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ORIGINAL ARTICLE

CeO_2-NPs Attenuates Apoptosis and EMT in Rat Lung Exposed to Hyperoxia by Inhibiting M1 Macrophage Polarization

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ABSTRACT

Oxygen therapy is widely used therapeutically in the treatment of acute and chronic hypoxemia. However, hyperoxia—elevated inhaled oxygen—causes hyperoxic acute lung injury (HALI). HALI is mainly related to excessive production of reactive oxygen species (ROS). Effective treatment strategies for HALI remain limited. Apoptosis and epithelial-to-mesenchymal transition (EMT) are significant signs of lung damage after exposure to hyperoxia. HALI Responds to Inflammatory Response. Suppressing inflammation induced by M1 macrophage polarization. protects against HALI.

CeO_2-NPs are representative nano-antioxidants. In this study, we clarified the role of CeO_2-NPs in lung tissues of hyperoxia-exposed rats and the effect of CeO_2-NPs on macrophage polarization. CeO_2-NPs attenuated lung injury induced by hyperoxia exposure. In addition, CeO_2-NPs reduced the apoptosis, increased the expression of Bcl-2 and decreased expression of Bax and cleaved-caspase 3. CeO_2-NPs also reduced EMT in the lung of hyperoxia-exposed rats. CeO_2-NPS reduced the expression of IL-1β, TNF-α, IL-6. Our study showed that hyperoxia promoted M1 macrophage polarization, which was reduced by CeO_2-NPs treatment.

In conclusion, hyperoxia induced lung injury and promoted apoptosis and EMT in rats, and CeO_2-NPs had a therapeutic effect on HALI. CeO_2-NPs reduced the release of inflammatory factors and M1 macrophage polarization in rats following hyperoxia exposure. CeO_2-NPs attenuates apoptosis and EMT in rat lungs exposed to hyperoxia by inhibiting M1 macrophage polarization.

INTRODUCTION

Oxygen therapy is widely used therapeutically in the treatment of acute and chronic hypoxemia. However, hyperoxia—elevated inhaled oxygen—causes hyperoxia-induced acute lung injury (HALI) Tibboel et al. (2013), Lord et al. (2021), it is associated with increased mortality Chu et al. (2018). The development of HALI involves excessive ROS production Resseguie et al. (2015). The ROS-induced inflammatory response in the lung,

which is primary to the pathogenesis, is defined by the release of pro-inflammatory cytokines such as interleukin 1β (IL-1β), interleukin 6 (IL-6), and tumor necrosis factor α (TNF-α), followed by an influx of inflammatory cells that intensify the response and cause lung tissue damage Shahzad et al. (2022). There are several different types of macrophages in every bodily cavity. The organism activates or inactivates them through various signals in response to microbial invasion, tissue damage, and metabolic disorders; promotes polarization of adaptive T cell responses Locati et al. (2020). To establish a host defense or heal tissue, macrophage polarization to proinflammatory M1-like or anti-Inflammatory M2-like cells is essential Liu et al. (2021). Hyperoxia has well-documented detrimental consequences on lung biology, including cell apoptosis Bhandari et al. (2006) and the epithelial-to-mesenchymal transition (EMT) Vyas-Read et al. (2014). EMT is commonly understood to be the process by which epithelial cells become mesenchymal cells by losing their polarity and junctions Pan et al. (2017), Lamouille et al. (2014).

Hyperoxia promotes macrophage recruitment and triggers M1-like macrophage polarization and increased interleukin release to exacerbate lung injury Hirani et al. (2022).

Cerium oxide nanoparticles (CeO_2-NPs) have reversible redox properties due to the interconvertibility between

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Ce3+ and Ce4+ Dowding et al. (2014), which can scavenge a wide range of ROS Lord et al. (2021), Kajjumba et al. (2022) and be used for the treatment of oxidative stress-related diseases Li et al. (2020). It has been discovered that CeO_2-NPs efficiently reduce symptoms of hepatic IRI by scavenging reactive oxygen species and preventing Kupffer cell and monocyte/macrophage cell activation. Significantly less pro-inflammatory cytokines were generated, and there was less neutrophil recruitment and infiltration, all of which suppressed the ensuing inflammatory response that implicated the liver Ni et al. (2019). The benefits of CeO_2-NPs on HALI and the underlying biological mechanism are still unknown. Due to the strong anti-inflammatory and antioxidant damage effects of CeO_2-NPs, we hypothesized that CeO_2-NPs have therapeutic effects on HALI.

In this study, we clarified the role of CeO_2-NPs in lung tissues of hyperoxia-exposed rats and the effect of CeO_2-NPs on macrophage polarization.

MATERIALS AND METHODS

Animals

Three to four-week-old male Sprague–Dawley (SD) rats were purchased from Lanzhou University (Lanzhou, China). All experiments were approved by the Animal Ethics Committee of the First Hospital of Lanzhou University (LDYYLL2022-216). Rats were humanely cared for during the experiments. Rats were kept under controlled conditions: free access to food and water, 12-hour light/dark cycle, constant temperature (22 °C) and humidity (45-55%).

Animal treatment

Rats were randomly divided into four groups (n=7): (1) RA group; (2) HALI group; and (3); HALI + CeO_2-NPs group. Rats in the CeO_2-NPs group were intraperitoneally injected with CeO_2-NPs (0.5 mg/kg) following hyperoxia exposure once daily for 7 days.

Hyperoxia-induced lung injury model

Cages (6 rats per cage) were placed in a hyperoxia exposure chamber for 7 days. Sodium absorbent was used to line the bottom of the chamber to collect CO2. Continuous delivery of sufficient oxygen to the chamber, with the oxygen concentration regulated to 90% and monitored with oxygen monitors.

Bronchoalveolar Lavage Analysis

Alveolar lavage of rat lungs with saline was performed twice at the end of the experiment. Bronchoalveolar lavage fluid (BALF) was centrifuged at 200 × g for 10 min.

Histopathology of lung tissue

At the end of the experiment, the left lung was fixed with 4% paraformaldehyde for 24 hours at room temperature and embedded in paraffin. The lung tissue was cut into 4-micron sections and stained with hematoxylin-eosin (H&E). A scale ranging from 0 to 3 was used to measure the degree of lung injury: In the first grade, there is normal pulmonary appearance; in the second grade, there is perivascular edema formation, partial leukocyte infiltration, and moderate neutrophil leukocyte infiltration; in the third grade, there is severe structural destruction of the lungs and massive neutrophilic infiltration.

Immunohistochemistry and immunofluorescence

The lung tissue slices underwent a series of procedures including xylene deparaffinization, rehydration in graded alcohol, boiling in 0.01 M sodium citrate buffer (pH 6.0), room temperature cooling, and three PBS washes. Sections were blocked for 30 minutes at room temperature using 1% goat serum and 3% hydrogen peroxide for endogenous peroxidase.

After that, sections were incubated for an entire night at 4 °C with the matching primary anti-caspase3 antibody (19677-1-AP, Proteintech), anti-Bax antibody (T40051, Abmart), anti-Bcl-2 antibody (T40056, Abmart), anti-E-cadherin antibody (TA0131, Abmart), anti-N-cadherin antibody (T55015, Abmart), and anti-vimentin antibody (T55134, Abmart).

After that, sections were cleaned and treated for 30 minutes with rat-specific horseradish peroxidase polymer anti-rabbit antibody. Next, horseradish peroxidase substrate was added and incubated for an additional three minutes.

Hematoxylin was then used to stain the lung slices. After washing, enzyme-labeled secondary antibodies were added and the mixture was incubated for 50 minutes at room temperature to achieve immunofluorescence. After that, 4',6-diamidino-2-phenylindole (DAPI) was used to stain the sections. Images were examined with fluorescence microscope, and Image J software was used to compute the average OD values and fluorescence intensities.

ELISA

IL-1β, IL-6 and TNF-α were assayed based on kit instruction (RuixinBiotech, China) and the plate was then transferred to a microplate reader (synergy H1, BioTek Instruments, USA) and measured at 450 nm optical density.

Statistical analysis

ImageJ was used to analyze the images. GraphPad Prism 9 was utilized to evaluate the experimental data. The mean ± standard deviation (SD) is used to express normally distributed data, and one-way analysis of variance

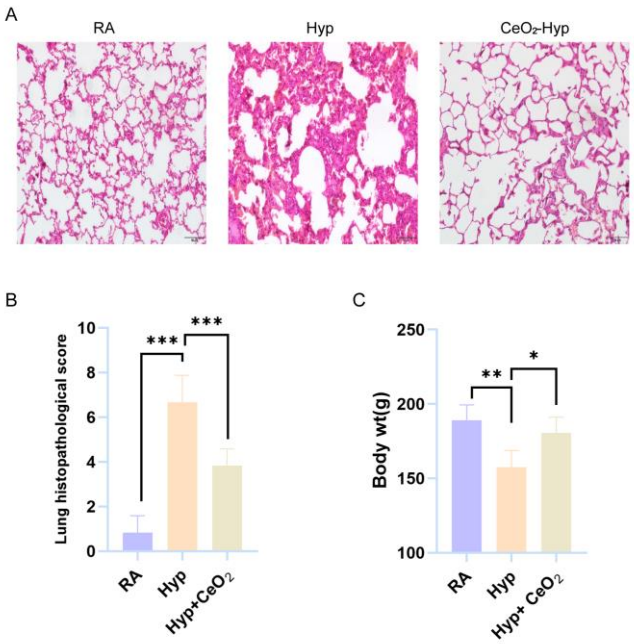
(ANOVA) was used to compare the groups. $P < 0.05$ is considered statistically significant. The number of biological replicates is presented in each figure.

RESULTS

CeO_2-NPs attenuates hyperoxia-induced lung injury in rat

HE showed that lung injury was evident in hyperoxia-exposed rats, whereas CeO_2-NPs treatment attenuated lung injury in hyperoxia-exposed rats (Fig1, A-B). Rats following hyperoxia exposure showed significant weight loss compared to controls, and CeO_2-NPs treatment increased the body weight of hyperoxia-exposed rats (Fig1C).

Figure 1: CeO_2-NPs attenuates hyperoxia-induced lung injury in rat.

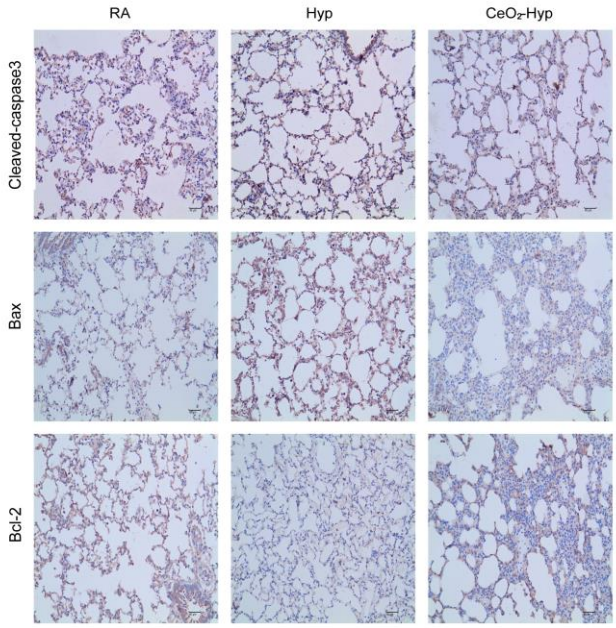


(A) HE staining in lung tissues of different treatment groups (Bar = 100 μm, magnification 200 ×, ±SD, n=6). (B) HE staining score of different treatment groups. (C) Body weight of rats in each group (±SD, n=6).

CeO_2-NPs attenuates hyperoxia-induced apoptosis in Sprague–Dawley rat lung tissues.

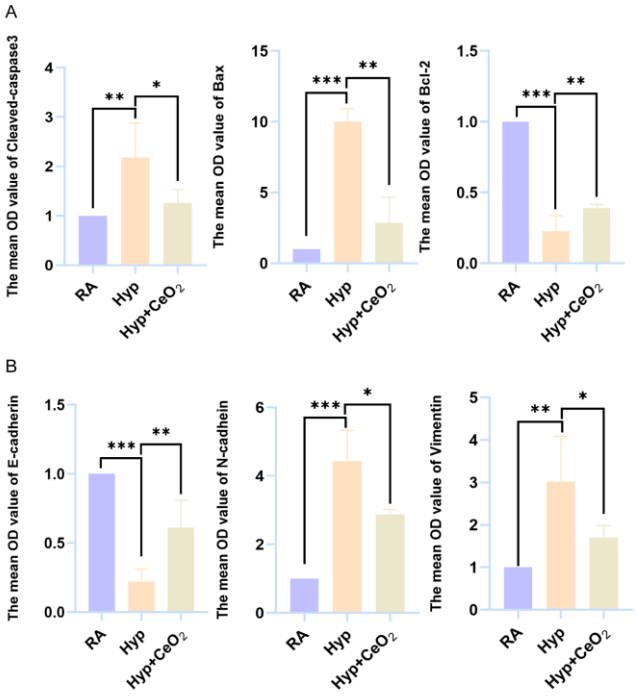
Immunohistochemistry showed that apoptosis was significantly increased in lung tissues of rats following hyperoxia exposure, as evidenced by an increase in the expression of cleaved-caspase3 and Bax and a decrease in the expression of Bcl-2, whereas CeO_2-NPs treatment attenuated apoptosis in lung tissues of rats following hyperoxia exposure (Fig2, Fig3A).

Figure 2: CeO_2-NPs attenuates hyperoxia-induced apoptosis in rat.



(A) Immunohistochemical staining of Cleaved-caspase3, Bax and Bcl-2 in lung tissues of different treatment groups, (Bar = 40 μm, magnification 200 ×, ±SD, n=6).

Figure 3: CeO_2-NPs attenuates hyperoxia-induced apoptosis in rat.

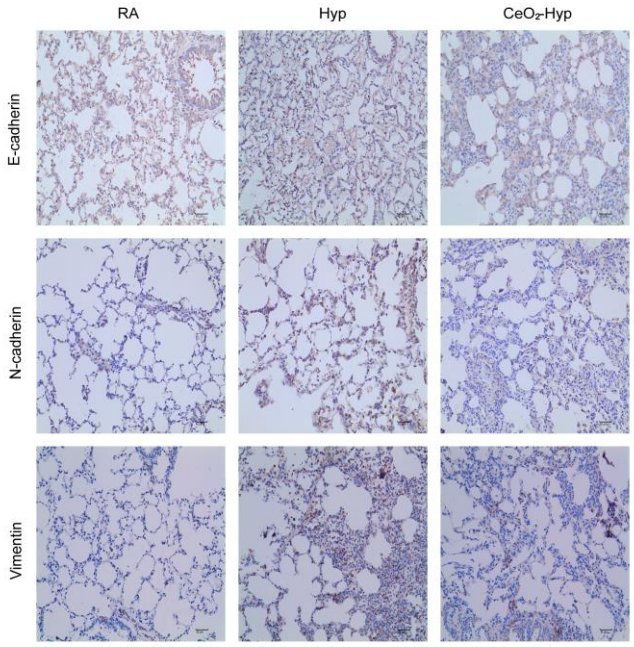


(A) Immunohistochemical quantification of Cleaved-caspase3, Bax, Bcl-2. (B) Immunohistochemical quantification of E-cadherin, N-cadherin, and Vimentin.

CeO_2-NPs attenuates hyperoxia-induced EMT in rat lung tissues

Immunohistochemistry showed that EMT was significantly increased in lung tissues of hyperoxia-exposed rats, as evidenced by an increase in the expression of N-cadherin and Vimentin and a decrease in the expression of E-cadherin, whereas CeO_2-NPs treatment attenuated EMT in lung tissues of hyperoxia-exposed rats (Fig3B, (Fig4)).

Figure 4: CeO_2-NPs attenuates hyperoxia-induced EMT in rat.

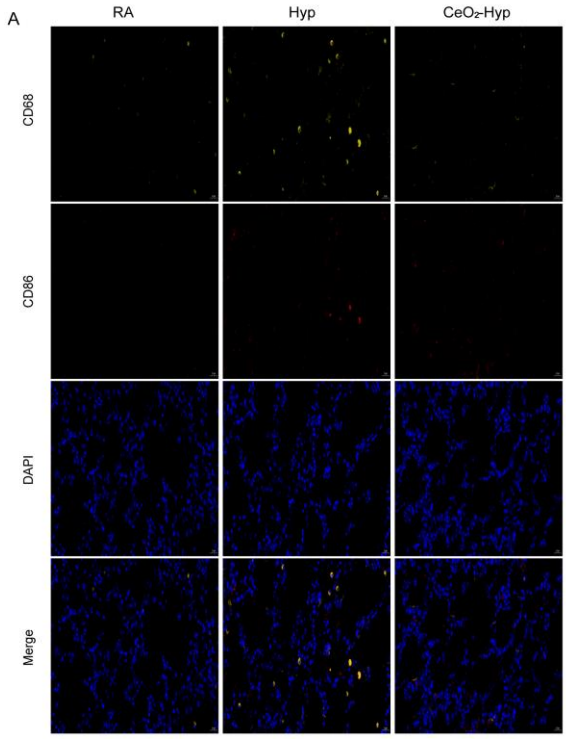


Immunohistochemical staining of E-cadherin, N-cadherin, and Vimentin in lung tissues of different treatment groups, (Bar = 40 μm, magnification 200 ×, ±SD, n=6).

CeO_2-NPs attenuates hyperoxia-induced M1 macrophage polarization in Sprague–Dawley rat lung tissues.

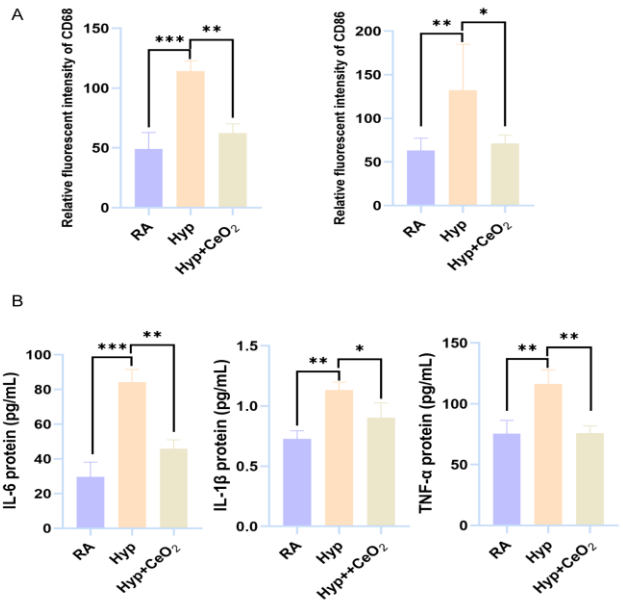
M1 polarization of macrophages promotes the expression of inflammatory factors. Immunofluorescence results showed that hyperoxia promoted macrophage and M1 macrophage polarization, CeO_2-NPs decreased macrophage and M1 macrophage polarization (Fig5A). Hyperoxia increased the expression of inflammatory factors in alveolar lavage fluid of rats, with increased expression of IL-1, IL-1β, and TNF-a, whereas CeO_2-NPs treatment significantly reduced the expression of inflammatory factors (Fig3A). It suggests that CeO_2-NPs attenuates the expression of inflammatory factors after hyperoxia exposure.

Figure 5: CeO_2-NPs attenuates hyperoxia-induced M1 macrophage polarization in rat.



(A) Immunofluorescence analysis of CD68 and CD86. Macrophages were labelled using CD68 and MI-type macrophages were labelled using CD86 (Bar = 20 μm, magnification 400 ×, ±SD, n=6).

Figure 6: CeO_2-NPs attenuates hyperoxia-induced inflammation.



(A) Quantitative immunofluorescence analysis of CD68 and CD86. (B) IL-1β, IL-6 and TNF-αexpression in alveolar lavage fluid.

DISCUSSION

Long-term high oxygen concentration inhalation can cause lung injury and finally result in HALI. This is mainly related to excessive production of reactive oxygen species (ROS) Singer et al. (2024). Oxygen (O_2) toxicity remains a concern, especially to the lung, and effective treatment strategies for HALI remain limited. We investigated the mechanism of HALI and possible preventative measures in our previous research Wang et al. (2020). CeO_2-NPs are representative nano-antioxidants. The strong ROS scavenging ability of cerium oxide has therapeutic effects on a variety of diseases, such as respiratory syncytial virus infection Patel et al. (2022), Ischemic heart disease Wang et al. (2023), acute kidney injury Yu et al. (2020), ulcerative colitis Liu et al. (2023) and Parkinson's disease Gao et al. (2024). To the best of our knowledge, this study is the first to report the anti-inflammatory effects of CeO_2-NPs in HALI. In our study, CeO_2-NPs attenuated lung structural damage in rats after hyperoxia exposure. Reduced body weight in hyperoxia-exposed rats was increased by CeO_2-NPs treatment. This demonstrates the therapeutic effect of cerium oxide nanoparticles on hyperoxia-exposed rats.

Apoptosis and EMT are significant signs of lung damage after exposure to hyperoxia Zhang et al. (2016). Prolonged hyperoxia results in acute lung injury and apoptosis in rat lung to hyperoxia. The expression of Bax and cleaved-caspase 3 was increased and the expression of Bcl-2 was decreased in rat lung following hyperoxia exposure. CeO_2-NPs reduced the apoptosis, increased the expression of Bcl-2 and decreased expression of Bax and cleaved-caspase 3. EMT refers to the process wherein epithelial cells lose their polarity and junctions and take on mesenchymal cell traits. CeO_2-NPs also reduced EMT in the lung of hyperoxia-exposed rat. EMT was increased in hyperoxia-exposed rats, as evidenced by a decrease in E-Cadherin and an increase in the expression of N-cadherin and Vimentin, whereas CeO_2-NPs treatment reduced EMT in rat following hyperoxia exposure.

HALI Responds to Inflammatory Response. Reducing inflammation in macrophages protects against acute lung injury induced by oxidative stress Guo et al. (2021). This was in line with the idea that hyperoxia induces the production of mediators linked to the oxidative stress response. Rats following hyperoxia exposure showed higher levels of inflammatory factors (IL-1β, TNF-α, IL-6) than rats exposed to air Zhang et al. (2021). This result is consistent with the findings of our study. In our study, the expression of inflammatory factors was increased in hyperoxia-exposed rats, as evidenced by increased the expression of IL-1β, TNF-α, IL-6. CeO_2-NPs reduced the expression of IL-1β, TNF-α, IL-6. M1 macrophage polarization promotes elevation of IL-1β, IL-6, TNF-α, CXCL9 and CXCL10 Wu et al. (2020). Macrophage-derived inflammatory factors were related to HALI, hyperoxia triggered M1-like polarization, IL-6 Hirani et al. (2022), IL-1 Cardenas-Diaz et al. (2023), IL1-β Mian et al. (2019), and TNF-α Lin et al. (2003) inhibiting lung growth and inducing cell death. Our study showed that hyperoxia promoted M1 macrophage polarization, which was reduced by CeO_2-NPs treatment.

CONCLUSION

In conclusion, hyperoxia induced lung injury and promoted apoptosis and EMT in rats, and CeO_2-NPs had a therapeutic effect on HALI. CeO_2-NPs reduced the release of inflammatory factors and M1 macrophage polarization in rats following hyperoxia exposure. CeO_2-NPs attenuates apoptosis and EMT in rat lungs exposed to hyperoxia by inhibiting M1 macrophage polarization.

LIMITATIONS

First, this study did not investigate the therapeutic effect of different concentrations of CeO_2-NPs; in addition, the issue of the time course of changes in injury endpoints in rats may be very important and is not addressed.

DECLARATIONS

Competing interests

The authors affirm that they have no known financial or interpersonal conflicts that would have appeared to have an impact on the research presented in this study.

Author Contributions

Hong Guo: designed the research and conducted the most of the experiments.

Xin Zhao: collated data, Data curation.

Ying Yao: collated data, Data curation.

Kai-Hua Yu: assisted experiments.

Su-Heng Chen: assisted experiments.

Yu-Lan Li: coordinated and directed the project.

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