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Research Article

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Endoplasmic reticulum stress-induced NLRP3 inflammasome activation as a novel mechanism of polystyrene microplastics (PS-MPs)-induced pulmonary inflammation in chickens

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Abstract: Microplastics (MPs) have attracted growing attention worldwide as an increasingly prevalent environmental pollutant. In addition, chicken meat is currently the most widely consumed kind of poultry in the global market. Consumer demand for chicken is on the rise both at home and abroad. As a result, the safety of chicken raising has also received significant attention. The lungs play an essential role in the physiological activities of chickens, and they are also the most vulnerable organs. Lung injury is difficult to repair after the accumulation of contaminants, and the mortality rate is high, which brings huge economic losses to farmers. The research on the toxicity of MPs has mainly focused on the marine ecosystem, while the mechanisms of toxicity and lung damage in chickens have been poorly studied. Thus, this study explored the effects of exposure to polystyrene microplastics (PS-MPs) at various concentrations for 42 d on chicken lungs. PS-MPs could cause lung pathologies and ultrastructural abnormalities, such as endoplasmic reticulum (ER) swelling, inflammatory cell infiltration, chromatin agglutination, and plasma membrane rupture. Simultaneously, PS-MPs increased the expression of genes related to the heat shock protein family (*Hsp60*, *Hsp70*, and *Hsp90*), ER stress signaling (activating transcription factor 6 (*ATF6*), *ATF4*, protein kinase RNA-like ER kinase (*PERK*), and eukaryotic translation initiation factor 2 subunit α (*eIF2α*)), pyroptosis-related genes (NOD-, LRR- and pyrin domain-containing protein 3 (*NLRP3*), apoptosis-associated speck-like protein containing a caspase recruitment domain (*ASC*), interleukin-1β (*IL-1β*), cysteinyl aspartate-specific proteinase 1 (*Caspase1*), and gasdermin-D (*GSDMD*)), and the inflammatory signaling pathway (nuclear factor-κB (*NF-κB*), inducible nitric oxide synthase (*iNOS*), and cyclooxygenase-2 (*COX-2*)). The above results showed that PS-MP exposure could result in lung stress, ER stress, pyroptosis, and inflammation in broilers. Our findings provide new scientific clues for further research on the mechanisms of physical health and toxicology regarding MPs.

Key words: Polystyrene microplastics; Endoplasmic reticulum stress; Lung; NLRP3 inflammasome; Inflammation

1 Introduction

In recent years, microplastics (MPs, plastic particles 1–10 μm in diameter) have become a research hotspot (Akdogan and Guven, 2019; Hou et al., 2022). In recent years, MPs were listed as the second largest scientific problem to be studied in environmental and ecological science (Xu et al., 2020). Polystyrene microplastics (PS-MPs) as harmful substances interfere with chemical signals in the body, affecting endocrine function (Watts et al., 2015). Scientists have pointed out that once PS-MPs enter the environment, they are ingested by organisms, pass through the cell membrane, and infiltrate organs to cause inflammatory reactions (Moore, 2008), potentially causing bodily harm (Imran et al., 2019). The small size of MP particles, which can be directly or indirectly consumed by raptors, mammals, and fish, affects the growth, development, and reproduction of organisms (Yin et al., 2018). According to reports, MPs have been detected in more than 40 organisms. MPs ingested in the body could accumulate and migrate among different tissues, resulting in changes in behavioral ability, growth and development, metabolism, reproduction, immunity and inflammation, and induced toxicology and genotoxicity

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(Chi et al., 2021), eventually leading to chronic harm. However, less attention has been paid to lung toxicity in the field of avian toxicity of MPs. PS-MPs with 5 μm diameter are usually considered to penetrate the liver, intestinal and blood barrier, and enter the gastrointestinal system, liver, kidney, heart, and brain (Auta et al., 2017). Therefore, we suspected that PS-MPs may cause lung toxicity in chickens.

The heat shock protein family (Hsp) is an essential molecular chaperone to maintain cell survival. Previous studies have shown that Hsp members are continuously activated and can kill tumor cells through the endoplasmic reticulum (ER) stress protein kinase RNA-like ER kinase (PERK)-C/EBP homologous protein (CHOP) pathway (Dudeja et al., 2009). ER is a particular organelle that coordinates protein synthesis, folding, and transport in straight nucleus cells. Under the influence of external factors, the function of correctly folded proteins in ER is interfered with, causing ER stress and further activating cellular unfolded protein response (UPR). If ER stress persists or aggravates, UPR will induce cell pyroptosis (Oslowski et al., 2012). Many researchers have reported that ER stress can activate NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammatory bodies. In a study of diabetes, ER stress promoted the activation of downstream NLRP3 inflammatory bodies and induced islet cell pyroptosis by activating inositol-requiring enzyme type 1 (IRE1) and PERK (Lerner et al., 2012).

As part of the systemic inflammatory process, lung injury is characterized by progressive inflammation, edema, and lung neutrophil accumulation (Peng et al., 2016). At the same time, it can release some inflammatory mediators, like NLRP3, tumor necrosis factor-α (TNF-α), and interleukins (ILs) (including IL-1β, IL-6, and IL-8) (Jin et al., 2018), leading to cell death (Shi et al., 2017; Lu et al., 2022). Recent studies have confirmed that PS-MPs promote lung inflammation, further leading to pyroptosis and bringing about lung injury (Mo et al., 2023). Nonetheless, the effect of MP aggregation on lung injury in chickens or the relevant molecular mechanism has not been elucidated.

Animal studies have shown that MPs less than 10 μm in diameter can pass through the cell membrane through the blood into the circulatory system and reach the lungs (Wu et al., 2022). MPs destroy the metabolism of lung cells, leading to lung dysfunction (Shi et al., 2021). The exact mechanism by which MPs induce this kind of pulmonary toxicity is unclear. Therefore, we chose PS-MPs with 5-μm diameter and constructed an experimental chicken model of exposure to different concentrations of PS-MPs for 42 d. We hypothesized that PS-MPs induce pulmonary toxicity in chickens, which may involve lung stress, ER stress, pyroptosis, and inflammation. This study aims to provide theoretical data for comprehensively studying the detailed damage mechanism of PS-MP exposure in chickens.

2 Materials and methods

2.1 Chemicals used for treatment

All chemicals were purchased from Sigma Aldrich (St. Louis, USA) unless otherwise stated. Paraformaldehyde solution (4%, volume fraction) was obtained from Solar Biological Life Sciences Company (Beijing, China). Hematoxylin and eosin (H&E) were purchased from the Beijing Solaibao Technology Co., Ltd. (Beijing, China). PS-MPs (5 μm) were purchased from Tesulang Chemical Company (China) and stored at room temperature. Prior to use, it was prepared into an aqueous solution of the required concentration for the experiment. One-day-old specific-pathogen-free broilers were acquired from Weiwei Ltd. (Harbin, China).

2.2 Experimental design

Chicks are high trophic-level poultry and may be more affected by MPs. Thus, we chose chicks for our study model to explore a higher level of MP toxicity in chicken lungs. In our experiment, broilers were used as experimental animals to explore the effect of PS-MPs on the lungs of chickens. After purchase, broilers were adapted to standard temperature (22–25 ℃) and light (12-h light/dark cycle) conditions for a week with free access to food and water. Broilers were randomly divided into four groups with or without PS-MPs: (1) the control (Con) group was given ultra-pure water; (2) environment-related concentration group, 1 mg/L PS-MPs (L-MPs); (3) medium-dose group, 10 mg/L PS-MPs (M-MPs); (4) high-dose group, 100 mg/L PS-MPs (H-MPs). The concentration of PS-MPs was based on the study of the concentration of MPs in the water environment (Sun T et al., 2021a, 2021b). After

42 d of PS-MP exposure, the broilers in each group were sacrificed. The lung tissues were removed, frozen in liquid nitrogen, and stored in a −80 ℃ refrigerator for the follow-up experiments.

The histopathological and biochemical measurements are fully described in the supplementary materials and methods.

3 Results

3.1 Lung injury in chickens caused by PS-MPs

As shown in Fig. 1a, the pathological results of the lung were observed. In the Con group, the lung tissue structure was typical, the tissue structure was distinct, and the cell morphology was normal. Tissues in the L-MPs group exhibited lymphocytosis, inflammatory infiltration, and tissue necrosis. In the M-MPs group, there was obvious lymphocytosis and inflammatory infiltration of a large number of cells, edematous fluid of lung tissue, mitosis, and tissue necrosis. In the H-MPs group, in addition to cell swelling and inflammatory infiltration, we observed many inflammatory cells gathered into clusters, tissue structure necrosis, diffuse lesions, and mitosis.

3.2 Lung pyroptosis and ER stress in chickens by enhanced PS-MPs

The electron microscope section of the lung tissue was observed (Fig. 1b). In the Con group, the nuclear morphology was normal. In the L-MPs group, most of the nuclei were irregular. In the M-MPs group, chromatin agglutination was seen and the plasma membranes were ruptured. In the H-MPs group, the ER was swollen. The cell ultrastructure results indicated that PS-MPs can cause pyroptosis and ER stress in chicken lung tissue.

3.3 Changes in the expression of stress response genes

In order to discuss the stress response of lung tissue during PS-MP exposure, we further tested the expression level of Hsps. It was found that the expression levels of Hsp60, Hsp70, and Hsp90 were increased to different degrees in the experimental group (P<0.01, P<0.05; Figs. 2a–2c and 2e–2h), with the change in Hsp60 being the most obvious (Figs. 2a, 2e, and 2f). In contrast to the Con group, the protein expression of Hsp90 was decreased while messenger RNA (mRNA) expression was increased in the M-MPs group (P<0.01,

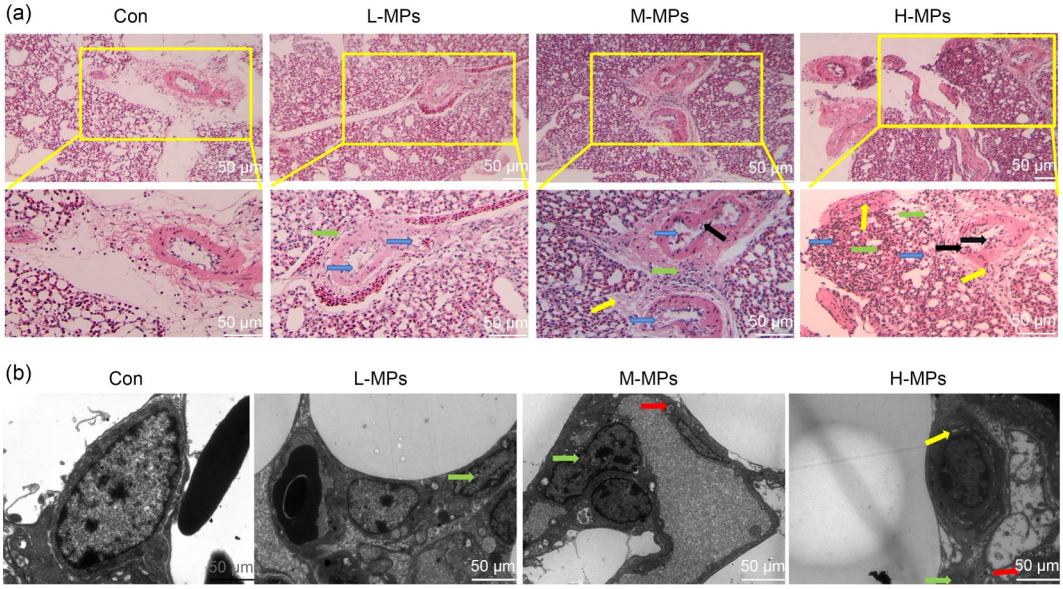

Fig. 1 Impacts of PS-MPs on the histopathological and ultrastructural integrity of lung tissue. Chickens were fed drinking water with and without PS-MPs at various concentrations for 42 d. (a) Histopathological sections of the lung. Blue arrow: inflammatory cell necrosis and inflammatory invasion; Green arrow: lymphocytes; Yellow arrow: edema fluid; Black arrow: nuclear fission. (b) Ultrastructural observation of the lung. Red arrow: chromatin agglutination, and the plasma membrane was ruptured; Green arrow: nuclei have an irregular shape; Yellow arrow: endoplasmic reticulum swelling. PS-MPs: polystyrene microplastics; Con: control; L-MPs: 1 mg/L PS-MPs; M-MPs: 10 mg/L PS-MPs; H-MPs: 100 mg/L PS-MPs (Note: for interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

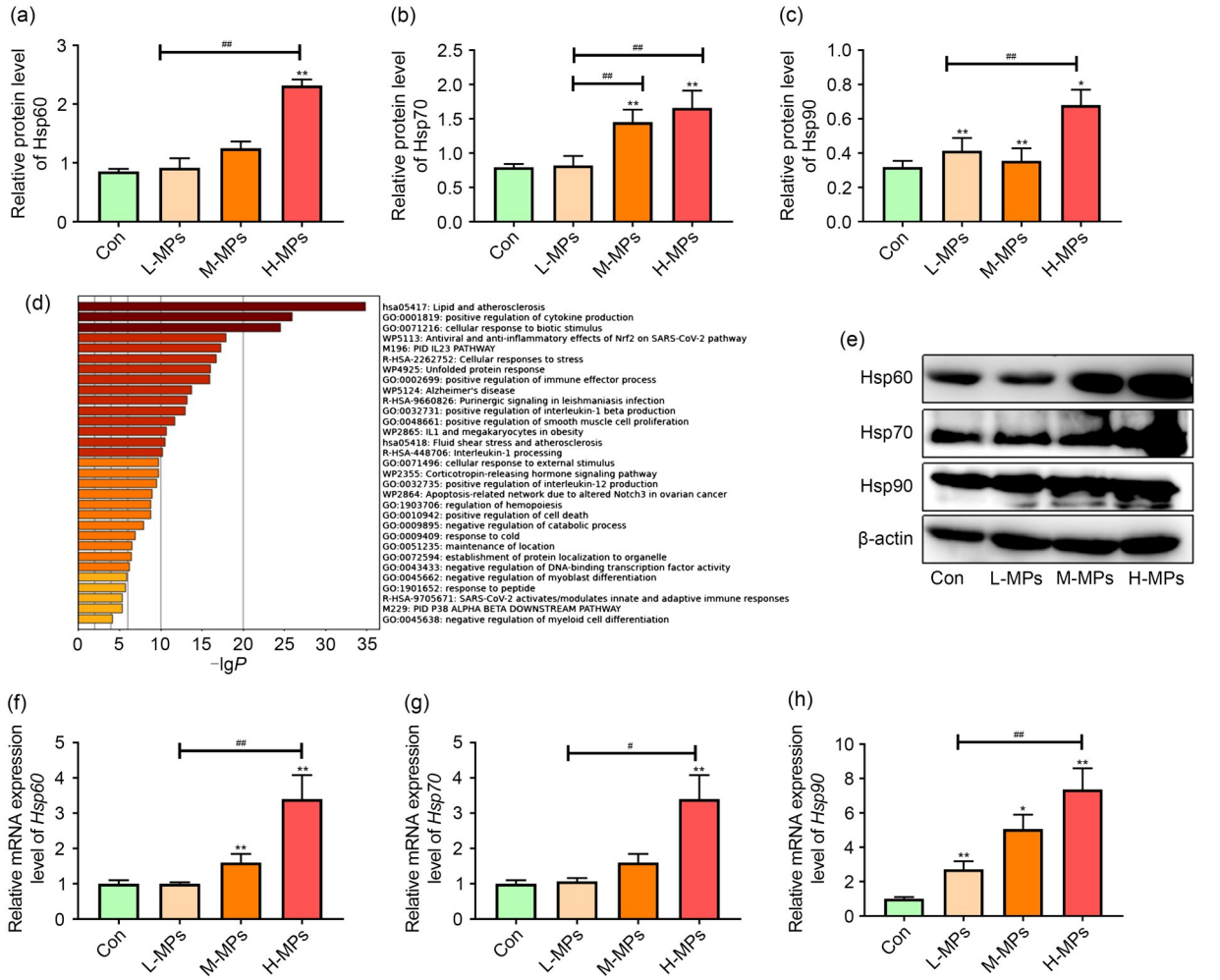

Fig. 2 Effects of PS-MPs on the expression of Hsps in lung tissue. Chickens were fed drinking water with and without PS-MPs at various concentrations for 42 d. (a–c) Relative protein expression levels of Hsp 60, Hsp70, and Hsp90. (d) GO enrichment analysis. (e) Western blot results of Hsp expression. (f–h) mRNA expression levels of *Hsp60*, *Hsp70*, and *Hsp90*. * indicates significant differences between the control (Con) group and experimental group ($^{*}P$<0.05 and $^{**}P$<0.01); $^{\#}$ indicates significant differences between the M-MPs, H-MPs groups and the L-MPs group ($^{\#}P$<0.05 and $^{\#\#}P$<0.01). Data are shown as mean±standard deviation (n≥3). PS-MPs: polystyrene microplastics; Hsps: heat shock proteins; L-MPs: 1 mg/L PS-MPs; M-MPs: 10 mg/L PS-MPs; H-MPs: 100 mg/L PS-MPs; GO: Gene Ontology; mRNA: messenger RNA.

P<0.05; Figs. 2c, 2e, and 2h). The term bar chart was enriched, and the results presented 20 term types mainly represented by UPR, inflammatory response, and cellular response to chemical stress (Fig. 2d). These findings suggested that the genes involved in this study are rich in biological processes, indicating that the major categories of genes involved do not exist independently but have a particular relationship. This further validated that PS-MPs at high concentration can stimulate apparent stress response in the lungs.

3.4 ER stress in the lungs caused by PS-MPs

The protein expression and mRNA expression of ER stress-related indexes in the lung tissue of broilers were detected. It can be seen from Fig. 3 that in all the experimental groups, PERK, phospho-PERK (p-PERK), eukaryotic translation initiation factor 2 subunit α (eIF2α), phospho-eIF2α (p-eIF2α), glucose-regulated protein 78 (GRP78), IRE1, X-box-binding protein 1 (XBP1), activating transcription factor 4 (ATF4), ATF6, and CHOP were increased compared with the Con group (P<0.01, P<0.05). The gene expression elevated with the increase in PS-MP concentration, suggesting that PS-MPs in the lung tissue of broilers activated the PERK, GRP78, and ATF6 signaling pathways of ER stress. The protein expression of p-eIF2α/eIF2α was increased in the M-MPs, albeit with no significant difference in concentration (P>

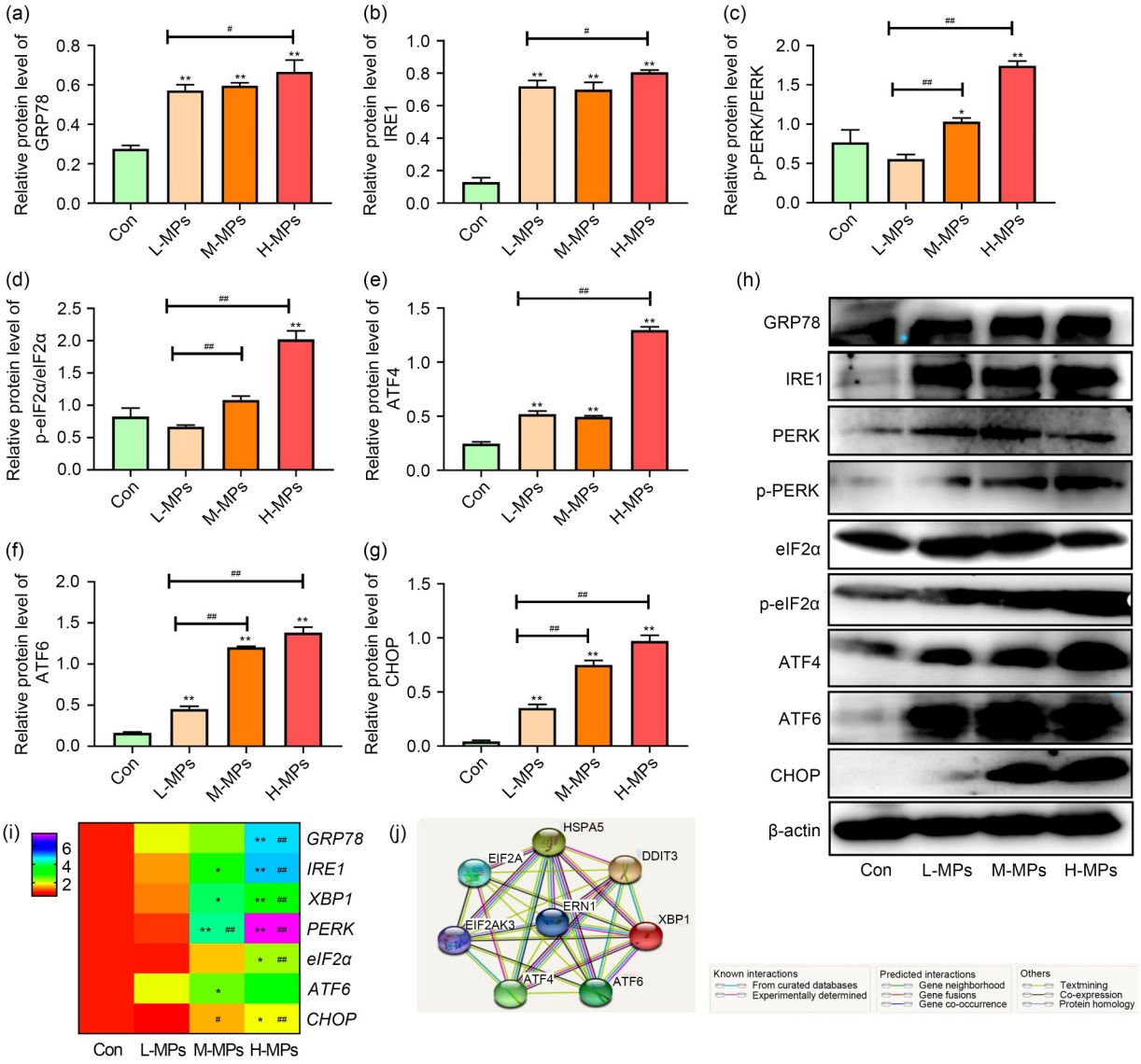

Fig. 3 Effects of PS-MP stimulation on endoplasmic reticulum (ER) stress in lung tissue. Chickens were fed drinking water with and without PS-MPs at various concentrations for 42 d. (a–g) Relative proteins expression levels of ER stress-related (GRP78, IRE1, p-PERK/PERK, p-eIF2α/eIF2α, ATF4, ATF6, and CHOP). (h) Western blot results of ER stress index. (i) ER stress index mRNA levels (*GRP78*, *IRE1*, *XBP1*, *PERK*, *eIF2α*, *ATF6*, and *CHOP*). (j) Protein–protein interaction analysis corresponding to genes. * indicates significant differences between the control (Con) group and experimental group ($^{*}P<0.05$ and $^{**}P<0.01$); $^{\#}$ indicates significant differences between the M-MPs, H-MPs groups and the L-MPs group ($^{\#}P<0.05$ and $^{\#\#}P<0.01$). Data are shown as mean±standard deviation ($n\geq3$). PS-MP: polystyrene microplastic; L-MPs: 1 mg/L PS-MPs; M-MPs: 10 mg/L PS-MPs; H-MPs: 100 mg/L PS-MPs; GRP78: glucose-regulated protein 78; IRE1: inositol-requiring enzyme type 1; PERK: protein kinase RNA-like ER kinase; p-PERK: phospho-PERK; eIF2α: eukaryotic translation initiation factor 2 subunit α; p-eIF2α: phospho-eIF2α; ATF4: activating transcription factor 4; CHOP: C/EBP homologous protein; XBP1: X-box-binding protein 1.

0.05; Fig. 3d). Although the expression of p-PERK/PERK protein was decreased slightly in the L-MPs group, the difference was also insignificant ($P>0.05$; Fig. 3c). High-dose PS-MP is suggested to activate the PERK signal pathway in lung ER stress. The results indicated that PS-MPs caused ER stress disorder in the lung tissue of broilers, and the three ER stress pathways were activated under a high concentration of PS-MPs.

3.5 ER stress triggered by PS-MPs to activate the NLRP3 inflammasome

Pyrolysis, considered as one of the most concerning ways of programmed pyroptosis in recent years,

has also been observed in the toxicity test of PS-MPs. The detected expression levels of pyroptosis-related protein and mRNA in the lung tissue of broilers are illustrated in Fig. 4. Compared with the Con group, the protein levels of NLRP3, IL-18, apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), IL-1β, cysteinyl aspartate-specific proteinase 1 (Caspase1), and gasdermin-D (GSDMD) were increased significantly in the PS-MP group (P< 0.01; Figs. 4a–4f). Figs. 4h and 4i present the relationship between the mRNA expression levels of scorch death-related genes and gene interaction in the lung tissue of broilers, respectively. According to the results of quantitative analysis, the transcriptional levels of *NLRP3*, *IL-17*, *IL-18*, *IL-1β*, *Caspase1*, and *GSDMD* were all increased to varying degrees (P<0.01, P<0.05). This confirmed that PS-MP exposure could trigger ER stress to activate the NLRP3 inflammasome and induce pyroptosis in the lungs of broilers.

3.6 Lung inflammation induced by PS-MPs

Apparent inflammatory lesions were found from the histopathological results of the lung, which were investigated to explore the injury mechanism of lung inflammation. The key indicators of the nuclear factor-κB (NF-κB) signaling pathway and the protein and gene expression levels of some inflammatory cytokines were detected. The results showed that the contents of NF-κB, inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), and TNF-α were increased significantly (P<0.01, P<0.05; Fig. 5). Simultaneously,

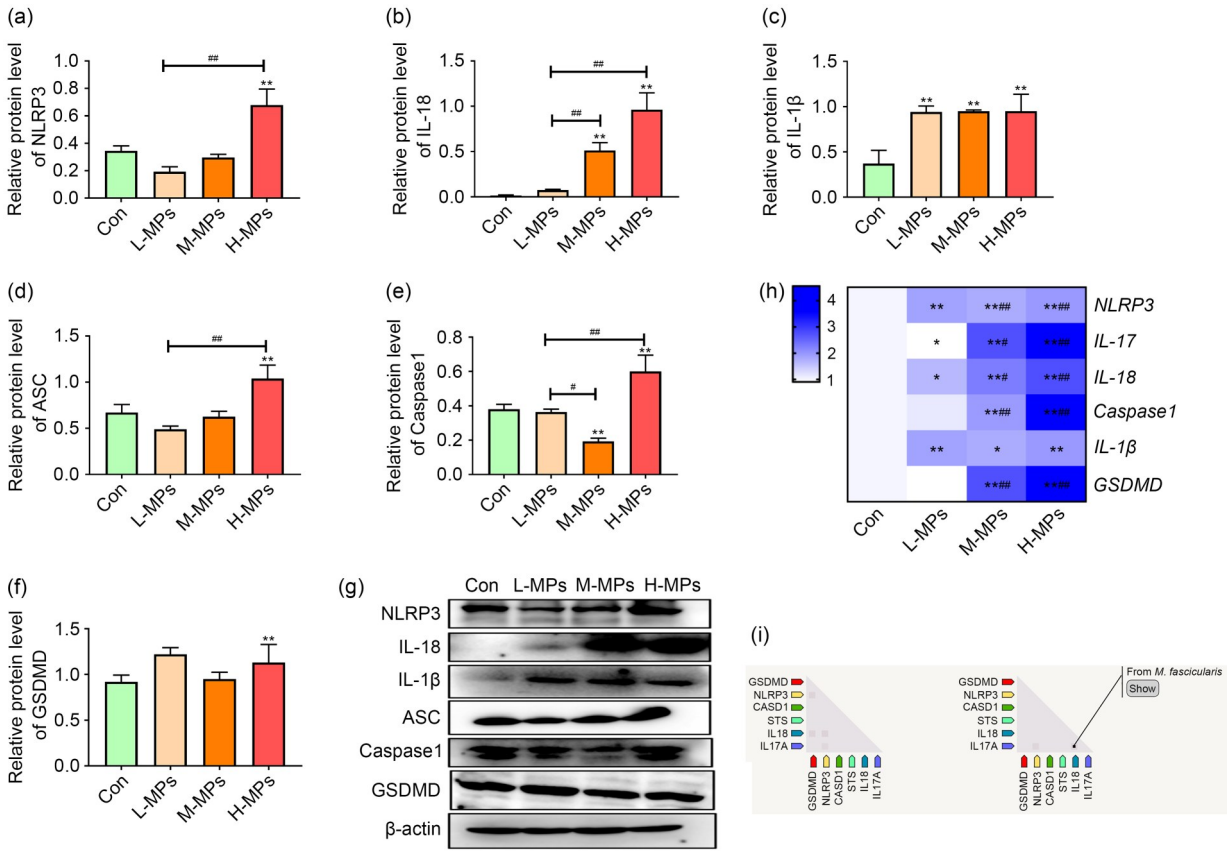

Fig. 4 Effects of PS-MP stimulation on pyroptosis in vivo. Chickens were fed drinking water with and without PS-MPs at various concentrations for 42 d. (a–f) Relative protein expression levels of pyroptosis markers (NLRP3, IL-18, IL-1β, ASC, Caspase1, and GSDMD). (g) Western blot results of pyroptosis index. (h) mRNA levels (*NLRP3*, *IL-17*, *IL-18*, *Caspase1*, *IL-1β*, and *GSDMD*). (i) Gene co-expression. * indicates significant differences between the control (Con) group and experimental group ($^{*}P$<0.05 and $^{**}P$<0.01); $^{\#}$ indicates significant differences between the M-MPs, H-MPs groups and the L-MPs group ($^{\#}P$<0.05 and $^{\#\#}P$<0.01). Data are shown as mean±standard deviation (n≥3). PS-MP: polystyrene microplastic; L-MPs: 1 mg/L PS-MPs; M-MPs: 10 mg/L PS-MPs; H-MPs: 100 mg/L PS-MPs; NLRP3: NOD-, LRR- and pyrin domain-containing protein 3; IL-18: interleukin-18; ASC: apoptosis-associated speck-like protein containing a caspase recruitment domain; Caspase1: cysteinyl aspartate-specific proteinase 1; GSDMD: gasdermin-D.

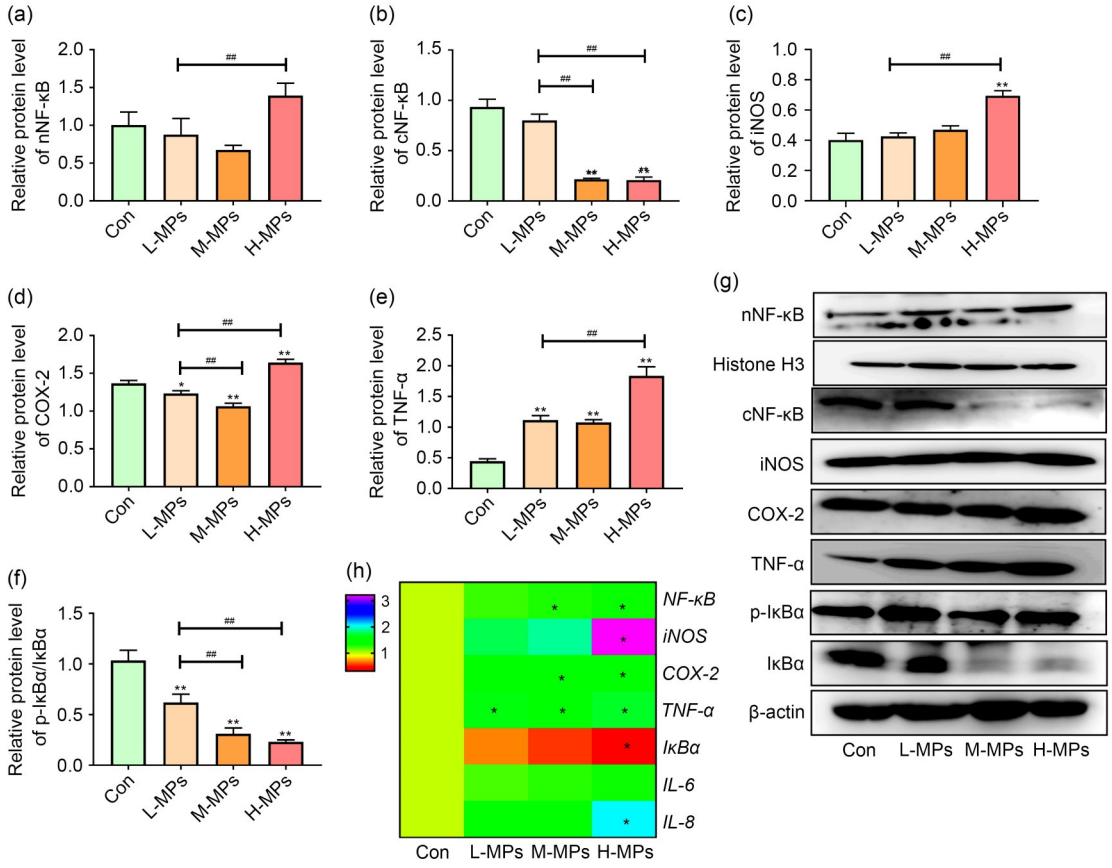

Fig. 5 Effects of PS-MPs on lung inflammation indices. Chickens were fed drinking water with and without PS-MPs at various concentrations for 42 d. (a–f) Relative expression levels of inflammation-related proteins (nNF-κB, cNF-κB, iNOS, COX-2, TNF-α, and p-IκBα/IκBα). (g) Western blot results of inflammation indices. (h) mRNA level, an index related to inflammation (*NF-κB*, *iNOS*, *COX-2*, *TNF-α*, *IκBα*, *IL-6*, and *IL-8*). * indicates significant differences between the control (Con) group and experimental group ($^{*}P<0.05$ and $^{**}P<0.01$); $^{\#}$ indicates significant differences between the M-MPs, H-MPs groups and the L-MPs group ($^{\#\#}P<0.01$). Data are shown as mean±standard deviation ($n\geq3$). PS-MPs: polystyrene microplastics; L-MPs: 1 mg/L PS-MPs; M-MPs: 10 mg/L PS-MPs; H-MPs: 100 mg/L PS-MPs; NF-κB: nuclear factor-κB; nNF-κB: nuclear NF-κB; cNF-κB: cytoplasm NF-κB; iNOS: inducible nitric oxide synthase; COX-2: cyclooxygenase-2; TNF-α: tumor necrosis factor-α; IκBα: NF-κB inhibitor α; p-IκBα: phosphor-IκBα; IL-6: interleukin-6.

the protein content and phosphorylation level of phosphor-NF-κB inhibitor α (p-IκBα) were decreased significantly ($P<0.01$, $P<0.05$; Figs. 5f–5h), indicating that exposure to PS-MPs activated NF-κB and enhanced nuclear translocation in the lungs of broilers. The mRNA expression of inflammation-related factors (*NF-κB*, *IκBα*, *IL-8*, *iNOS*, *COX-2*, and *TNF-α*) was also increased ($P<0.05$), while the gene expression of *IL-6* was not significant (Fig. 5h).

4 Discussion

The environmental problems caused by MPs are becoming increasingly severe, and the relevant biotoxic effects and health risks have also attracted much attention (Yin et al., 2021). Therefore, studying typical species in affected terrestrial ecosystems is essential. Some studies have shown that chickens with lung injury will develop pulmonary edema and alveolar epithelial cell damage, and release a large number of inflammatory cells and inflammatory factors (Zhang et al., 2023), leading to death when the lung injury is severe (Shi et al., 2021). In our study, pathological lung tissue damage was observed. PS-MPs caused severe structural damage to the lung, including increased lymphocytes, inflammatory infiltration, ER swelling, and the appearance of pyroptosis corpuscles (Fig. 1). Similarly, Hou et al. (2021) found that PS-MPs might induce ER stress. Besides, PS-MPs induced pyroptosis and

inflammation of rat lung granulosa cells through the NF-κB/NLRP3 signaling pathway (Hou et al., 2021; Zhao et al., 2021). The above findings are in good agreement with our data. Simultaneously, we proved that PS-MPs induced lung pyroptosis in chickens by lung stress-triggered ER stress, accompanied by inflammatory magnification.

After exposure to external pressure, the highly conserved Hsp in animals will maintain internal balance in various ways (Dudeja et al., 2009). In animals, protective mechanisms usually develop during stress and the synthesis of Hsp is induced, including Hsp60, Hsp70, and Hsp90 (Gong et al., 2017). Our exposure experiment revealed that the expression levels of Hsp60, Hsp70, and Hsp90 in the PS-MPs group were significantly higher than those in the control group (Fig. 2). The activation of a survival mechanism called heat shock response may support cell self-protection through the accumulation of Hsp proteins. Surprisingly, the continuous increase in Hsp levels can lead to toxic protein stress, which mediates the rapid induction of molecular chaperones (such as Hsp), leading to ER stress and inflammation.

In ER, homeostasis is maintained by various regulatory mechanisms, which is significant for the average growth and proliferation of cells (Sun DD et al., 2021). Among the three classical signaling pathways of ER stress, in our study, IRE1 could promote mRNA splicing and directly or indirectly affect the translation level while promoting the emergence of XBP1 unconventional transcripts (Figs. 3b, 3h, and 3i) and affecting downstream gene expression (Overley-Adamson et al., 2014). The kinase activity of PERK can phosphorylate eIF2α and inhibit the subsequent translation process. Studies have shown that GRP78 (Song et al., 2021), as an intraluminal molecular chaperone of ER, is significantly upregulated during ER stress and plays an auxiliary role in three signaling pathways, correcting the unfolded/misfolded proteins in the ER lumen in time, restoring the normal function of ER, and maintaining ER homeostasis (Li, 2003). GRP78 binds to the dominant proteins of the three pathways and its complex remains inactive (Oyagbemi et al., 2022). However, under various stimulating factors, ER is separated from the complex formed by PERK, IRE1, and ATF6 (Gong et al., 2022). Recent study has found that ER stress is closely related to many lung diseases (Qu et al., 2019). In this study, chickens exposed to PS-MPs showed increased transcription and translation of ER-related regulatory genes (Fig. 3). The toxicity of PS-MPs to chicken lung tissue interfered with ER homeostasis, and excessive UPR occurred in the lung.

Some studies have reported that ER stress is closely related to the activation of NLRP3 inflammasome and subsequent GSDMD-mediated cell petrogenesis (Gong et al., 2022; Abd-Elmawla et al., 2023). Stress can induce pyroptosis (Jeremias et al., 2000; Ruan et al., 2020). In the liver, ER stress causes the activation of IRE1 and PERK, leading to CHOP overexpression. CHOP activates the NLRP3 inflammatory body, which initiates hepatocyte pyrolysis (Caspase1, ASC, and NLRP3 secretion). Similarly, in our experimental chicken model, PS-MP exposure led to ER stress (Fig. 3), which activated NLRP3, followed by lung pyroptosis (Fig. 4). NLRP3 forms an activation complex with the ASC protein (Liu et al., 2022), which causes Caspase1 to cleave into its active form, thus mediating the inflammation of mature cytokines IL-1β and IL-18 (Chi et al., 2021). Studies have established that NLRP3 inflammasome triggers an inflammatory response and pyroptosis, which is closely related to the pathogenesis of lung diseases and respiratory diseases. It is inferred that ER stress can activate the NLRP3 inflammasome (Li et al., 2020), thus initiating the pyroptosis pathway and acting on pathophysiological processes in the lung.

Pyroptosis is closely linked to the inflammatory response. NF-κB is a nuclear transcription factor that regulates the expression of multiple genes in the inflammatory response, immune response, cell proliferation and apoptosis (Wang et al., 2021; Li et al., 2022). PS-MP exposure activated the inflammatory body-related molecules NLRP3 and ASC, significantly increased Caspase1 and its downstream inflammatory factors IL-1β and IL-18, and stimulated NF-κB (Zhang et al., 2022; Lian et al., 2023). Furthermore, it triggered the production of cytokines such as iNOS, COX-2, IL-6, IL-8, IL-18, IL-1β, and TNF-α (Liu et al., 2022), resulting in an inflammatory response (Fig. 5). The expression of inflammatory factors disrupts inflammatory homeostasis and exacerbates inflammatory response. In turn, NLRP3 recruits ASC with pro-Caspase1 into the NLRP3 inflammasome (Wang et al., 2020), thereby promoting pyroptosis. Our results showed that PS-MPs caused inflammation and pyroptosis through ER stress.

The activation of NLRP3 inflammasome is usually divided into two steps. The first is to assemble and activate inflammasome triggered by ER stress, which promotes the assembly of NLRP3 and ASC with pro-Caspase1 into the NLRP3 inflammasome (Figs. 3 and 4) (Wang et al., 2020), while the second is to sensitize and upregulate the expression of NLRP3 inflammasome-related proteins (Huang et al., 2020; Liu et al., 2022). This means activating Caspase1 and promoting the secretion of IL-18 and IL-1β, which leads to pyroptosis. Therefore, in this study, we detected and verified the lung injury-related indexes of chickens exposed to PS-MPs. The above preliminary data support our view that PS-MPs trigger ER stress through lung stress to induce NLRP3 inflammasome activation, causing pyroptosis and aggravated inflammation.

5 Conclusions

To sum up, we discovered the potential mechanism of MPs-induced pulmonary toxicity in chickens for the first time. We established that PS-MPs trigger ER stress through lung stress to induce NLRP3 inflammasome activation, leading to pyroptosis. Therefore, lung stress and ER stress play an essential role in PS-MP-induced lung injury in chickens. This information fills the gap in the toxicology mechanism of lung injury caused by MPs in the terrestrial ecosystem and provides a direction for protecting chickens from MP poisoning.

Data availability statement

Data will be made available on request.

Acknowledgments

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Author contributions

Hongmin LU performed the conceptualization, data curation, software, formal analysis, writing – original draft, and writing – review & editing. Tiantian GUO performed the conceptualization and software. Yue ZHANG contributed to the formal analysis and investigation. Dewang LIU performed the visualization. Lulu HOU contributed to the data curation, writing – review & editing, and software. Chengxue MA performed the validation, visualization, and writing – review & editing. Mingwei XING contributed to the data curation, validation, resources, funding acquisition, and project administration. All authors have read and agreed the final version of the manuscript. The authors have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Hongmin LU, Tiantian GUO, Yue ZHANG, Dewang LIU, Lulu HOU, Chengxue MA, and Mingwei XING declare that they have no conflict of interest.

All institutional and national guidelines for the care and use of laboratory animals were followed. The Experimental Animal Committee of Northeastern Forestry University approved the study (Approval No. UT-31; June 20, 2014).

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Supplementary information

Materials and methods