Archives of Microbiology (2022) 204:441
https://doi.org/10.1007/s00203-022-03039-w

ORIGINAL PAPER

Modulatory apoptotic effects of sinomenine on *Mycoplasma pneumonia* through the attenuation of inflammation via ERK/JNK/NF-κB signaling pathway

Yao Chen[1] · Wen Zhang[1] · Lihong Xin[1] · Zhen Wang[1] · Mao Zheng[2] · Annamalai Vijayalakshmi[3]

Received: 1 February 2022 / Accepted: 1 June 2022 / Published online: 30 June 2022

Abstract

Mycoplasma pneumoniae (MPP) induced pneumonia is a common disease of children. Sinomenine (SIN) is an isoquinoline mainly sequestered from *Sinomenium acutum.* It is a promising drug for treating arthritis, lung, colon, liver and gastric cancer. Hence, the present study investigated the role and mechanism of SIN treatment in MPP induced pneumonia in experimental *in-vivo* mice model. The BALB/c male mice were separated into four groups ($n = 6$ mice/group): normal, MPP, MPP + SIN (20 mg/kg bw), and SIN (20 mg/kg bw) alone. Results were expressed as mean ± SD. Data were analyzed using one way Analysis of Variance (ANOVA) with the Dunnett's post hoc test using SPSS v 18.0. P value < 0.05 was considered significant. The total protein, cell count, inflammatory cytokines, MP–IgM, Monocyte chemo attractant protein-1 (MCP-1), and MP–DNA were measured. The protein expressions of Bax/Bcl–2, ERK, JNK, NF-κB were analyzed and histopathology of lungs was examined. SIN treatment significantly ($p < 0.05$) reduced the total proteins, cell counts in BALF, inflammatory cytokines, MP–IgM, MCP-1, MP–DNA and reversed the histological alterations. SIN attenuated the apoptotic pathway through the modulation of Bax/Bcl-2 expression. SIN alleviated pulmonary inflammatory mediators and apoptosis in MPP-infected mice via suppression of ERK/JNK/NF-κB signaling. SIN administration diminished inflammation and lung fibrosis by inhibiting apoptosis in MPP mice. Hence, SIN is a potential natural protective remedy for MPP.

Keywords Apoptosis · *Mycoplasma pneumonia* · cytokines · inflammation · NF-κB · Sinomenine

Introduction

Mycoplasma pneumoniae (MPP) is the commonest cause of pneumonia in neonates (Gao et al. 2013). As per the WHO, 19% of under-five mortality is attributed to MPP (Guo et al. 2015). MPP is an inflammatory condition of alveoli in the lungs. It is frequently caused by viruses or bacteria and rarely by other microorganisms, drugs, and autoimmune diseases conditions (Shangguan et al. 2014). Bacterial pneumonia is treated with antibiotics. MPP is resistant to antibiotics and is difficult to cure (Ferrer et al. 2016). Moreover, MPP can also cause severe complications, such as asthma and pulmonary fibrosis (Wood et al. 2017).

Studies have suggested that pneumonia symptoms caused by MP are due to the initiation of pro-inflammatory cytokine levels (Shimizu 2016). MPP stimulates immune responses to kill the pathogens by upregulation of the cytokines such as IL-10, IL-8, TNF-α, and IL-6 and increasing leucocytes and neutrophils at the site of infection, ultimately leading to lung impairment (Wang et al. 2014). Severity of lung disease caused by MPP is associated with the immune response of the host (Waites et al. 2014). In general, corticosteroids are used for treating MPP to reduce the inflammatory response and pulmonary injury. However, corticosteroids cause various side effects. Thus, natural, safer and effective compounds are required for MPP treatment (Shangguan et al. 2014).

Sinomenine (SIN) is sequestered from the Chinese herb *Sinomenium acutum* (Bordon et al. 2013). SIN has

Communicated by Erko Stackebrandt.

✉ Mao Zheng
zhengmao19810908@sina.com

[1] Department of Respiratory, Xi'an Children's Hospital, Xi'an City 710000, China

[2] Department of Emergency, Xi'an Children's Hospital, Xi'an City 710000, China

[3] Department of Biochemistry, Rabiammal Ahamed Maideen College for Women, Thiruvarur, Tamil Nadu 610001, India

anti-arrhythmic, anti-analgesic, anti-arthritic, apoptotic, and anti-inflammatory properties. SIN also prevents lung cancer, breast cancer, liver cancer, and other tumors (Zhao et al. 2012; Gao et al. 2013). However, not much is known about the anti-inflammatory and apoptotic action of SIN on MPP. Therefore, we explored the anti-inflammatory and apoptotic efficacy of MPP in the mice model.

Materials and methods

Chemicals

Sinomenine (purity > 95%) and mycoplasma pneumoniae (MPP) culture were purchased from ATCC, USA. Antibodies IL-6, TGF, IL-1, IL-8, and TNF-α were procured from Thermofischer Scientific, USA. Antibiotics, and all the other biochemicals were procured from Merck, Germany.

Mycoplasma pneumoniae (MPP) culture

MP strain ATCC15531 was cultured in Hayflick adapted medium comprising of PPLO broth, horse serum (25%), penicillin-G, yeast extract, glucose (0.5%), Thallium acetate (0.025%), and phenol red (0.002%). The cells were cultured at 37 °C for 1 week (Lavazza et al. 2022).

Animal maintenance

BALB/c male mice aged 3-week-old weighing 15 ± 1 gm was procured from the departmental animal house and preserved in a controlled temperature, humidity and kept in aseptic cage dust pathogen free environment. All mice were acclimatized for 1 week before experiments. The research was approved by Xi'an Children's Hospital animal ethical committee (Approval No: XCH202106726A).

Experimental design

Mice were divided into 4 groups with 6 numbers each ($n = 6$/group).

Group-I mice were considered as control (normal).

Group-II mice were induced with pneumonia through 100 µl MP (1×10^7 ccu/ml). MP was administered through nasal drops for 2 days.

Group-III mice were administered SIN (20 mg/kg bw) after 3 days of successively giving MPP. MPP was administered through nasal drops for 2 days.

Group-IV mice were treated with SIN (20 mg/kg bw) alone for 3 days.

Subsequently, all the mice were sacrificed at the end of the experiment. Samples were collected for further biochemical and histopathological analysis.

Broncho alveolar lavage fluid (BALF) isolation

Five separate diluents of 30 mL buffered saline (0.89%) were instilled in the right side of central lobe of lung by inserting a catheter in the trachea of terminally anesthetized mice. The instilled fluid was gently retracted and the pooled fluid was rapidly centrifuged for 10 min at 6000 rpm. The clear solution was transferred to tubes and stored at 75 °C for subsequent testing as per the Van Hoecke et al. 2017 method.

Estimation of complete protein in BALF

The total protein in the BALF was assessed in normal and experimental mice using a kit for protein assay based on the Bradford method. BALF protein was considered as a marker of lung penetrability and epithelial injury (Caraballo et al. 2013).

BALF—total cell count

Draining method was used to separate the slime from BALF. Total cells were counted using a hemocytometer with a 5 mL dilution of BALF. The cells were counted and the percentages were calculated using the Zhang et al. 2020 method.

Analysis of MP–IgM, MCP-1, and inflammatory cytokines by ELISA

MP–IgM in the lung tissues and MCP-1, cytokines (IL-6, TGF, IL-1, IL-8, and TNF-α) in the serum of the mice were determined using Kienast et al. 1996 method by ELISA kit (Cayman chemicals, USA) as per manufacturer's protocol.

Detection of MP–DNA by qRT–PCR

The DNA samples were extracted from the left lobe of the lung using a kit from Thermo Fisher Scientific (Waltham, USA), as per the manufacturer's instructions. The RT–PCR MP kit was used to detect MP–DNA (Thermo Fisher Scientific, Waltham, USA). As a fluorescent indicator for RT–PCR, SYBR green (Synergy Brands, Inc. (stock symbol)—dsDNA binding dye) was used as per Abdel Sater et al. 2022 method. This procedure was carried out using a qPCR apparatus (Bio-Rad, USA) in accordance with the manufacturer's instructions. The following were the primer sequences:

MP–DNA, F: 5'-CAGAAACACACACAGCAGCTATT-3' R: 5'-CACGTTGATCCGCAAAGGAAGT-3'; GAPDH,

5'-TCTCCCTCACAATTTCCATCCC-3' R: 5'-TTTTTG TGGGTGCAGCGAAC-3'

Histological analysis

On the fourth day, the inferior lobe of the right lung was insulated with 5 percent formaldehyde, fixed with paraffin, cut into sections and stained with hematoxylin and eosin (H & E) as per Zhou et al. 2017 method. After staining, tissues were examined for signs of lung injury and inflammation under a microscope.

Western blotting analysis

The removed lung tissues were washed twice with PBS before being placed on ice for 30 min with an ice cold lysis buffer containing 0.01 percent protease inhibitor (Lichtenstein 2015). The lysate was centrifuged at 13,000 rpm for 10 min at 4 °C. The resulting solution was then processed through SDS–PAGE (Sodium Dodecyl Sulphate–Polyacrylamide Gel Electrophoresis) (10%) and transferred to PVDF–(Polyvinylidene fluoride) film. After that, 5% fat-free milk was used to block the membrane and added with specific primary antibodies for Bax, Bcl-2, ERK, JNK, and NF-κB. The β-actin was then used as a standard and treated with horseradish peroxidase-conjugated secondary antibodies. Enhanced chemiluminescence was used to see the membrane (ECL, Millipore).

Statistical analysis

Results were expressed as mean ± SD. Data were analyzed using one way Analysis of Variance (ANOVA) with the Dunnett's post hoc test. All statistical analysis were performed using software SPSS version 18.0. P value < 0.05 was considered statistically significant.

Results

Effect of SIN on the protein in BALF

The protein level in BALF was significantly increased in the MPP-induced experimental mice significantly as compared to the control group ($p < 0.05$). SIN treatment significantly reduced ($p < 0.05$) the total protein levels in BALF in SIN + MPP group ($p < 0.05$). Protein levels in SIN alone groupwere significantly ($p < 0.05$) similar to protein levels in the control mice. Thus, SIN proved to have ameliorative effects (Fig. 1a).

Effect of SIN on the cells in BALF

There was a BALF were analyzed in the experimental mice, the significant increase in the cells in BALF of the MP-induced group as compared to control group ($p < 0.05$). SIN treatment reversed and significantly reduced the increased total cell count in the SIN + MPP group ($p < 0.05$). SIN alone group retained similar cell count as the control group (Fig. 1a).

Effect of SIN on MP–IgM and MCP-1

MP–IgM and MCP-1 in the MPP-infected mice were significantly elevated in contrast to control mice. SIN significantly reversed the MP–IgM and MCP-1 levels in the MPP + SIN group ($p < 0.05$). The control group and SIN alone group showed similar values (Fig. 1b).

Effect of SIN on MPP

The relative expression of MP–DNA increased significantly in the MPP-group as compared to the control group ($p < 0.05$). SIN significantly reversed the augmented MPP–DNA expression in the MPP + SIN group. The MP–DNA expressions in control and SIN were similar (Fig. 1c).

Effect of SIN on inflammatory cytokines

The inflammatory cytokines play a dynamic role in the protection and inflammation reactions. The levels of cytokines (IL-6, TGF, IL-8, IL-1, and TNF-α) were significantly ($p < 0.05$) elevated in the MPP infected mice as compared to the control mice. However, SIN treatment significantly reduced ($p < 0.05$) these cytokines in the MPP + SIN group. The control and SIN alone mice had similar cytokines levels (Fig. 2).

Effect of SIN on lung histopathology

Figure 3 depicts the lung histopathology in control and experimental mice. The MPP lung tissues of the mice displayed extreme damage to the tissues, increased inflammatory cells penetration, and vasodilation. Conversely, MPP + SIN treatment reversed the inflammation and tissue architecture towards normal. SIN-alone treated mice also preserved the normal tissue architecture.

Effect of SIN on Bax/Bcl-2 protein expression

The control and SIN alone treated mice had reduced intensities of Bax and elevated intensities of Bcl-2. MPP-induced mice up-regulated the Bax expression and

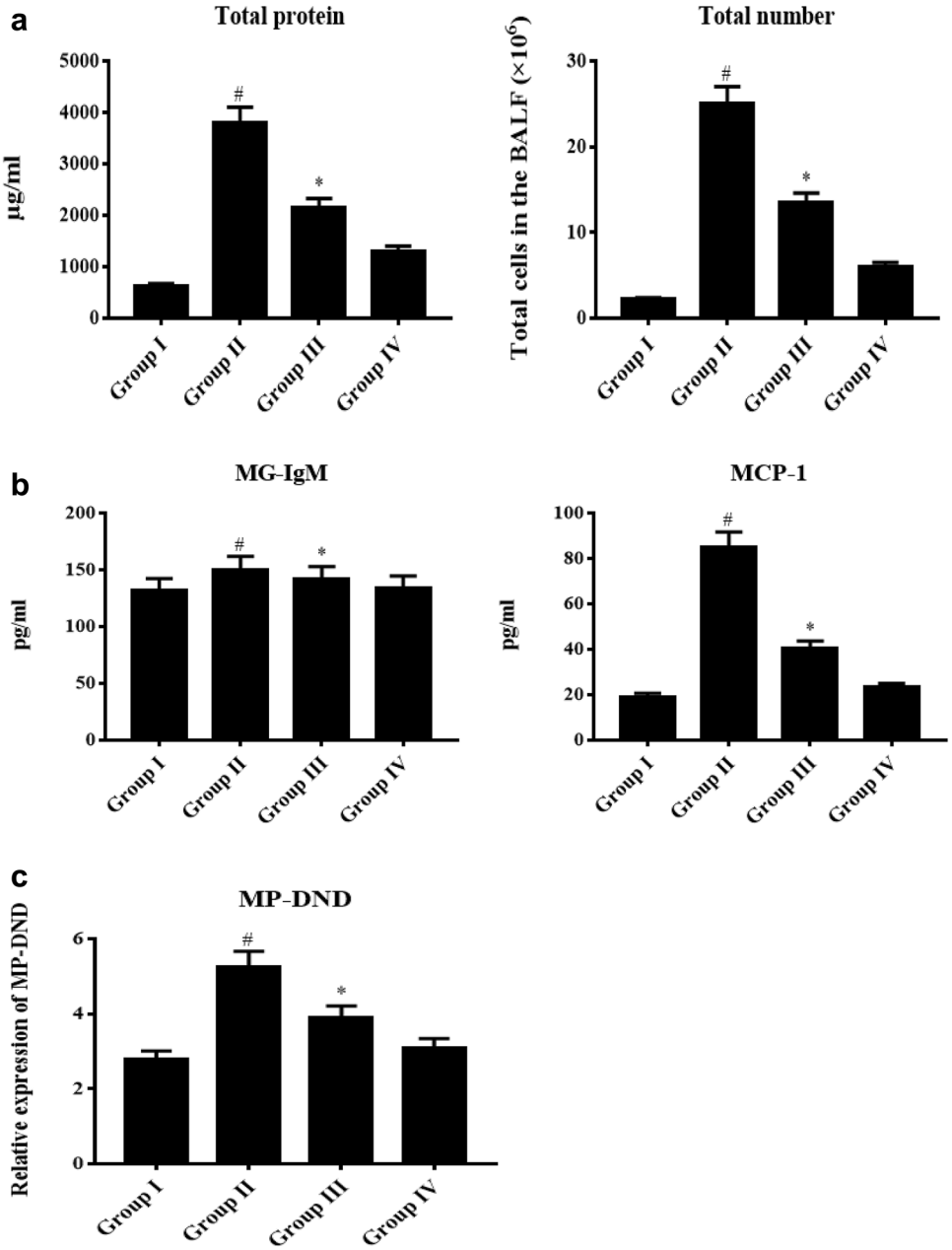

Fig. 1 **a** Effect of SIN on whole proteins and total cells in BALF of experimental MPP mice. Results were expressed as mean ± SD of six observations.*$P < 0.05$ indicates the values not sharing a common superscript letter differ significantly. **b** Effect of SIN on MP–IgM in lung tissue and MCP-I in serum were measured by ELISA. Results were expressed as mean ± SD of six observations.*$P < 0.05$ indicates the values not sharing a common superscript letter differ significantly. **c** Anti-MPP action of SIN on mice model. The MPP–DNA's relative expression was measured by qRT–PCR. Results were expressed as mean ± SD of six observations. *$P < 0.05$ indicates the values not sharing a common superscript letter differ significantly

down-regulated Bcl-2. These findings indicated that SIN inhibits MPP-induced apoptosis via diminishing Bax and enhancing Bcl-2 levels (Fig. 4).

Effect of SIN on ERK/JNK/NF-κB protein expression

The effect of SIN on ERK, JNK, and NF-κB expressions was analyzed (Fig. 5). Protein expressions of ERK and NF-κB were significantly increased and JNK significantly decreased in the MPP group versus control group.

Discussion

MPP can cause damage to the respiratory tract, and other pulmonary problems. MPP causes acute bronchitis, acute asthma, and community-acquired pneumonia in children and adults (Xu et al. 2019). Multiple antibiotics have recently been found to be ineffective against MPP infection. MPP patients develop serious lung complications and require intensive care (Maselli et al. 2018). SIN is an isoquinoline that is being studied for the treatment of

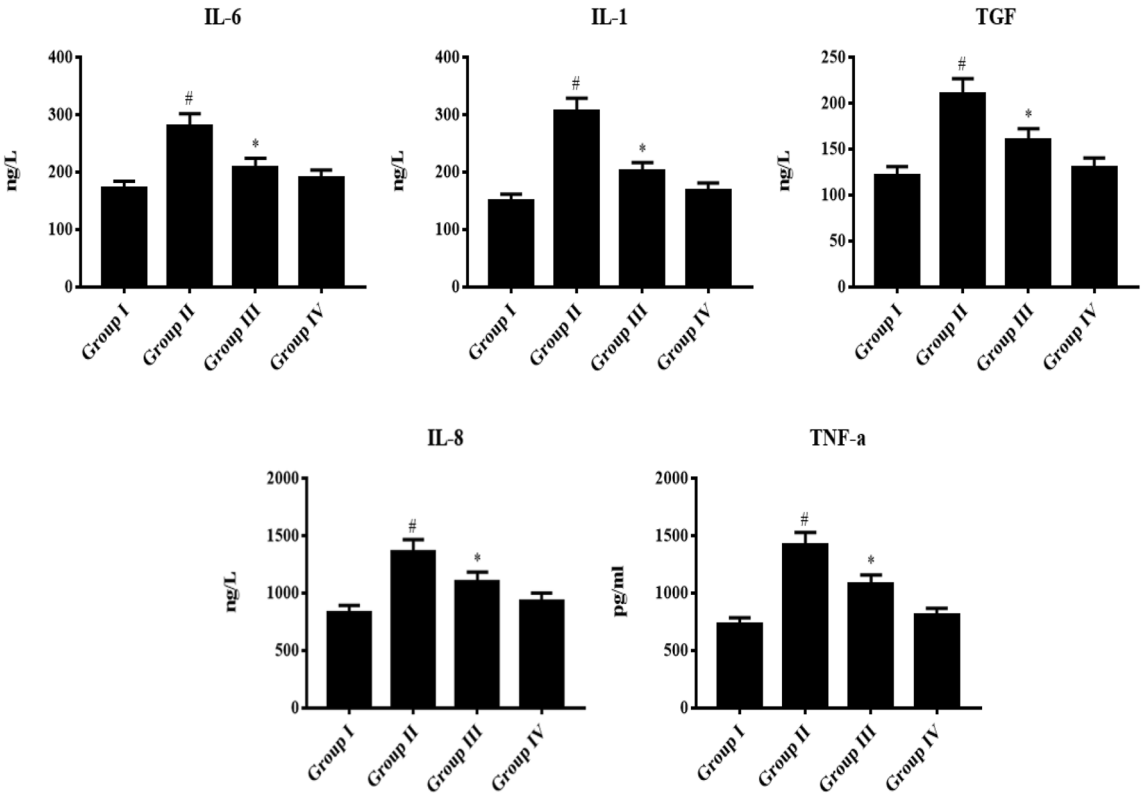

Fig. 2 Effect of SIN on the determination of pro-inflammatory cytokines in serum. Results were expressed as mean ± SD of six observations. *i < 0.05 indicates the values not sharing a common superscript letter differ significantly

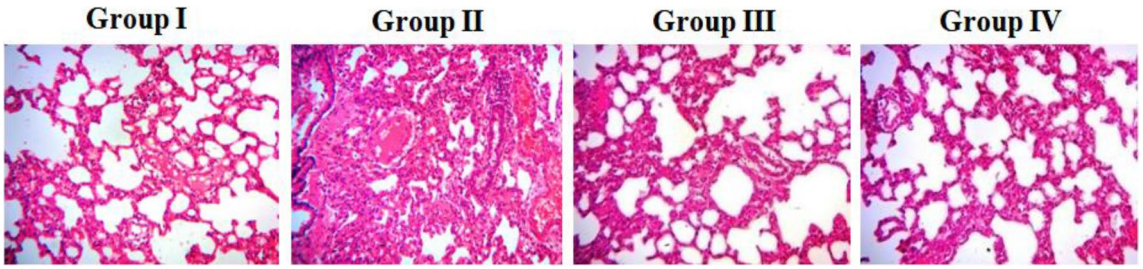

Fig. 3 Effect of SIN on the histopathology of lung tissues. **A** Control mice show normal lungs histology. **B** MPP infected mice show impairment of lung tissues, vasodilation, and inflammation. **C** MPP + SIN treated mice show lessened inflammation and reversal of the lung cells arrangements to normal **D** SIN alone treated mice display normal lungs architecture. (Stained with H&E, magnification X40)

rheumatoid arthritis, inflammation, and several cancer models in the laboratory (Wood et al. 2017). The current study examines the effects of SIN on MPP in mice. The anti-inflammatory and anti-MPP mechanisms of action were also studied. Research suggests that MPP is linked to the cytokines expression and the initiation of pulmonic fibrosis (Zhu et al. 2016). MPP infection causes the production of cytokines, such as IL-6 and TNF-α, which excite many leukocytes congregated at the contamination site, resulting in respiratory impairment (Kurai et al. 2013). Furthermore, the critical inflammatory mediators IL-1 and TNF-α have been implicated in the advancement of primary inflammation, which can lead to the formation of lymphocytes and neutrophils (Lin et al. 2018). These MPP induced inflammatory mediators play a key role in the depletion of the alveolar medium (Boddeke et al. 2001). In the current investigation, SIN reduced the inflammatory mediators in serum, such as IL-8, IL-6, TNF-α, IL-1, TGF, and MCP-1. Thus, the study implies that SIN has anti-inflammatory properties (Wang et al. 2006). The NF-κB signaling pathway has been used to control inflammatory responses in the lungs (Wang et al.

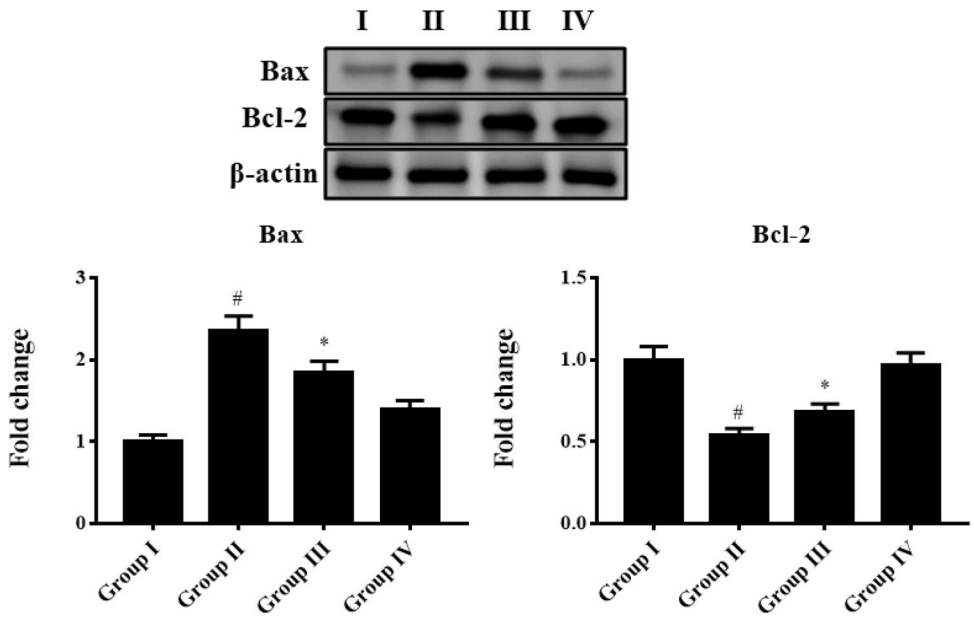

Fig. 4 Effect of SIN on apoptosis by Bax/Bcl-2 protein expression. These protein expressions were analyzed by western blotting. Results were expressed as mean ± SD of six observations. *$P < 0.05$ indicates the values not sharing a common superscript letter differ significantly

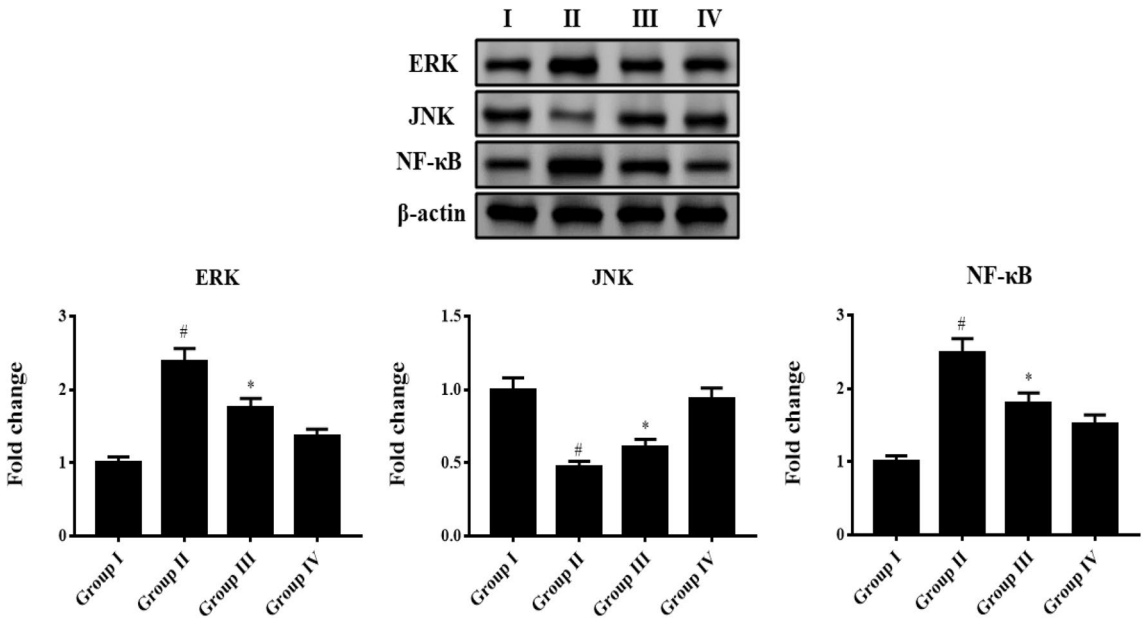

Fig. 5 Effect of SIN on ERK/JNK/NF-kB signaling pathway. These protein expressions were analyzed by western blotting. Results were expressed as mean ± SD of six observations. *$P < 0.05$ indicates the values not sharing a common superscript letter differ significantly

2005). TNF-α, IL-1, IL-6, IL-8, and TGF are all required for the highest transcription of various cytokines (Wang et al. 2006). As a result, NF-κB inhibitors may be useful as an anti-inflammatory (Tornatore et al. 2012). MPP can be stopped by inhibiting phosphorylation of JNK and ERK. SIN has proven anti-inflammatory properties by inducing NF-κB (Liu et al. 2013). Our data also revealed that SIN inhibited JNK, ERK, and NF-κB phosphorylation. This implied that SIN is a useful forMPP treatment.

SIN inhibited the pulmonary damage through inhibiting MPP by apoptosis and pulmonic fibrosis protein degradation. The anti-apoptosis Bcl-2 family includes Bax and Bcl-2. These can inhibit caspase-3 induction and are implicated in apoptosis control (He et al. 2005). SIN has been shown as apoptosis regulator via Bax/Bcl2 expressions (Mu et al. 2016). Our findings also revealed that MP–DNA expression was higher in the lung tissues of MPP-infected mice. SIN treatment reversed the DNA expressions. MPP-induced

mice had a high MP–IgM. These were significantly reduced with SIN treatment. The findings indicate that SIN inhibits MPP through the attenuation of inflammation via ERK/JNK/NF-κB signaling pathway.

Conclusions

Our findings reveal that MP–DNA expression was higher in the lung tissues of MPP-infected mice. SIN had inhibitory effect on MPP–DNA. MPP-induced mice had increased MP–IgM. SIN + MPP group had significant reduction in MPP–IgM levels indicating that SIN treatment inhibited MPP–IgM. These findings suggest that SIN inhibits MPP in mice by the suppression of the IgM reaction and attenuation of inflammation via ERK/JNK/NF-κB signaling pathway.

Author contributions YC: Methodology, formal analysis, investigation, manuscript drafting, review and editing. WZ: Methodology, investigation, manuscript drafting, review and editing. LX: Conceptualization, methodology, formal analysis, manuscript drafting. ZW: Conceptualization, methodology, formal analysis, investigation, manuscript Drafting. MZ: Conceptualization, methodology, formal analysis, investigation, manuscript drafting, review and editing. AV: Conceptualization, methodology, formal analysis, manuscript drafting, review and editing.

Funding The authors declare no funding for this research.

Data availability statement The data sets generated during and/or analyzed during the current study can be procured from the corresponding author on reasonable request.

Declarations

Conflict of interest No conflicts of interest were reported.

Ethical approval This research was approved by Xi'an Children's Hospital animal ethical committee, (Approval No. XCH202106726A).

Informed consent All the authors provided consent to publish this research.

References

Abdel Sater F, Younes M, Nassar H, Nguewa P, Hamze K (2012) A rapid and low-cost protocol for the detection of B.1.1.7 lineage of SARS-CoV-2 by using SYBR Green-based RT-qPCR. Mol Biol Rep 48(11):7243–7249

Boddeke EW (2001) Involvement of chemokines in pain. Eur J Pharmacol 429(1–3):115–119

Bordon J, Aliberti S, Fernandez-Botran R, Uriarte SM, Rane MJ, Duvvuri P, Peyrani P, Morlacchi LC, Blasi F, Ramirez JA (2013) Understanding the roles of cytokines and neutrophil activity and neutrophil apoptosis in the protective versus deleterious inflammatory response in pneumonia. Int J Infect Dis 17(2):e76-83

Caraballo JC, Borcherding J, Thorne PS, Comellas AP (2013) Protein kinase C-ζ mediates lung injury induced by diesel exhaust particles. Am J Respir Cell Mol Biol 48(3):306–313

Ferrer BE, Webster J, Bruce J, Narh-Bana SA, Narh CT, Allotey NK, Glover R, Bart-Plange C, Sagoe-Moses I, Malm K, Gyapong M (2016) Integrated community case management and community-based health planning and services: a cross sectional study on the effectiveness of the national implementation for the treatment of malaria, diarrhoea and pneumonia. Malar J 15(1):340

Gao LW, Yin J, Hu YH, Liu XY, Feng XL, He JX, Liu J, Guo Y, Xu BP, Shen KL (2006) The epidemiology of paediatric Mycoplasma pneumoniae pneumonia in North China: 2006 to 2016. Epidemiol Infect 147:e192

Gao T, Hao J, Wiesenfeld-Hallin Z, Wang DQ, Xu XJ (2013) Analgesic effect of sinomenine in rodents after inflammation and nerve injury. Eur J Pharmacol 721(1–3):5–11

Guo L, Liu F, Lu MP, Zheng Q, Chen ZM (2015) Increased T cell activation in BALF from children with Mycoplasma pneumoniae pneumonia. Pediatr Pulmonol 50(8):814–819

He X, Wang J, Guo Z, Liu Q, Chen T, Wang X, Cao X (2005) Requirement for ERK activation in sinomenine-induced apoptosis of macrophages. Immunol Lett 98(1):91–96

Kienast K, Knorst M, Müller-Quernheim J, Ferlinz R (1996) Modulation of IL-1 beta, IL-6, IL-8, TNF-alpha, and TGF-beta secretions by alveolar macrophages under NO2 exposure. Lung 174(1):57–67

Kurai D, Nakagaki K, Wada H, Saraya T, Kamiya S, Fujioka Y, Nakata K, Takizawa H, Goto H (2013) Mycoplasma pneumoniae extract induces an IL-17-associated inflammatory reaction in murine lung: implication for mycoplasmal pneumonia. Inflammation 36(2):285–293

Lavazza C, Budelli S, Montelatici E, Viganò M, Ulbar F, Catani L, Cannone MG, Savelli S, Groppelli E, Lazzari L, Lemoli RM, Cescon M, La Manna G, Giordano R, Montemurro T (2022) Process development and validation of expanded regulatory T cells for prospective applications: an example of manufacturing a personalized advanced therapy medicinal product. J Transl Med 20(1):14

Lichtenstein DA (2015) BLUE-protocol and FALLS-protocol: two applications of lung ultrasound in the critically ill. Chest 147(6):1659–1670

Lin Y, Tan D, Kan Q, Xiao Z, Jiang Z (2018) The Protective effect of naringenin on airway remodeling after mycoplasma pneumoniae infection by inhibiting autophagy-mediated lung inflammation and fibrosis. Mediators Inflamm 2018:8753894

Liu F, Sun GQ, Gao HY, Li RS, Soromou LW, Chen N, Deng YH, Feng HH (2013) Angelicin regulates LPS-induced inflammation via inhibiting MAPK/NF-κB pathways. J Surg Res 185(1):300–309

Maselli DJ, Medina JL, Brooks EG, Coalson JJ, Kannan TR, Winter VT, Principe M, Cagle MP, Baseman JB, Dube PH, Peters JI (2018) The immunopathologic effects of mycoplasma pneumoniae and community-acquired respiratory distress syndrome toxin. A primate model. Am J Respir Cell Mol Biol 58(2):253–260

Mu S, Kang B, Zeng W, Sun Y, Yang F (2016) MicroRNA-143-3p inhibits hyperplastic scar formation by targeting connective tissue growth factor CTGF/CCN2 via the Akt/mTOR pathway. Mol Cell Biochem 416(1–2):99–108

Shangguan Z, Sun Q, Zhang M, Ding J, Yi L, Gao Y, Zhan A, Zhao R, Ci X (2014) Mycoplasma pneumoniae infection in hospitalized adult patients with community-acquired pneumonia in China. J Infect Dev Ctries 8(10):1259–1266

Shimizu T (2016) Inflammation-inducing factors of mycoplasma pneumoniae. Front 7:414

Tornatore L, Thotakura AK, Bennett J, Moretti M, Franzoso G (2012) The nuclear factor kappa B signaling pathway: integrating metabolism with inflammation. Trends Cell Biol 22(11):557–566

Van Hoecke L, Job ER, Saelens X, Roose K (2017) Bronchoalveolar lavage of murine lungs to analyze inflammatory cell infiltration. J vis Exp 123:55398

Wang Y, Sheibani N (2006) PECAM-1 isoform-specific activation of MAPK/ERKs and small GTPases: implications in inflammation and angiogenesis. J Cell Biochem 98(2):451–468

Wang Y, Fang Y, Huang W, Zhou X, Wang M, Zhong B, Peng D (2005) Effect of sinomenine on cytokine expression of macrophages and synoviocytes in adjuvant arthritis rats. J Ethnopharmacol 98(1–2):37–43

Wang M, Wang Y, Yan Y, Zhu C, Huang L, Shao X, Xu J, Zhu H, Sun X, Ji W, Chen Z (2014) Clinical and laboratory profiles of refractory Mycoplasma pneumoniae pneumonia in children. Int J Infect Dis 29:18–23

Wood PR, Kampschmidt JC, Dube PH, Cagle MP, Chaparro P, Ketchum NS, Kannan TR, Singh H, Peters JI, Baseman JB, Brooks EG (2017) Mycoplasma pneumoniae and health outcomes in children with asthma. Ann Allergy Asthma Immunol 119(2):146-152.e2

Xu F, Li Q, Wang Z, Cao X (2019) Sinomenine inhibits proliferation, migration, invasion and promotes apoptosis of prostate cancer cells by regulation of miR-23a. Biomed Pharmacother 112:108592

Zhang M, Gu L, Zheng P, Chen Z, Dou X, Qin Q, Cai X (2020) Improvement of cell counting method for Neubauer counting chamber. J Clin Lab Anal 34(1):e23024

Zhao XX, Peng C, Zhang H, Qin LP (2012) Sinomenium acutum: a review of chemistry, pharmacology, pharmacokinetics, and clinical use. Pharm Biol 50(8):1053–1061

Zhou X, Moore BB (2017) Lung section staining and microscopy. Bio Protoc 7(10):e2286

Zhu Q, Sun Y, Mao L, Liu C, Jiang B, Zhang W, Li JX (2016) Antinociceptive effects of sinomenine in a rat model of postoperative pain. Br J Pharmacol 173(10):1693–1702