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Matrine Attenuates Mitochondrial Fragmentation in Ovalbumin-Induced Asthmatic Mice by Activating the AMPK/Nrf2 Pathway

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Background: Asthma, a prevalent respiratory condition, is characterized by hyperresponsiveness and airway inflammation, and mitochondrial dysfunction and inflammation can exacerbate these symptoms. Therefore, this study aims to investigate whether matrine, known for its anti-inflammatory properties, attenuates mitochondrial fragmentation in asthmatic mice by activating the adenosine 5′-monophosphate-activated protein kinase (AMPK)/Nuclear Factor Erythroid 2-related Factor 2 (Nrf2) pathway.
Methods: An asthma model was induced in BALB/c mice through sensitization with ovalbumin (OVA). The mice were given matrine (50 mg/kg, 100 mg/kg) and dexamethasone (2 mg/kg) through gavage feeding. Serum, lung tissue, and bronchoalveolar lavage fluid (BALF) were collected. Hematoxylin-eosin (H&E), Periodic Acid-Schiff (PAS) and immunohistochemistry, enzyme-linked immunosorbent assay (ELISA), flow cytometry, and western blot analyses were used to assess changes in inflammation and oxidative stress levels in the airways and lung tissues. Furthermore, the expression levels of AMPK-dynamin-related protein 1 (DRP1)-NOD-like receptor family, pyrin-domain-containing-3 (NLRP3) pathway proteins were evaluated in the lung tissues.
Results: Matrine treatment significantly reduced airway inflammation and mucus secretion in OVA-induced asthmatic mice ($p < 0.05$). Notably, inflammatory cell infiltration around the airway and mucus secretion in the airway, as evidenced by H&E and PAS staining, was substantially decreased in matrine-treated mice compared to the OVA group. Additionally, matrine significantly reduced T-helper cell type 2 (Th2) cytokine levels in mediastinal lymph nodes and BALF, as well as serum immunoglobulin (Ig)E levels ($p < 0.05$). Moreover, matrine exhibited antioxidant effects by enhancing superoxide dismutase (SOD) and catalase (CAT) activity while reducing malondialdehyde (MDA) expression and reactive oxygen species (ROS) accumulation in lung tissues ($p < 0.05$). Furthermore, the suppression of Caspase-1, NLRP3, and interleukin (IL)-1β by matrine indicated its anti-inflammatory properties ($p < 0.05$). Mechanistically, matrine increased AMPK phosphorylation, inhibited DRP1-mediated mitochondrial fission, and promoted mitochondrial fusion, suggesting its potential to alleviate airway inflammation ($p < 0.05$).
Conclusions: Matrine alleviates allergic airway inflammation in OVA-induced asthmatic mice by regulating the AMPK-DRP1-NLRP3 pathway.

Keywords: asthma; matrine; NOD-like receptor family, NLRP3; AMP-activated protein kinases; DRP1

Introduction

Asthma is characterized by chronic inflammation and hypersensitivity of the airways, leading to symptoms like chest tightness, shortness of breath, dyspnea, coughing, and wheezing [1]. Recognizing symptoms early and managing triggers such as allergens or infections are essential for timely diagnosis and better treatment outcomes [2]. Globally, approximately 358 million people are affected by asthma, which has resulted in a 12.6% rise in the overall prevalence rate since 1990 [3]. About 10% of asthma cases present severe or uncontrollable symptoms [4]. Pathologically, asthma involves mucus hyperproduction, airway obstruction, and increased inflammatory cell infiltration [5], with uncontrolled inflammation playing a significant role in the pathogenesis of respiratory diseases. Disruption of mitochondrial homeostasis contributes to oxidative stress and inflammation, linking to apoptosis, ion balance, innate immunity, and metabolism [6]. Therefore, maintaining mitochondrial function and alleviating inflammation are crucial for managing asthma.

Mitochondrial homeostasis is essential for maintaining cellular energy production and growth, achieved through the dynamic balance of fission and fusion processes [7]. During stress, continuous fusion and fission of mito-

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chondrial tubules sustain normal metabolism [8]. Hyperactivation of dynamin-related protein 1 (DRP1) and abnormalities in mitochondrial dynamics are associated with various lung diseases [9,10], leading to an imbalance in mitochondrial homeostasis involving proteins like DRP1, Mitochondrial fission 1 protein (Fis1), Mitofusin (MFN) 1, MFN2, Optic atrophy 1 (OPA1), and Mitochondrial transcription factor A (Tfam) [11]. Mitochondrial fission is mediated by DRP1 which is primarily located in the cytoplasm, and phosphorylation at serine 616 promotes fragmentation, excessive reactive oxygen species (ROS) production, and structural damage [12,13]. Pharmacological activation of adenosine 5′-monophosphate-activated protein kinase (AMPK) inhibits DRP1 activation, thereby hindering mitochondrial fission and mitigating tissue damage [14]. In asthma induced by particulate matter (PM), decreased mitochondrial fusion marker (OPA-1), mitochondrial antioxidant manganese-superoxide dismutase (Mn-SOD), and p-AMPK, alongside elevated NOD-like receptor family, pyrin-domain-containing-3 (NLRP3), interleukin (IL)-1β, and the fission marker (DRP1) exacerbate inflammation [15]. Excessive ROS disrupt mitochondrial bioenergetics, activating NLRP3 inflammasome and aggravating inflammation [16]. Inhibition of ROS-triggered NLRP3 inflammasome by aryl hydrocarbon receptor reduces Muc5ac expression in cockroach allergen-induced asthma [17]. Excessive production of ROS induced by tumor necrosis factor (TNF)-α decreases the expression of fusion proteins and increases mitochondrial fission proteins, leading to mitochondrial fragmentation and mitophagy [18]. Carbon tetrachloride (CCl4) induces liver injury by upregulating ROS-associated mitochondrial fission proteins and downregulating fusion proteins [19]. Ovalbumin (OVA) induces NLRP3 inflammasome assembly and pro-inflammatory cytokines production in respiratory diseases [20]. Therefore, activating AMPK and reducing ROS can inhibit DRP1-mediated mitochondrial fission, thereby alleviating airway inflammation in asthma.

Sophora flavescens, a traditional Chinese herbal medicine, is renowned for its efficacy in treating various conditions associated with heat toxins and damp-heat syndromes, such as skin itching and carbuncle sores [21]. Matrine (molecular weight: 248.36 g/mol; molecular formula: $C_{15}H_{24}N_2O$), an alkaloid extracted from Sophora flavescens, exhibits diverse pharmacological effects, such as anticancer, anti-oxidative, antiviral, antibacterial, antifibrotic, anti-allergic, anti-inflammatory, cardioprotective, and neuroprotective properties [22]. Research suggests that matrine exhibits efficacy in treating various conditions, including asthma, rheumatoid arthritis, encephalomyelitis, Alzheimer's syndrome, myocardial ischemia, and osteoporosis, primarily due to its anti-inflammatory and anti-apoptotic mechanisms [23].

Moreover, matrine has been effective in reducing goblet cell hyperplasia, airway hyperresponsiveness, inflammatory response, and eosinophil infiltration in asthmatic mice. It decreases the levels of T-helper cell type 2 (Th2) cytokines and chemokines, inhibits OVA-immunoglobulin (Ig)E production, and regulates the expression of cytokines such as interleukin (IL)-4, interferon (IFN)-γ, IL-13, and TNF-α [24]. In ischemic diseases, matrine inhibits oxidative stress, reducing apoptosis in nerve cells and cardiomyocytes. Its mechanisms involve AMPK and the Nuclear Factor Erythroid 2-related Factor 2 (Nrf2)/HO-1 pathway [25]. Additionally, metformin and matrine activate the AMPK/Nrf2-dependent pathway to attenuate oxidative stress-induced damage [26]. Matrine inhibits NF-κB expression, reduces apoptosis and inflammation, and enhances normal cell survival in stressed bronchial epithelial cells [23]. Matrine facilitates apoptosis in colorectal cancer cells by inducing mitochondrial division associated with MIEF1 through the LATS2-Hippo pathway [27]. Nonetheless, exploring its role in maintaining mitochondrial homeostasis in asthma requires further investigation.

We hypothesize that matrine attenuates mitochondrial fragmentation in OVA-induced asthmatic mice by activating the AMPK/Nrf2 pathway. Thus, we aim to investigate whether the matrine-triggered AMPK/Nrf2 signaling is correlated with the antioxidant response following OVA exposure. Additionally, considering the significant association between mitochondrial dysfunction and airway inflammation in asthma, we aim to explore the role of matrine in mitochondrial homeostasis in OVA-induced asthmatic mice. In summary, this study offers essential insights into both the understanding and potential treatment of asthma.

Materials and Methods

Experimental Animals

Female BALB/c mice (n = 35), aged 8 weeks and weighing 20–22 g, were obtained from the Feeding Department of Yanbian University Health Science Center (China Yanji Animal License [JI] 2020-00093). The mice were housed in a specific pathogen-free (SPF) controlled environment with relative humidity of 50%–60%, temperature at 22 ± 2 °C, supplied with sterilized water and food, and a 12-hour light/dark cycle. The study design adhered to the Regulations on the Administration of Experimental Animals and was approved by the Ethics Committee of Yanbian University School of Medicine, China (approval number: SYXK(JI)2020-0009).

Construction of Asthmatic Mice Models

Mice were randomly divided into 5 groups (n = 7): the control group, the OVA group (ovalbumin, 40 µg, S7951, Sigma, St. Louis, MO, USA), the low-dose matrine group (OVA + matrine (Mat) 50 mg/kg), the dexamethasone group (OVA + Dexamethasone (Dex), 2 mg/kg, positive control drug), and the high-dose matrine group (OVA + Mat 100 mg/kg). The OVA group received intraperitoneal injections

of a solution (0.2 mL) containing 1 mg aluminum hydroxide (sc-214529, Santa Cruz, Dallas, TX, USA) as an adjuvant and 10 μg OVA on 0, 7th, and 14th days for sensitization. The sensitized mice underwent a challenge with nebulized 1% OVA-in-saline (15 mL) for 6 consecutive days on day 21, once daily for 30 minutes each time. The matrine and dexamethasone groups were administered matrine solution via gavage and dexamethasone solution (2 mg/kg) via injection, respectively, 30 minutes before the challenge. Matrine ($C_{15}H_{24}N_2O$; molecular weight: 248.36; purity ≥98%) was obtained from PUSH Biological Technology (519-02-8, Chengdu, China) and dissolved in normal saline. However, the control group received normal saline.

Sample Collection and Processing

The day after the final experiment, eyeball blood was collected under anesthesia using an intraperitoneal injection of 1% sodium pentobarbital (40 mg/kg). Subsequently, mouse bronchoalveoli were lavaged with 1 mL of PBS, and approximately 800 μL of bronchoalveolar lavage fluid (BALF) was collected. Upon centrifugation of the BALF cells at 400 g and 4 °C for 10 minutes, the supernatant was collected and stored at –80 °C for subsequent cytokine analysis. BALF cells in the centrifuged cell pellet were counted by Diff-quik staining. Other cell pellets were analyzed for cytokines using flow cytometry, as detailed below. After euthanasia of mice by cervical dislocation, the lower lobe of the right lung was collected and utilized for pathological and histological examinations, while additional lung tissues were preserved at –80 °C for subsequent protein analysis. Flow cytometry analysis was conducted on single-cell suspensions derived from draining lymph nodes to assess cytokine levels in mediastinal lymphocytes.

Histological Analysis

Lung tissue sections underwent hematoxylin-eosin (H&E) staining (Cat. No. G1120, Solarbio, Beijing, China) for analysis of inflammatory cells. Airway goblet cells and mucus secretion were determined using Periodic Acid-Schiff (PAS) staining (Cat. No. G1281, Solarbio, Beijing, China). Moreover, for immunohistochemical analysis of DRP1, paraffin-embedded lung tissue sections were initially deparaffinized and hydrated. Antigen retrieval was conducted using citrate, followed by incubation with 3% H_2O_2 and 5% goat serum for 10 and 15 minutes, respectively, at ambient temperature. Subsequently, tissue sections underwent incubation with rabbit DRP1 antibody (Cat. No. DF7037, 1:200, Affinity, Liyang, China) and goat anti-rabbit IgG H&L (HRP) antibody (Cat. No. 6721, 1:2000; Abcam, Boston, MA, USA). Finally, images were captured using an optical microscope (Eclipse Ni-U, Nikon, Tokyo, Japan).

Enzyme-Linked Immunosorbent Assay (ELISA)

The cytokines in the BALF supernatant and serum IgE levels were quantified using corresponding ELISA kits. IL-4 (Cat. No. M4000B, R&D Systems, Minneapolis, MN, USA), IL-13 (Cat. No. M1300CB, R&D Systems, Minneapolis, MN, USA), IL-5 (Cat. No. M5000, R&D Systems, Minneapolis, MN, USA), and IgE (Cat. No. ab157718, Abcam, Cambridge, UK) were assessed with a sensitivity of 2.0 pg/mL. Additionally, ELISA assays were conducted to determine catalase (CAT), (Cat. No. AF3398, R&D Systems, Minneapolis, MN, USA) and superoxide dismutase (SOD, Cat. No. DYC3419-2, R&D Systems, Minneapolis, MN, USA) activity, as well as malondialdehyde (MDA, Cat. No. A003-1-2, Nanjing Jiancheng Bioengineering Institute, Nanjing, China) following the manufacturer's instructions. Finally, absorbance values at 450 nm were determined by a microplate reader (Cat. No. 1681130, Bio-Rad, Hercules, CA, USA).

Dihydroethidium (DHE) Staining

We prepared a 50 mL volume of fresh 5 μm DHE staining solution (Cat. No. S0063, Biyuntian Biotechnology, Shanghai, China) following the instructions provided in the reagents and stored it at –20 °C in the dark until use. Subsequently, we washed the slide gently and incubated it with the 5 μm DHE staining solution in the dark at 37 °C for 30 minutes. Finally, we carefully rinsed the slide and immediately captured fluorescence images using Cytation 5 (BioTek, Suwanee, VT, USA).

Western Blot

Total protein was extracted using RIPA buffer, and subsequently quantified utilizing a bicinchoninic acid assay (BCA). Following denaturation, 20 μg of protein sample was prepared on 12% SDS-PAGE and transferred onto polyvinylidene fluoride (PVDF) membranes (03010040001, Millipore Sigma, Burlington, MA, USA). After this, the membranes were blocked with 5% skim milk powder for 1 hour, followed by overnight incubation with the primary antibodies at 4 °C. The primary antibodies included anti-Nrf2 (1:1000 dilution; Cat. No. SAB4501984, Sigma-Aldrich, St. Louis, MO, USA), anti-p-DRP1 (1:2000 dilution; Cat. No. DF2980, Affinity Biosciences, Cincinnati, OH, USA), anti-DRP1 (1:1000 dilution; Cat. No. DF7037, Affinity Biosciences, Cincinnati, OH, USA), anti-Mitofusin (MFN) 1 (1:1000 dilution; Cat. No. DF7543, Affinity Biosciences, Cincinnati, OH, USA), anti-MFN2 (1:1000 dilution; Cat. No. DF8106, Affinity Biosciences, Cincinnati, OH, USA), anti-NLRP3 (1:1000 dilution; Cat. No. A126974, ABclonal, Woburn, MA, USA), anti-Caspase-1 (1:1000 dilution; Cat. No. A0964, ABclonal, Woburn, MA, USA), anti-Heme oxygenase-1 (HO-1; 1:1000 dilution; Cat. No. ab13243, Abcam, Cambridge, UK), anti-p-AMPK (1:1000 dilution; Cat. No. 2535T, CST, Danvers, MA, USA), anti-p-

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AMPK (1:1000 dilution; Cat. No. 2535T, CST, Danvers, MA, USA), anti-AMPK (1:1000 dilution; Cat. No. ab32047, Abcam, Cambridge, UK), anti-IL-1β (1:1000 dilution; Cat. No. 12242s, Cell Signaling Technology, Danvers, MA, USA), and anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:1000 dilution; Cat. No. 2118S, CST, Danvers, MA, USA). The next day, the membranes underwent a 2-hour incubation with the secondary antibody, goat anti-rabbit antibody (1:5000 dilution; Cat. No. 5151, CST, Danvers, MA, USA) at room temperature (25 °C). Ultimately, immunoblots were developed using an enhanced chemiluminescence (ECL) substrate (34580, Thermo Fisher Scientific, Waltham, MA, USA) and protein bands were visualized through Quantity One software (BioRad, Hercules, CA, USA). The greyscale values of protein bands were determined using ImageJ software (version 1.8.0, NIH, Bethesda, MD, USA). GAPDH was used as an internal control protein.

Flow Cytometry

We utilized the FITC-CD4 antibody (Cat. No. 11-0041-82; Invitrogen, Carlsbad, CA, USA) and Percp-Cy5.5 CD3e antibody (Cat. No. 45-003182; Invitrogen, Carlsbad, CA, USA) to determine the percentages of IL-13$^+$CD4$^+$ cells and IL-4$^+$CD4$^+$ cells in mediastinal lymph nodes (mLN). Subsequently, the cells were treated with Permeabilization Kit (Cat. No. 88-8824-00; Invitrogen, Carlsbad, CA, USA) and Intracellular Fixation, followed by staining with intracellular antibodies comprising anti-APC-IL-13 (32007; Invitrogen, Carlsbad, CA, USA) and anti-PE-Cy7 IL-4 (25-7042-41; Invitrogen, Carlsbad, CA, USA). Gating for IL-13$^+$CD4$^+$ cells and IL-4$^+$CD4$^+$ cells was performed on the CD3$^+$CD4$^+$ cell subset. Finally, the cells were analyzed using Cytoflex flow cytometry (CytoFLEX S, Beckman Coulter, Inc., Pasadena, CA, USA).

Immunofluorescent Staining of DRP1

For immunofluorescent staining of DRP1, BALF cells smeared on slides underwent initial fixation in ice-cold methanol, followed by permeabilization in PBS containing 0.25% Triton X-100 at ambient temperature for 10 minutes. Subsequently, the cells were rinsed three times with PBS. After this, antigen retrieval was conducted using proteinase K (S302080, Dako, Glostrup, Denmark) for 15 minutes at 37 °C. The smeared BALF cells were treated with 1% bovine serum albumin (BSA; A1933, Sigma-Aldrich, St. Louis, MO, USA) in PBS with 0.05% Tween 20 (0.05% PBS-T) for one hour. The cells were then incubated with Mitotraker Red for 30 minutes at 37 °C in the dark, followed by three washes with serum-free medium. The cells underwent overnight incubation with anti-DRP1 antibody (1:100 dilution; #DF7037, Affinity Biosciences, Cincinnati, OH, USA) at 4 °C. Following a gentle wash, the smeared BALF cells were exposed to Alexa Fluor 488 (green)-labeled goat anti-Rabbit IgG (A-11008; Invitrogen, Carlsbad, CA, USA) in 1% BSA for 2 hours at ambient temperature in the dark. Following another washing, the nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI; 00-4959-52, Invitrogen, Carlsbad, CA, USA). Finally, the cells were observed and their images were captured using Cytation 5 (BioTek, Suwanee, VT, USA).

Statistical Analysis

SPSS 19.0 software (IBMCo., Armonk, NY, USA) was adopted for statistical analysis. Each experiment was independently repeated at least three times. A two-tailed unpaired Student's *t*-test was utilized for comparisons between the two groups, and one-way analysis of variance (ANOVA) followed by Turkey's post hoc test was applied for multiple group comparisons. The data were expressed as means ± standard deviations (SD) or medians with ranges. A p-value < 0.05 represented the statistical significance threshold.

Results

Matrine Significantly Attenuates Airway Inflammation and Mucus Secretion in OVA-Induced Asthmatic Mice

We assessed the impact of matrine on peri-airway inflammatory cell infiltration and mucus secretion in asthmatic mice using H&E staining and PAS staining (Fig. 1A). Compared to the control group, the infiltration of inflammatory cells around the airway and mucus secretion in the airway were significantly increased in the OVA group. However, mice who received high doses of matrine treatment exhibited substantially reduced numbers of inflammatory cells around the airway and mucus secretion compared to the OVA group. Additionally, we quantified BALF cells using Diff-quik staining (Fig. 1B) to monitor inflammatory cells around the airway. The results revealed that high doses of matrine significantly decreased the number of eosinophils, inflammatory cells, lymphocytes, and neutrophils around the airway ($p < 0.05$). These findings suggest that matrine may mitigate OVA-induced asthma by alleviating airway inflammation, and mucus secretion.

Matrine Reduces Serum IgE and Th2 Cytokine Levels in OVA-Induced Asthmatic Mice

Th2 cytokine levels in BALF were quantified using ELISA (Fig. 2A). Compared to the control group, the OVA group exhibited a substantial increase in the expression levels of IL-13, IL-5, and IL-4 ($p < 0.05$). Conversely, relative to the OVA group, the levels of Th2 cytokines were notably down-regulated in the high-dose matrine group ($p < 0.05$). Furthermore, flow cytometry analysis illustrated that the percentages of IL-4$^+$CD4$^+$ cells (Fig. 2B,C) and IL-13$^+$CD4$^+$ cells (Fig. 2D,E) in the mLN of the OVA group were significantly increased compared to those in the control group ($p < 0.05$). In contrast, the percentages

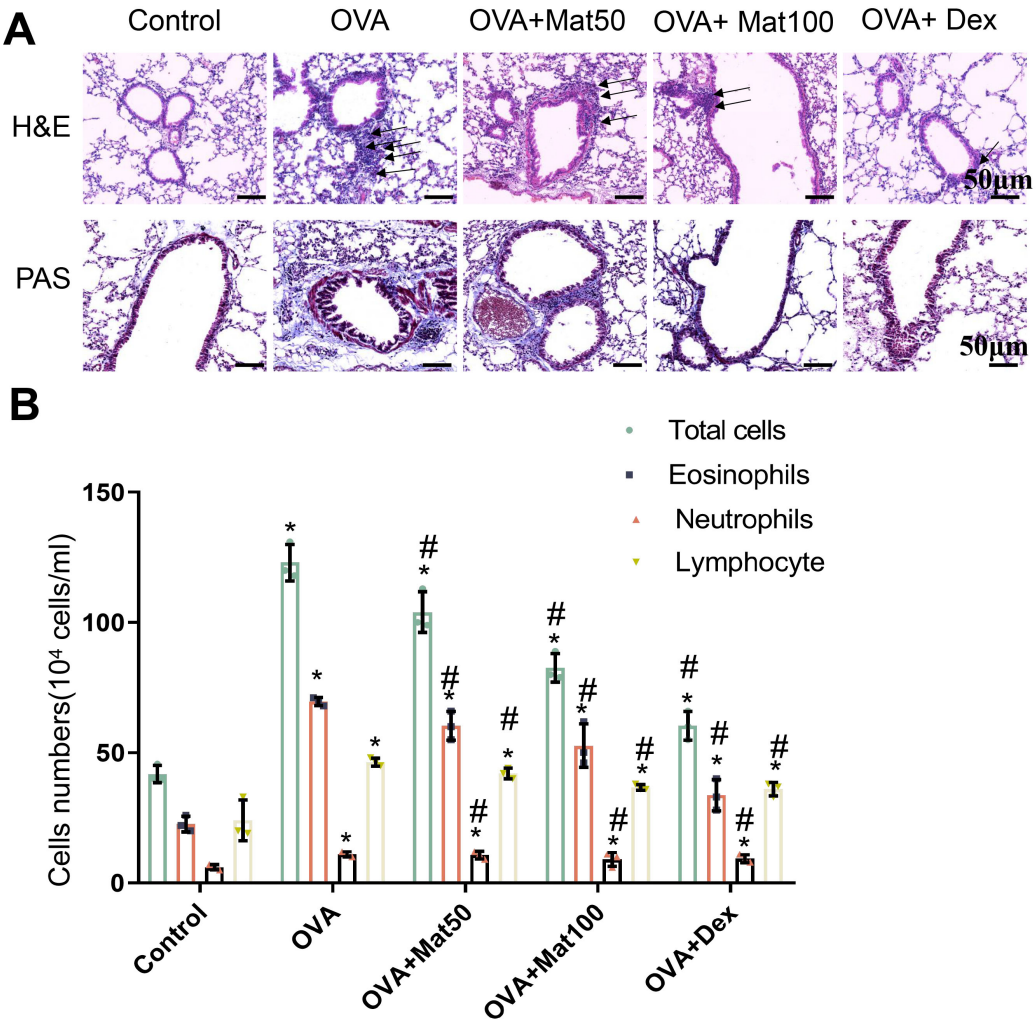

Fig. 1. Impact of matrine on airway inflammation and mucus secretion. (A) Arrows indicate inflammatory cells around the airway in the hematoxylin-eosin (H&E) staining of the lung section; PAS staining of the lung section; arrows indicate mucus secretion. Scale bar = 50 μm, magnification = 100×. (B) Diff-quik staining for bronchoalveolar lavage fluid (BALF) counting. N = 3. $^{*}p < 0.05$ *vs.* control; $^{\#}p < 0.05$ *vs.* OVA. OVA, ovalbumin; Mat, matrine; Dex, dexamethasone; PAS, Periodic Acid-Schiff.

of IL-4^{+}CD4^{+} cells (Fig. 2B,C) and IL-13^{+}CD4^{+} cells (Fig. 2D,E) in mLN were significantly decreased after treatment with high-dose matrine.

Furthermore, total serum IgE and specific IgE levels were assessed using ELISA (Fig. 2F). Compared to the control group, there was a significant elevation in Serum IgE levels in the OVA group ($p < 0.05$). However, compared to the OVA group, the mice treated with high-dose matrine showed a considerable decrease in IgE expression ($p < 0.05$). These findings suggest that matrine potentially mitigates OVA-induced asthma by reducing Th2 and IgE levels.

Impact of Matrine on Oxidative Stress in the Lungs of Asthmatic Mice

Considering that excessive ROS production can lead to mitochondrial damage, we assessed the expression levels of SOD, CAT, and MDA in BALF using ELISA (Fig. 3A). Compared to the control group, the OVA group exhibited a significant decrease in SOD and CAT activity, accompanied by a substantial elevation in MDA expression ($p < 0.05$). Conversely, treatment with high-dose matrine resulted in opposite outcomes relative to the OVA group ($p < 0.05$). Additionally, we analyzed the ROS level in lung tissue (Fig. 3B) and observed that ROS expression was significantly higher in the OVA group compared to the control group. However, high-dose matrine led to a remarkable reduction in ROS production around the airway in lung tissue, which differs from the finding observed in the OVA group. These findings suggest that matrine may prevent mitochondrial damage by enhancing SOD and CAT activity, reducing MDA expression, and decreasing ROS accumulation.

Impact of Matrine on DRP1-Mediated NLRP3

We evaluated the impacts of matrine on the NLRP3 inflammasome in asthmatic mice using western blot analy-

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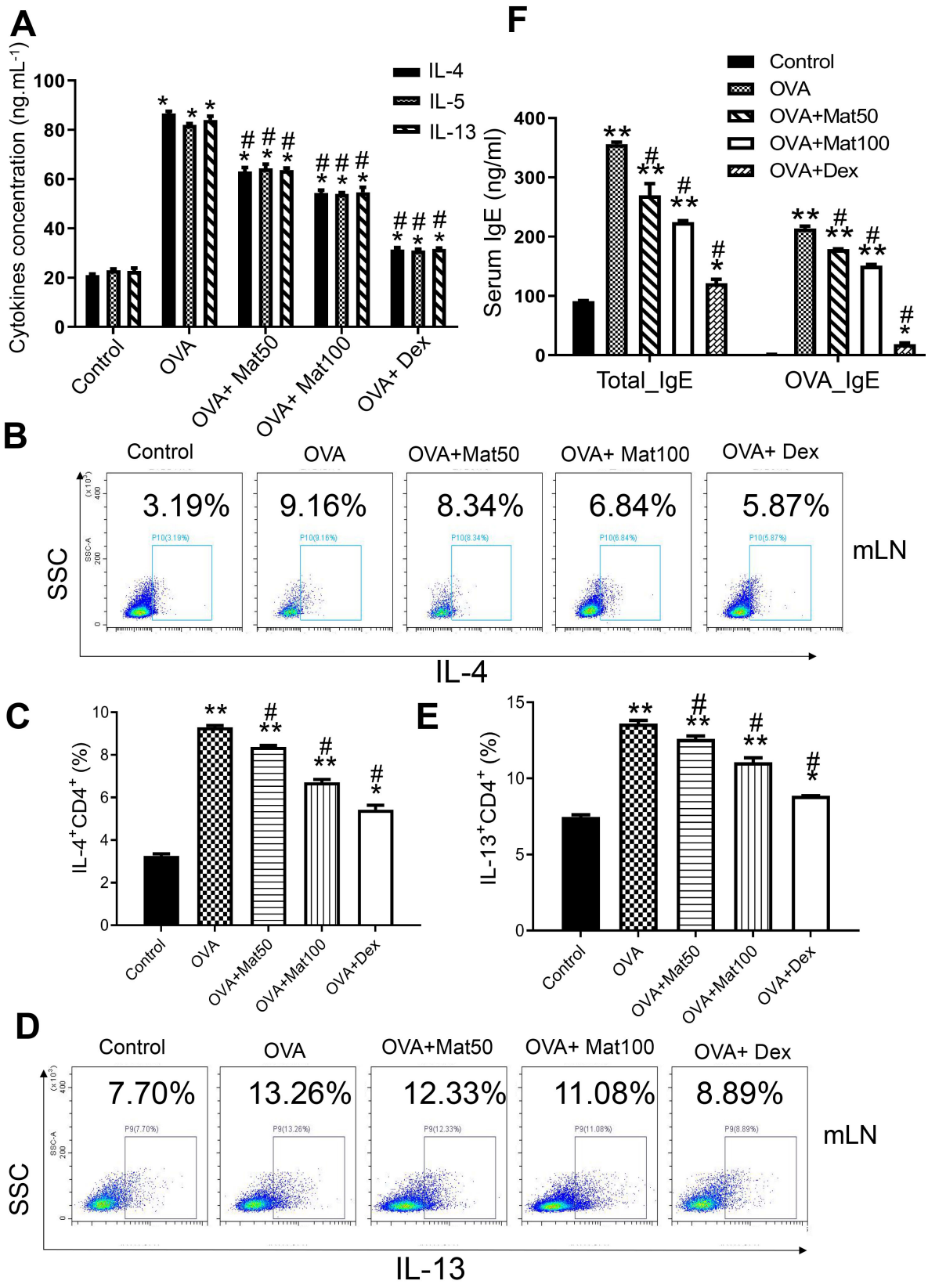

Fig. 2. Effects of matrine on BALF inflammatory cell infiltration, cytokine, and serum immunoglobulin (Ig)E levels in asthmatic mice. (A) Interleukin (IL)-13, IL-5, and IL-4 in BALF supernatant. (B,C) The percentage and number of mLN IL-4$^+$CD4$^+$ cells were assessed using flow cytometry. (D,E) Flow cytometry analysis was used to determine the number and percentage of mLN IL-13$^+$CD4$^+$ cells. (F) IgE level in mouse serum. N = 3, $^*p < 0.05$, $^{**}p < 0.01$ *vs.* Control; $^{\#}p < 0.05$ *vs.* OVA. mLN, mediastinal lymph nodes.

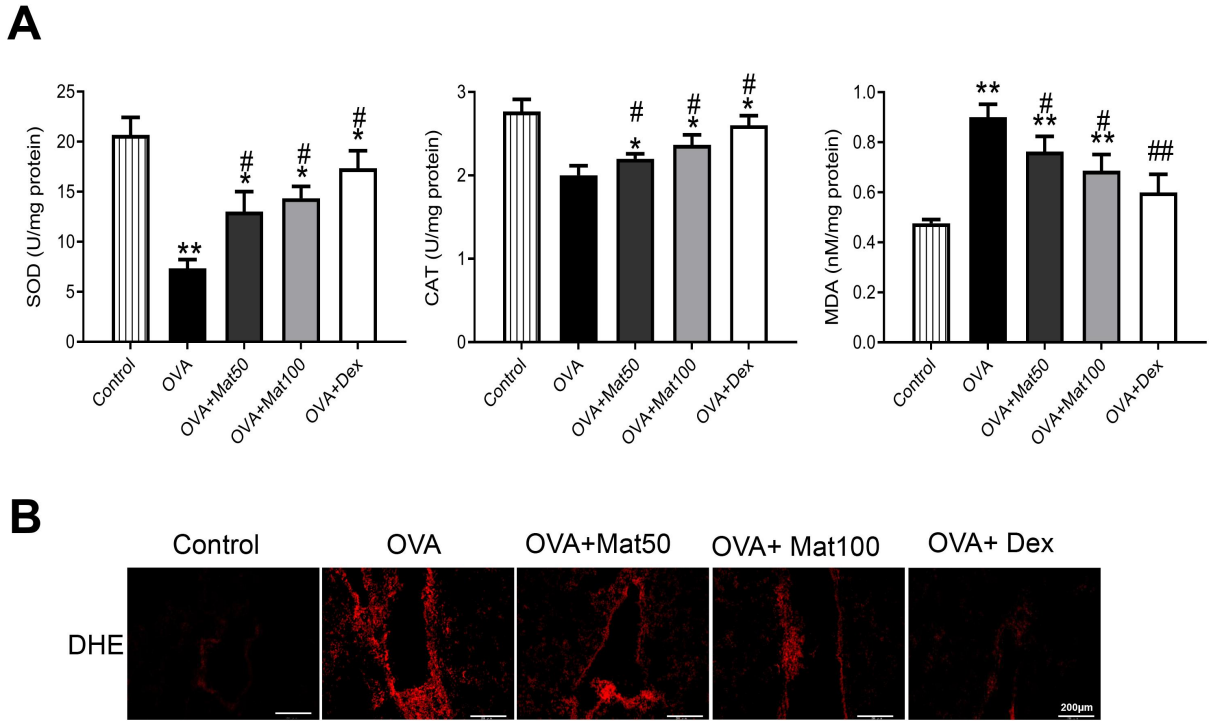

Fig. 3. Impact of matrine on oxidative stress in the lungs of asthmatic mice. (A) SOD, CAT, and MDA expression levels were measured using enzyme-linked immunosorbent assay (ELISA). N = 3, $^{*}p < 0.05$, $^{**}p < 0.01$ *vs.* Control; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ *vs.* OVA. (B) ROS production in lung tissue sections was assessed using DHE staining. Scale bar = 200 μm, magnification = 100×. SOD, superoxide dismutase; CAT, catalase; MDA, malondialdehyde; ROS, reactive oxygen species; DHE, dihydroethidium.

sis to examine the expression levels of NLRP3, IL-1β and Caspase-1 (Fig. 4A,B). Compared to the Control group, a significant up-regulation was observed in the levels of Caspase-1, NLRP3, and IL-1β expression in the OVA group ($p < 0.05$). Conversely, high-dose matrine effectively suppressed the expressions of IL-1β, Caspase-1, and NLRP3 compared to the OVA group ($p < 0.05$). Furthermore, immunohistofluorescent staining of NLRP3 revealed a substantial increase in NLRP3 expression in the OVA group (Fig. 4C), while high-dose matrine displayed a remarkable reduction in NLRP3 expression. These findings demonstrate that matrine effectively attenuates the NLRP3-mediated inflammatory response in asthmatic mice.

Impact of Matrine on AMPK-DRP1 Pathway

We evaluated the expression levels of p-AMPK, AMPK, p-DRP1, DRP1, MFN1, and MFN2 using western blot analysis (Fig. 5A,B). The OVA group exhibited a substantial decrease in the phosphorylation level of AMPK and the expression of MFN1 and MFN2, along with a significant elevation in levels of phosphorylated DRP1 and total DRP1 expression relative to the control group ($p < 0.05$). Conversely, compared to the OVA group, high-dose matrine significantly enhanced the expression levels of MFN1, p-AMPK, and MFN2 while reducing AMPK, DRP1, and p-DRP1 expression ($p < 0.05$). Additionally, immunohistochemical staining of DRP1 (Fig. 5C) revealed that matrine exposure suppressed DRP1 expression. Furthermore, immunofluorescence analysis of lung tissue sections demonstrated a substantial reduction in DRP1 expression in the high-dose matrine group compared to the OVA group (Fig. 5D). These findings suggest that matrine may alleviate airway inflammation in asthmatic mice by facilitating AMPK phosphorylation and mitochondrial fusion while inhibiting DRP1-mediated mitochondrial fission.

Discussions

This study investigated the role of matrine in inhibiting DRP1-mediated mitochondrial fission during allergic airway inflammation by modulating AMPK and ROS. Our findings revealed that matrine attenuated OVA-induced peri-airway eosinophil infiltration, inflammatory cell count in BALF, and IgE levels in Th2 cytokines and serum. Additionally, matrine suppressed IL-13 and IL-4 expression in mLN, elevated CAT and SOD activity in BALF, and reduced ROS and MDA expression in lung tissue. Furthermore, matrine enhanced AMPK phosphorylation, as well as MFN1 and MFN2 expression, while decreasing Caspase-1, DRP1, NLRP3, and IL-1β expression in OVA-induced asthmatic mice.

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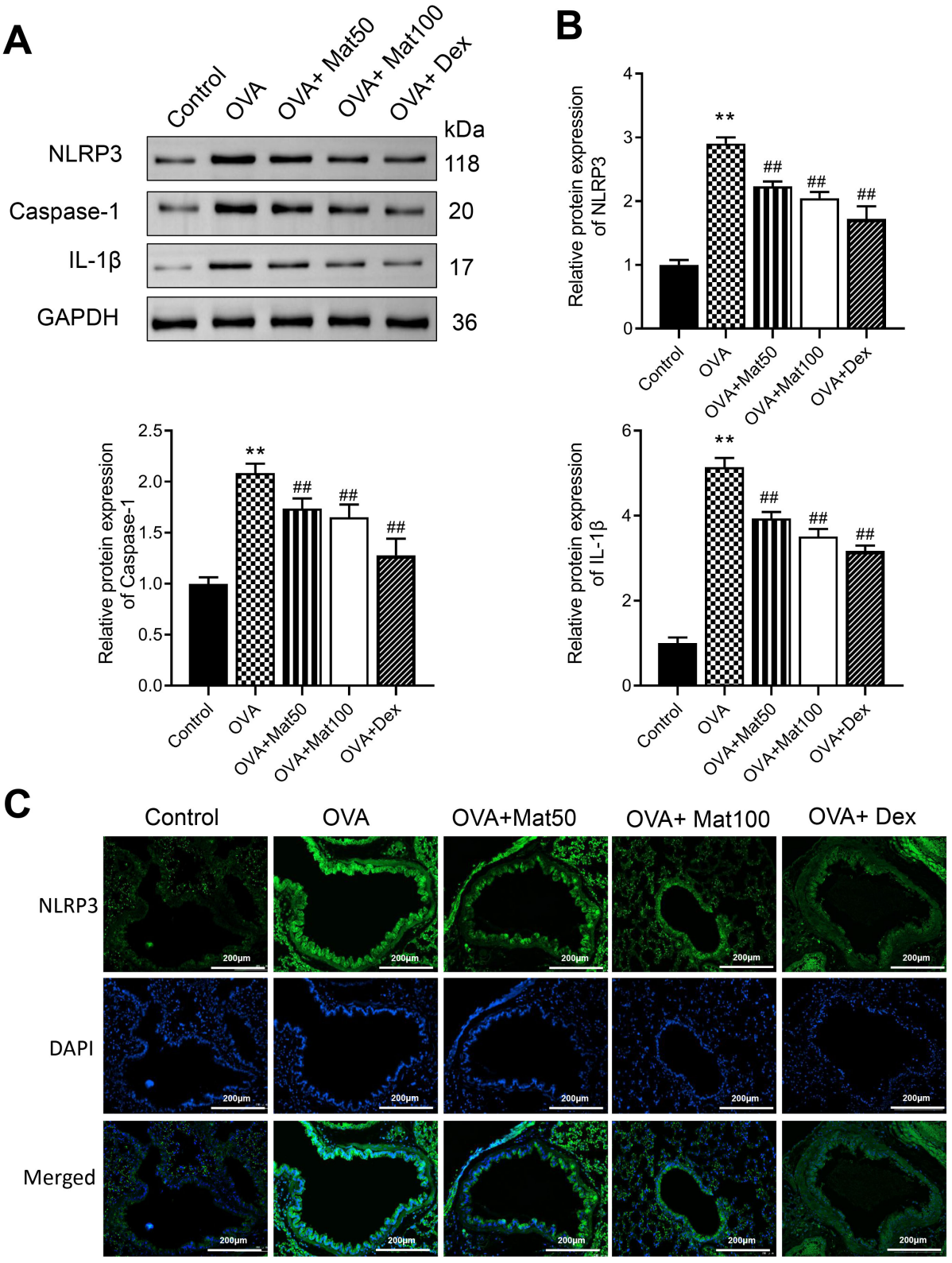

Fig. 4. Impact of matrine on DRP1-mediated NLRP3. (A) Western blot analysis for Caspase-1, NLRP3, IL-1β, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). (B) Quantitative western blot results (Panel A). $^{**}p < 0.01$ *vs.* control. $^{\#\#}p < 0.01$ *vs.* OVA. (C) Immunofluorescent staining of NLRP3. N = 3, Magnification = 100×, scale bar = 200 μm. NLRP3, NOD-like receptor family, pyrin-domain-containing-3; DRP1, dynamin-related protein 1; DAPI, 4′,6-diamidino-2-phenylindole.

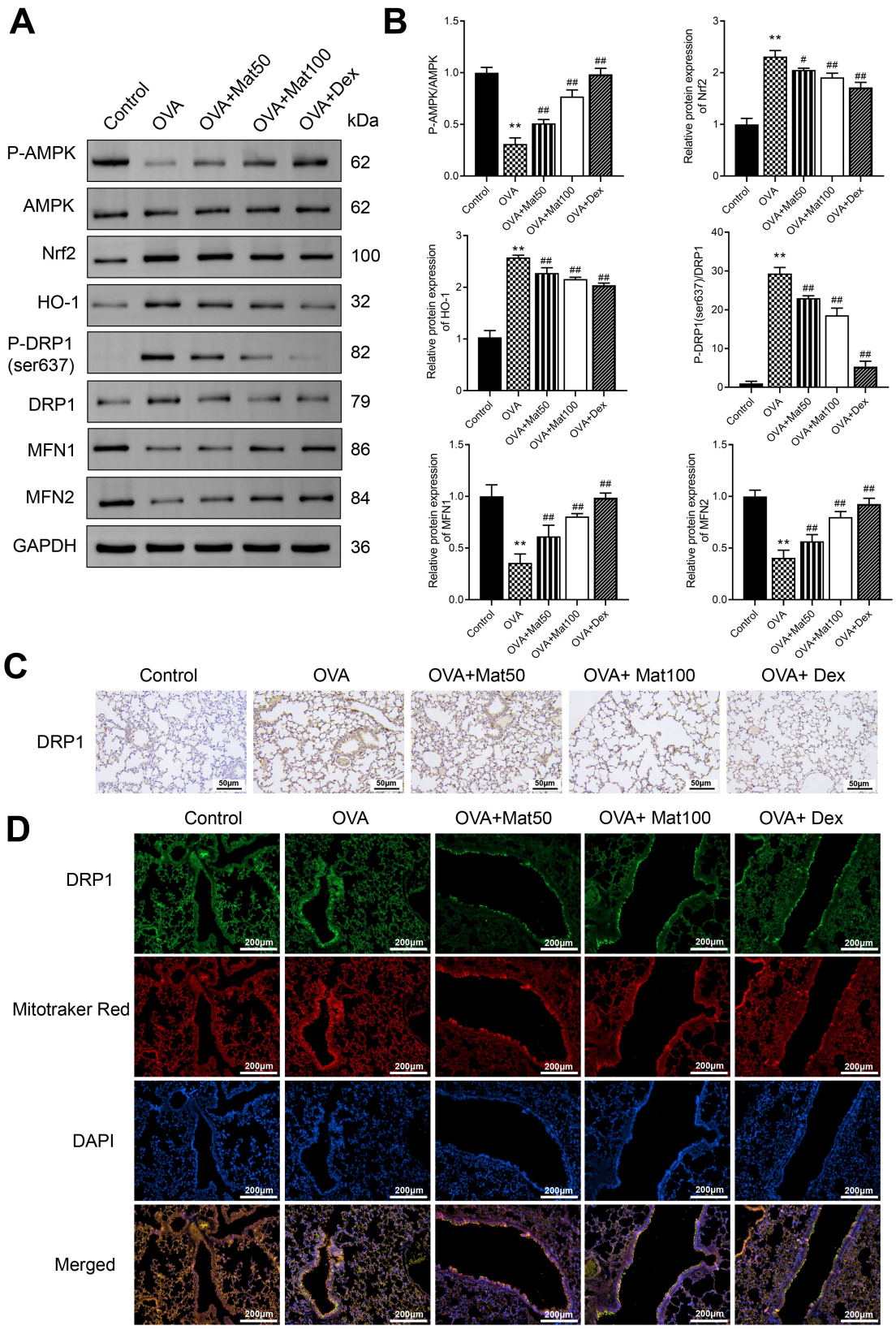

Fig. 5. Impact of matrine treatment on AMPK/DRP1 pathway. (A) Western blot analysis of p-AMPK, AMPK, HO-1, Nrf2, p-DRP1 (Ser616), MFN1, MFN2, DRP1, and GAPDH. (B) Quantitative western blot results (Panel A). $^{**}p < 0.01$ *vs.* Control, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ *vs.* OVA. (C) Immunohistochemical staining of DRP1. Scale bar = 50 μm. (D) Immunofluorescent staining of DRP1. N = 3, Scale bar = 200 μm, magnification = 100×. AMPK, adenosine 5′-monophosphate-activated protein kinase; Nrf2, Nuclear Factor Erythroid 2-related Factor 2; MFN, Mitofusin; HO-1, Heme oxygenase-1.

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Excessive ROS production and oxidative stress contribute to asthma development and bronchial epithelial cell injury triggered by OVA, playing pivotal roles in immune response development, airway epithelium-mediated sensing, airway remodeling, and hyperresponsiveness [28]. Antioxidant defense mechanisms protect against airway inflammation and oxidative lung injury triggered by OVA in asthmatic mice. Excessive ROS production can induce cellular oxidative stress, activate various pathways, and trigger the mitochondria-mediated intrinsic apoptosis pathway, leading to apoptosis [29,30]. Matrine, an alkaloid known for its antioxidant and anti-inflammatory properties, has been extensively studied in various diseases [31,32]. According to Zhao *et al*. [33], seven consecutive days of matrine administration significantly decreased MDA expression and increased the expression of Bcl-2, glutathione peroxidase (GSH-Px), SOD, total antioxidant capacity (T-AOC), and CAT in a focal cerebral ischemia-reperfusion (I/R) injury model, thereby mitigating cerebral I/R damage. Furthermore, matrine can activate the p38 mitogen-activated protein kinase/nuclear factor E2-related factor-2/antioxidant response element (MAPK/Nrf2/ARE) antioxidant signaling pathway, reducing advanced glycation end products-induced ROS production, and preventing ROS-mediated apoptosis [34]. Sun *et al*. [35] observed that matrine increased T-SOD, T-AOC, and CAT expression in D-galactose-induced aging mouse models while decreasing MDA expression, thus reducing oxidative stress injury in the plasma, liver, and brain. Similarly, our study found that matrine elevated SOD and CAT expression while reducing MDA expression in OVA-induced asthma, suggesting its potential to suppress asthma inflammation by inducing antioxidant enzymes, thereby preventing oxidative stress and tissue damage. Mitochondria are recognized as a major source of ROS, and airway inflammation may be suppressed by reduced mitochondrial ROS (mtROS) expression. Interestingly, this study indicated that matrine attenuated ROS production in the airways of asthmatic mice. Based on these findings, we suggest that matrine might alleviate airway inflammation by inhibiting oxidative stress and ROS production.

Studies have demonstrated that phosphorylation of DRP1 (Ser616) induces mitochondrial fission. Furthermore, matrine has been reported to ameliorate cardiac injury through SIRT1/AMPK signaling [36] and mitigate non-alcoholic fatty liver via AMPK [37]. Additionally, melatonin has been found to reduce VSMC calcification through AMPK/DRP1 [38], whereas Sfrp5 can improve post-myocardial infarction remodeling by increasing p-AMPK and mitochondrial fusion while decreasing p-DRP1 (Ser616) [39], and mangiferin can inhibit vascular smooth muscle cell proliferation through AMPK/DRP1 [40]. Moreover, matrine upregulates Nrf2-mediated antioxidative stress genes in colitis [26], and metformin improves lead-induced mitochondrial fragmentation via AMPK/Nrf2 [26]. SMS ameliorates liver injury triggered by heat stress (HS) through DRP1-dependent mitophagy mediated by AMPK [41]. Upregulation of NR4A1 promotes chondrocyte mitochondrial fission through AMPK [42]. Resveratrol alleviates BCP by activating AMPK and reducing mitochondrial fission [43].

Our findings confirm that matrine reverses abnormal mitochondrial fragmentation through AMPK and Nrf2. ROS can trigger mitochondrial fission. Moreover, H2O2-induced periodontitis damages periodontal ligament cells (hPDLCs) through ROS-DRP1 crosstalk, which can be mitigated by N-acetylcysteine (NAC) and mitochondrial fission inhibitor (Mdivi-1) [44]. DRP1 interacts with ROS in mitochondrial fission and apoptosis induced by paraquat (PQ) in mouse alveolar type II (AT-II) cells [45]. Plasma protein, Caveolin-1 (Cav-1), moderates mitochondrial morphology and function via DRP1 [46]. Suppression of DRP1 or mitochondrial ROS alleviates NOX2/4-induced inflammation [47]. Furthermore, our findings indicate that ROS abundance and DRP1 translocation to mitochondria trigger fission in OVA-induced inflammation, suggesting that matrine likely improves mitochondrial function by regulating ROS-DRP1 to reduce fission in mitochondria.

Findings indicate that glycolysis in oligodendrocytes downregulates NLRP3 inflammasome activation and inflammatory response induced by DRP1 fission protein, thereby improving Alzheimer's disease (AD) [48]. DRP1-mediated mitochondrial fission induces ROS production in retinal cells, forming NLRP3 inflammasome [49], thereby initiating inflammatory cascades. Suhuang can suppress DRP1 activation and mitochondrial fission to restore mitochondrial homeostasis, reducing ROS overproduction, mPTP opening, mitochondrial membrane potential collapse, and mtDNA release. Additionally, Suhuang limits the activation of NLRP3 inflammasome by facilitating ubiquitination degradation of NLRP3 and preventing NLRP3-ASC interaction, thereby alleviating the inflammatory response [50]. Oxidative stress and mitochondrial dysfunction activate inflammatory mediators such as the NLRP3 inflammasome [49]. Mitochondrial dysfunction disrupts ROS release, triggering inflammation, while increased ROS production activates the NLRP3 inflammasome [50], leading to IL-1β maturation and Caspase-1 activation, thus amplifying inflammation [36]. This suggests a close association between NLRP3 activation and mitochondrial damage [51]. NLRP3-induced inflammation is crucial in asthma pathogenesis [52,53].

Matrine promotes proliferation and inhibits apoptosis, inflammation, and fibrosis in normal cells. It also hinders eotaxin and Th2 cytokine production, alleviating allergic airway inflammation and eosinophil infiltration in asthmatic mice triggered by OVA [54]. Matrine inhibits airway inflammation by alleviating SOCS3 expression through NF-κB inhibition in asthmatic mice and airway epithelial cells (mLE-12 and BEAS-2B). Furthermore, our results

confirm the association between mitochondrial dysfunction and the NLRP3 inflammasome in asthmatic mice. Therefore, inhibiting ROS and maintaining mitochondrial function may impede Caspase-1, NLRP3, and IL-1β expression. These outcomes suggest that matrine may target the AMPK-DRP1-NLRP3 signaling pathway to alleviate airway inflammation in asthma.

However, there are limitations to consider, such as the generalizability of animal models to human asthma, the method of drug administration via gavage differing from typical clinical routes like inhalation, and the focus on short-term outcomes without assessing long-term effects and safety. Additionally, the study was conducted with a relatively small sample size, potentially affecting the statistical power of the results. The mechanisms involving the AMPK/Nrf2 pathway were explored, but a more detailed understanding of the molecular interactions remains necessary. Comparative studies involving other treatments would also help position matrine within the broader context of asthma therapies. Addressing these limitations in future research will be essential to validate and extend the findings of this study.

Conclusions

In summary, our study highlights the therapeutic potential of matrine in allergic airway inflammation by modulating the AMPK-DRP1-NLRP3 pathway. Matrine effectively mitigates airway inflammation and mucus secretion in OVA-induced asthmatic mice. Moreover, it also reduces Th2 cytokines and serum IgE levels, indicating its anti-allergic effects. Additionally, matrine enhances antioxidant defense by increasing SOD and CAT levels while reducing MDA and ROS levels in lung tissue. Moreover, it promotes mitochondrial fusion and inhibits the activation of NLRP3 inflammasome while suppressing DRP1-mediated mitochondrial fission. Collectively, these findings support matrine as a promising therapeutic option for bronchial asthma by targeting multiple implicated pathways. However, further investigations are needed to elucidate its molecular mechanisms and clinical potential.

Availability of Data and Materials

The data used to support the findings of this study are available from the corresponding author upon request.

Author Contributions

NR, JW and LMS designed the research study. NR, JW and LMS performed the research. JNG, CZC and YLC provided help and advice on experiments. JNG, CZC and YLC analyzed the data. LMS wrote the manuscript. All authors contributed to important editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The study design adhered to the Regulations on the Administration of Experimental Animals and was approved by the Ethics Committee of Yanbian University School of Medicine, China (approval number: SYXK(JI)2020-0009).

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Conflict of Interest

The authors declare no conflict of interest.

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