, glucocorticoid, cell factor, and various cell stress), it can rapidly induce the expression of SGK1.[4,29] SGK1 can regulate multiple signal transduction pathways to regulate cell proliferation, differentiation, survival, and apoptosis. Herbacetin intervention, different concentrations in epithelial cells, cells, and smooth muscle cells of bronchial asthma mice in submucosa SGK1 protein expression levels were significantly lower, its expression level is closely related to the severity of asthma. Studies have shown that after prolonged exposure to LPS, enhanced phosphorylation of SGK1 activates the nuclear factor NF-κB in airway epithelial cells, and inhibition of SGK1/ Nf-κB p-P65 can eliminate airway inflammation. Protect the autoimmune balance and alleviate the impairment of epithelial barrier function.[30]

The NO produced induced by LPS through iNOS can determine the degree of inflammation.[31] And it can inhibit the activity of iNOS or induced iNOS to assess drug effects on inflammatory process.[32,33] We demonstrated that inhibition of SGK1 expression could alleviate the degree of cell damage. By detecting the levels of TNF-α, IL-6, IL-1β and other inflammatory cytokines levels in the lung epithelial cell culture medium and SGK1 expression after silencing of SGK1, the expression of SGK1 in the lung epithelial cell supernatant was detected. The level of LPS-induced inflammation and ROS decreased, and the trend of Herbacetin intervention was inhibited. After silencing SGK1 expression combined with Herbacetin intervention, the level of LPS-induced apoptosis decreased and cell damage was alleviated. Western blot to detect the influence of our Herbacetin on the NF-κB transcription activity. Herbacetin inhibits LPS stimulation in a dose-dependent manner the NF-κB transcription activity. Furthermore, we found our Herbacetin inhibition in the form of a time-dependent inhibition of the NF-κB to phosphorylation of the cell nucleus and translocation. These results validate that the flavonoid pharmacophore of Herbacetin is a key structural feature of SGK1 binding and that Herbacetin inhibits LPS-induced inflammatory injury by inhibiting the NF-κB pathway activated by SGK1 phosphorylation.[34]

To explore the molecular mechanism of our Herbacetin-mediated inhibition of SGK1, observe asthma mice after expressing the lung tissue pathology specimen of SGK1, the expression of asthma mice SGK1 and inflammatory cytokines, pulmonary function index, and airway was positively related to the severity of vascular remodeling; Airway inflammation and hyperresponsiveness play an important role in asthma. The local accumulation of inflammatory cells in the airways, the release of inflammatory mediators and cytokines involved in the pathogenesis of asthma are the main reasons for the formation and maintenance of airway inflammation, which in turn cause tissue damage and airway dysfunction. Asthma immunomodulatory disorders, immune imbalances in IL-6, IL-1β cytokine production, inflammatory cytokines are involved in the pathogenesis of asthma. A central artery, mutual coordination and interaction between different cytokines, form a strong network of cytokines. It is directly or indirectly involved in the entire asthma process through multiple signal transduction pathways in vivo. Furthermore, NF-κB activation is thought to be critical in OVA-stimulated inflammation due to its ability to induce the expression of pro-inflammatory genes in macrophages; thus, we hypothesized that Herbacetin may at least partially inhibit the effects of NF-κB signaling. As the results of western blot and immunofluorescence double labeling showed, Herbacetin reversed the enhanced phosphorylation of SGK1 could

activate the nuclear factor NF-κB in airway epithelial cells, inhibit the expression of SGK1/ NF-κB p-P65, eliminate airway inflammation, and protect the autoimmune balance.[35]

Conclusion

The current study shows that Herbacetin inhibits LPS-stimulated apoptosis by reducing the production of inflammatory factors TNF-α and IL-6, IL-1β, and oxidative stress. Herbacetin mediates damage repair through the SGK1/NF-κB pathway, and future evaluation of the role of Herbacetin in humans is needed to determine its potential use as a therapeutic agent for inflammation-related diseases.

Abbreviations

SAP, severe acute pancreatitis; CGA, Chlorogenic acid; JHT, JinHong Tablet; AP, acute pancreatitis; ARDS, acute respiratory distress syndrome; DIC, disseminated intravascular coagulation; ARF, acute renal failure; AHF, acute heart failure; CXCL1, chemokine ligand 1; SPF, specific pathogen-free; GSDMD, Gasdermin-D; TLR4, toll-like receptor 4; MyD88, myeloid differentiation factor 88; IKK, kappa-B kinases; NF-κB, nuclear factor kappa-B; MIP-2, inflammatory protein 2; COX-2, cyclooxygenase-2; PGE2, prostaglandin E2. HE, hematoxylin and eosin; IHC, Immunohistochemistry; qRT-PCR, quantitative real-time PCR; CCK-8, Cell Counting Kit-8; NPS, nasal polyps; DHE, Dihydroergotamine.

Data Sharing Statement

All data are provided in this study, and raw data can be requested from the corresponding author.

Ethics Approval and Consent to Participate

The authors are responsible for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are adequately investigated and resolved. Animal care and experimental procedures were conducted following a protocol approved by the Animal Care and Use Committees of Medical Ethics Committee of the Shanghai University of Traditional Chinese Medicine (approval number: PZSHUTCM221017003).

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

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