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ORIGINAL ARTICLE

Acute Exposure to Diesel-Biodiesel Particulate Matter Promotes Murine Lung Oxidative Stress by Nrf2/HO-1 and Inflammation Through the NF-kB/TNF-α Pathways

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Abstract— Air pollution caused by fuel burning contributes to respiratory impairments that may lead to death. We aimed to investigate the effects of biodiesel (DB) burning in mouse lungs. DB particulate matter was collected from the exhaust pipes of a bus engine. Mice were treated with 250 μg or 1000 μg of DB particulate matter by intranasal instillation over 5 consecutive days. We demonstrated that DB particulate matter penetrated the lung in the 250-μg and 1000-μg groups. In addition, the DB particulate matter number in pulmonary parenchyma was 175-fold higher in the 250-μg group and 300-fold higher in the 1000-μg group compared to control mice. The instillation of DB particulate matter increased the macrophage number and protein levels of TNF-alpha in murine lungs. DB particulate matter enhanced ROS production in both exposed groups and the malondialdehyde levels compared to the control group. The protein expression levels of Nrf2, p-NF-kB, and HO-1 were higher in the 250-μg group and lower in the 1000-μg group than in control mice and the 250-μg group. In conclusion, DB particulate matter instillation promotes oxidative stress by activating the Nrf2/HO-1 and inflammation by p-NF-kB/TNF-alpha pathways.

KEY WORDS: inflammation; diesel/biodiesel mixture; oxidative stress; lung; mouse.

INTRODUCTION

Exposure to air pollution has been consistently associated with adverse health effects, especially in the respiratory and the cardiovascular systems [1]. Particulate matter that is mainly derived from vehicle exhaust pipes represents one of the most important contributors to air pollution [1]. Particulate matter generated by diesel engines consists of fine particles with an aerodynamic diameter smaller than 2.5 μm, which penetrates into bronchiolar and alveolar regions of lung [2, 3]. Therefore, particulate matter produced by diesel combustion contributes to air pollution-associated respiratory impairments [4].

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Particulate matter produced by diesel combustion mainly consists of metals and polycyclic aromatic hydrocarbons, which are toxic to the respiratory system [5, 6]. In rodent models, acute exposure to diesel particles increases the number of inflammatory cells in bronchoalveolar lavage fluid and the release of pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin 1β (IL-1β), in lung tissue [7, 8]. Additionally, different concentrations of diesel particles enhance the ROS synthesis in type II human alveolar epithelial cells [9]. The city of Rio de Janeiro has the second largest concentration of people, vehicles, and industries in Brazil and it has a high level of air pollution [10]. One of the strategies to reduce air pollutant emission by vehicles (mainly busses and trucks) is the inclusion of 7% of biodiesel in petroleum-derived fuels as diesel [11] because the mixture of diesel-biodiesel (DB) fuel may produce a lower level of fine particulate matter [12]. However, no study has thus far demonstrated the lung burden of the exposure to DB particulate matter obtained from vehicle exhaust pipes. Therefore, we aimed to investigate the outcomes of acute exposure of murine lungs to DB particulate matter resulting from fuel burning from public transport in Rio de Janeiro City.

MATERIAL AND METHODS

Animals

Sixty female C57BL/six mice (8–10 weeks) were obtained from the Bio Rio Foundation (Rio de Janeiro, Brazil). Mice were acclimatized for 2 weeks before the experiments began. The animals were housed in plastic cages and maintained on a 12-h daylight cycle. Food and water were provided *ad libitum*. All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guiding Principles in the Care and Use of Animals" approved by the Council of the American Physiological Society, USA, and the National Council for Controlling Animal Experimentation, Ministry of Science, Technology and Innovation (CONCEA/MCTI), Brazil. The study was approved by the Ethics Committee on the Use of Animals, Health Sciences Center, Federal University of Rio de Janeiro (CEUA no. 01200.001568/2013-87).

Particle Collection and Preparation

Particles from DB combustion were gently collected with a very soft toothbrush off exhaust pipes of busses belonging to the public transportation fleet of Rio de Janeiro City. They were equipped with Mercedes Benz Engines MB OM 924 LA, running with DB 500 type B containing 500 ppm sulfur. DB particle was weighed and suspended in a solution of saline (0.9% NaCl 98% *v*/*v*) and dimethyl sulfoxide (2% *v*/*v*), which yielded a suspension of 10 μg/μL.

Analysis of DB Particle Composition and Size

The size of DB particle was evaluated by dynamic light scattering (ZetaPals, Brookhaven Instruments Corporation, Holtsville, NY, USA), and the composition was analyzed by gas chromatography and mass spectrometry by Laboratório Oceanus-Hidroquímica (Rio de Janeiro, Brazil).

Experimental Design

Animals were randomly allocated to the following three groups (20 animals per group): control group treated with 25 μL of vehicle (98% saline and 2% dimethyl sulfoxide); 250-μg group treated with 25 μL of DB particle suspension; and 1.000-μg group treated with 100 μL of DB particle suspension. All animals were intranasally instilled daily for 5 consecutive days. For such purposes, they were anesthetized with sevoflurane (Laboratório Cristália, São Paulo, Brazil) *via* a nasal cone.

Pulmonary Mechanics

Twenty hours after the end of the instillations, ten animals per group were mechanically ventilated. We determined the lung resistive (ΔP_1), viscoelastic/inhomogeneous (ΔP_2) pressures, static elastance (E_{st}), and viscoelastic component of elastance (ΔE) [13]. Detailed procedures are explained in supplementary file.

Tissue Harvesting and Microscopic Analyses

On the sixth day, the remaining ten animals per group were anesthetized with sevoflurane and killed by cervical dislocation. The bronchoalveolar lavage fluid was collected and stored. The right lungs were collected for lysate and the left lungs were collected for the histology analysis. Detailed procedures are explained in supplementary file.

Immunofluorescence for Nuclear Factor Erythroid-Derived-Like 2 (Nrf2) or Phosphorylated Nuclear Factor Kappa B (p-NF-kB) p65

To determine the localization of Nrf2 or p-NF-kB on murine lungs, sections were immunolabeled with rat anti-Nrf2 or goat anti-p-NF-kB, both purchased from Santa Cruz Biotechnology. After antigen retrieval and washing, sections were treated with an anti-rabbit secondary antibody conjugated with Alexa Fluor 488 or an anti-goat secondary antibody conjugated with Alexa Fluor 647 (Invitrogen, Carlsbad, CA, USA). The slides were viewed on a fluorescence microscope (Microscope Axio Observer.A1, Zeiss-Vision, Oberkochen, Germany) using a × 40 objective lens.

Biochemical Analysis

The ROS were measured in bronchoalveolar lavage by the method adapted from Choi et al. [14]. Lipid peroxidation was measured as described by Draper and Hadley [15]. Detailed procedures are explained in supplementary file.

Enzyme-Linked Immunosorbent Assays (ELISA)

The TNF–α levels were measured in bronchoalveolar lavage using an ELISA assay (BD Biosciences Pharmingen, San Diego, CA, USA) according to the instructions of the manufacturers. Data are presented as pg/mL of TNF–α.

Immunoblotting

Lung lysate proteins were separated by SDS-PAGE, transferred to nitrocellulose membranes and probed with the following antibodies: rabbit polyclonal to matrix metalloproteinase-12 (MMP-12), goat polyclonal to catalase, goat polyclonal to superoxide dismutase 3 (SOD-3), rabbit polyclonal to glutathione peroxidase-1/2 (GPx-1/2), rabbit polyclonal to nitrotyrosine, rabbit polyclonal to Nrf2, rabbit polyclonal to kelch-like ECH-associated protein 1 (Keap-1), goat polyclonal to heme oxygenase-1 (HO-1), goat polyclonal to p-NF-κB (p65) purchased by Santa Cruz Biotechnology, and rabbit polyclonal to glutamate cysteine ligase catalytic subunit (GCLC), rabbit polyclonal to glutamate cysteine ligase modifier subunit (GCLM), or mouse monoclonal to β-actin purchased by Sigma-Aldrich, St. Louis, MO. Then, samples were incubated with the appropriate horseradish peroxidase-conjugated secondary antibodies. The antigen-antibody complexes were detected using enhanced chemiluminescence (Santa Cruz Biotechnology). β-actin was used as a loading control and data are expressed as arbitrary units.

Zymography

To determine the gelatinolytic activity of active MMP-2, lung lysate protein was separated by SDS-PAGE gel containing 1 mg/mL gelatin (Sigma-Aldrich) [16]. Data are expressed as arbitrary units. Detailed procedures are explained in supplementary file.

Statistical Analysis

All data are presented as the mean ± standard deviation (SD). The data were compared using one-way ANOVA followed by Bonferroni's post-hoc test or Kruskal-Wallis test with Dunn's posttest using Graph Pad Prism software (Graph Pad Software, San Diego, CA, USA). In all instances, $p < 0.05$ was considered statistically significant.

RESULTS

Composition and Distribution of DB Particulate Matter in the Mouse Lung

Macroscopically, the lungs of mice treated with DB particulate matter had black spots mainly in the apical region. However, the 1000 μg group had more black spots than the 250-μg group (Fig. 1a). Microscopically, the 250 and 1000-μg groups had a high number of black particles distributed in pulmonary septa and alveoli (Fig. 1b, c). To confirm these macroscopic and microscopic observations, the number of DB particles was counted. The number of DB particulates was 175-fold higher in the 250-μg group than in the control group ($p < 0.001$). Moreover, the 1000-μg group had 300-fold higher DB particulate matter than the control group ($p < 0.001$) and it was 1.71-fold greater than in the 250-μg group ($p < 0.001$) after 5 days (Fig. 1d). In addition, DB particulate matter had an average diameter of 0.39 μm, and it mainly consisted of fluoranthene, naphthalene, and pyrene (Table 1).

DB Particulate Matter Instillation Altered Pulmonary Mechanics

The lung resistive pressure (DP_1) was 2.28-fold higher in animals in the 250-μg group and 2.14-fold higher in the 1000-μg group compared to the control

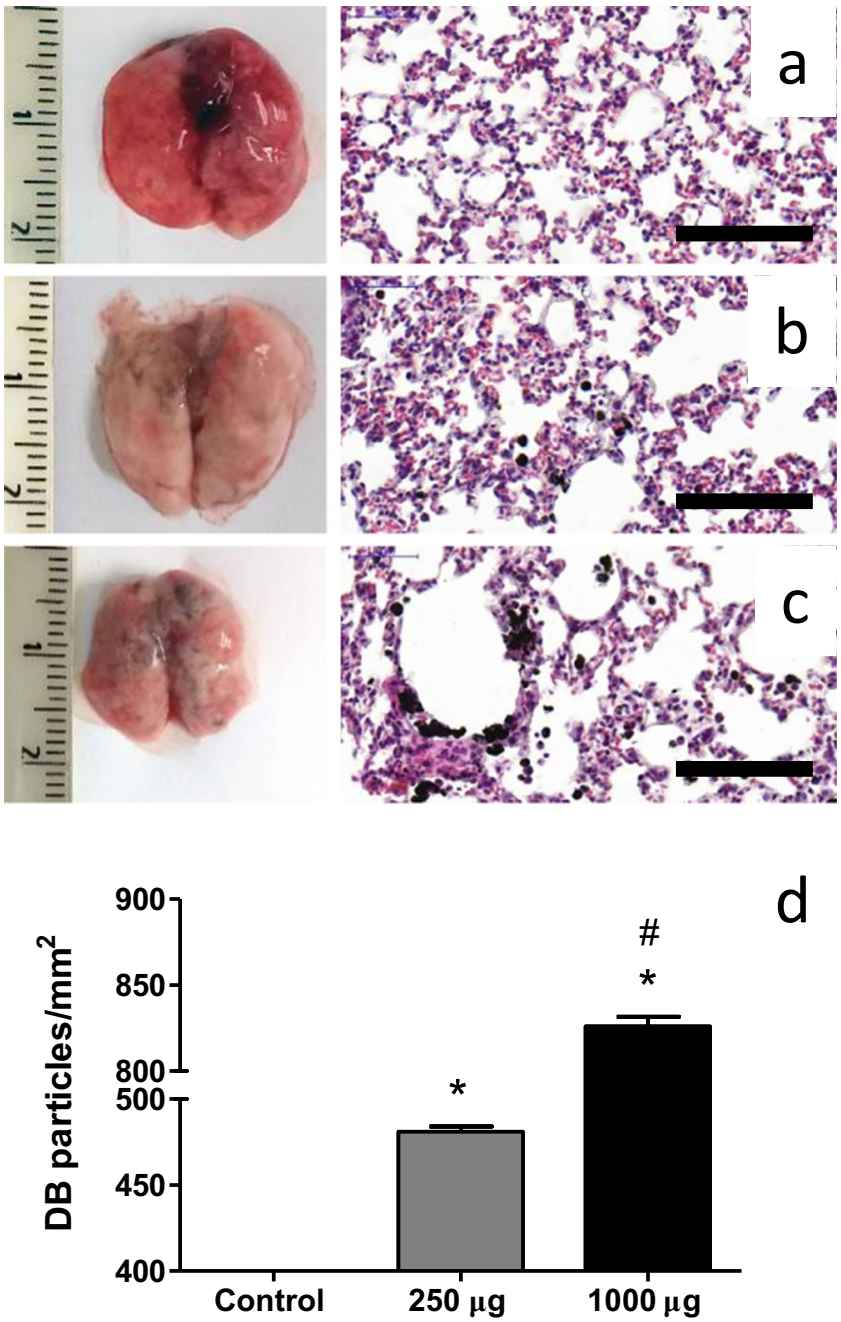

Fig. 1. Macroscopic and microscopic analyses of lungs in the control group and in those exposed for 5 days to diesel-biodiesel particulate matter produced by bus engines. **a** Representative macro and microscopic images of control murine lungs after 5 days of exposure to vehicle. **b** Representative macro and microscopic images of murine lungs after 5 days of exposure to 250-µg diesel-biodiesel particles. **c** Representative macro and microscopic images of mouse lung after 5 days of exposure to 1000-µg diesel-biodiesel particles. **d** The number of diesel-biodiesel (DB) particles per mm^2 in murine lungs after 5 days of exposure. Data ($n = 10$) are expressed as the mean ± SD. *$p < 0.05$ vs. the control group and # vs. the 250-µg group. Bar = 100 µm.

group after 5 days (Table 2). The static elastance (E_{st}) was 1.2-fold of increased in the animals in the 250-µg group and 1.19-fold higher in the 1000-µg group compared to the control group after 5 days (Table 2).

Table 1. Contents of Polycyclic Aromatic Hydrocarbons (PAHs) of Particles Biodiesel/Diesel Burning

Parameters	Values
Acenaphthene	< 0.002
Acenaphthylene	0.011
Anthracene	< 0.002
Benz[a]anthracene	< 0.002
Benzo[a]pyrene	< 0.002
Benzo[b]fluoranthene	< 0.002
Benzo[k]fluoranthene	< 0.002
Benzo [g, h, i] perylene	< 0.002
Chrysene	< 0.002
Dibenzo (a, h) anthracene	< 0.002
Phenanthrene	< 0.002
Fluoranthene	0.047
Fluorene	< 0.002
Indeno (1, 2, 3-cd) pyrene	< 0.002
Naphthalene	0.098
Pyrene	0.033

The values are expressed in mg/kg

DB Particulate Matter Instillation Effects on Extracellular Matrix Proteins and Inflammation

Microscopically, the 250 and 1000-μg groups had macrophages distributed in alveolar parenchyma (Fig. 2b, c). For quantification, we counted the number of macrophages in the alveolar parenchyma. The number of macrophages in alveolar parenchyma was 1.63-fold higher in the 250-μg group ($p < 0.001$) and 1.7-fold higher in the 1000-μg group ($p < 0.001$) compared to the control group after 5 days (Fig. 2d). The gelatinolytic activity of active MMP-2 was 38% lower in the 1000-μg group compared to the 250-μg group ($p < 0.05$) after 5 days (Fig. 3a), while the protein expression of MMP-12 was 4.13-fold greater in the 1000-μg group alone compared to the control group ($p < 0.01$) after 5 days (Fig. 3b). The protein levels of TNF-α in bronchoalveolar lavage fluid were 1.62-fold higher in the 250-μg group ($p < 0.05$) and 1.72-fold higher in the 1000-μg group compared to the control group ($p < 0.001$) after 5 days (Fig. 3c).

DB Particulate Matter Altered the Redox Profile in Murine Lungs

The lung levels of ROS were 2.45-fold higher in the 250-μg group ($p < 0.01$) and 2.18-fold higher in 1000-μg group ($p < 0.05$) than in the control group after 5 days (Fig. 4a). The protein SOD-3 expression was 72% reduced in the 1000 μg group compared to the control group and it was 69% lower compared to the 250-μg group ($p < 0.05$) after 5 days (Fig. 4b). The protein expression of catalase was 48% lower in the 1000-μg group compared to the control group ($p < 0.001$) and 54% reduced compared to the 250-μg group ($p < 0.01$) after 5 days (Fig. 4c). The protein levels of GPx 1/2 were 55% lower in the 1000-μg group compared to the control group ($p < 0.01$) and 50% compared to the 250-μg group ($p < 0.01$) after 5 days (Fig. 4d). The protein levels of GCLC were similar to the control, 250-μg and 1000-μg groups (Fig. 4e). On the other hand, the protein levels of GCLM were 72% reduced in the 250-μg group compared to the control group ($p < 0.001$) after 5 days (Fig. 4f). In addition, the protein levels of GCLM were 52% lower in the 1000-μg group compared to the control group ($p < 0.001$) and 72% lower compared to the 250-μg group ($p < 0.01$) after 5 days.

DB Particulate Matter Caused Oxidative Damage in Murine Lungs

The protein levels of nitrotyrosine were 1.6-fold higher in the 250-μg group ($p < 0.01$) (Fig. 5a) and 1.68-fold higher in the 1000-μg group ($p < 0.01$) (Fig. 5a) compared to the control group after 5 days. The intranasal instillation of DB particulate matter during 5 days increased the malondialdehyde levels 2.9-fold and 2.1-fold,

Table 2. Pulmonary Mechanics

	Groups		
Parameters	Control	250 μg	1000 μg
DP_1 (cmH_2O)	0.07 ± 0.02	0.16 ± 0.05*	0.15 ± 0.04*
DP_2 (cmH_2O)	0.91 ± 0.12	0.97 ± 0.13	0.85 ± 0.08
E_{st} (cmH_2O/mL)	20.45 ± 1.52	24.63 ± 2.97 *	24.49 ± 1.58*
DE (cmH_2O/mL)	4.56 ± 0.62	4.83 ± 0.67	4.16 ± 0.34

DP_1, lung resistive pressure; DP_2, lung viscoelastic/inhomogeneous pressure; E_{st}, lung static elastance; DE, lung viscoelastic component of elastance. Data ($n = 10$) are expressed as mean ± SD. *$p < 0.05$ vs. control group

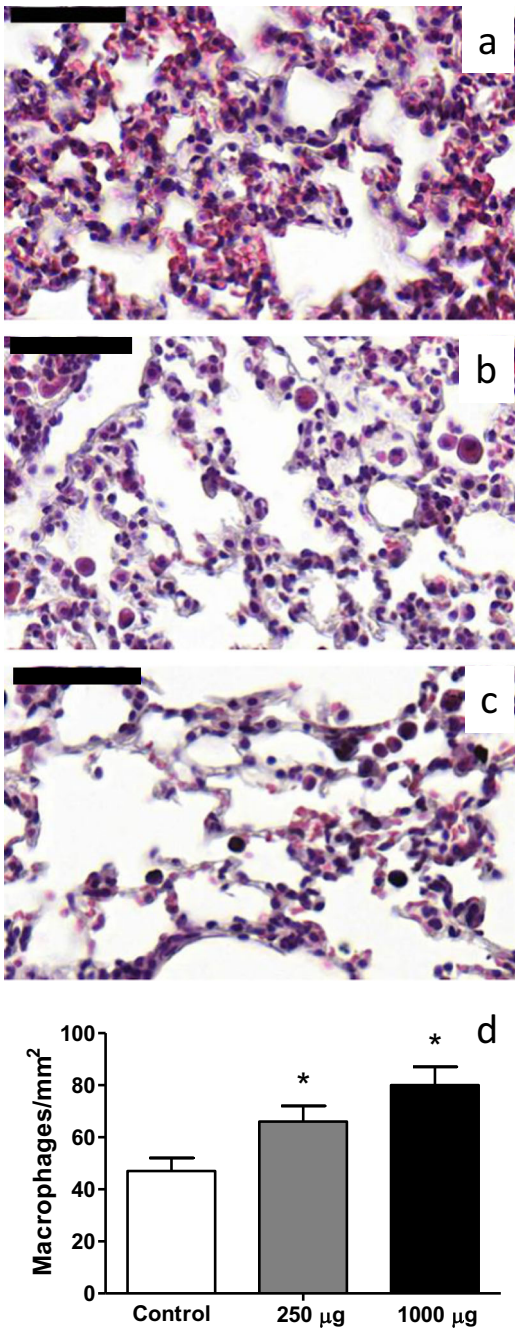

Fig. 2. Microscopic analyses of inflammatory responses of the control group and in those exposed for 5 days to diesel-biodiesel particulate matter produced by bus engines. **a** Representative images of macrophage distribution on murine lungs in the control group. **b** Representative images of the macrophage distribution in murine lungs in the 250-μg group. **c** Representative images of macrophage distribution in murine lungs in the 1000-μg group. Sections are stained with hematoxylin-eosin and the bar is equal to 50 μm. **d** The number of macrophages per mm^2 on murine lungs after 5 days of exposure to diesel-biodiesel particles. Data ($n = 10$) are expressed as the mean ± SD. *$p < 0.05$ vs. control group.

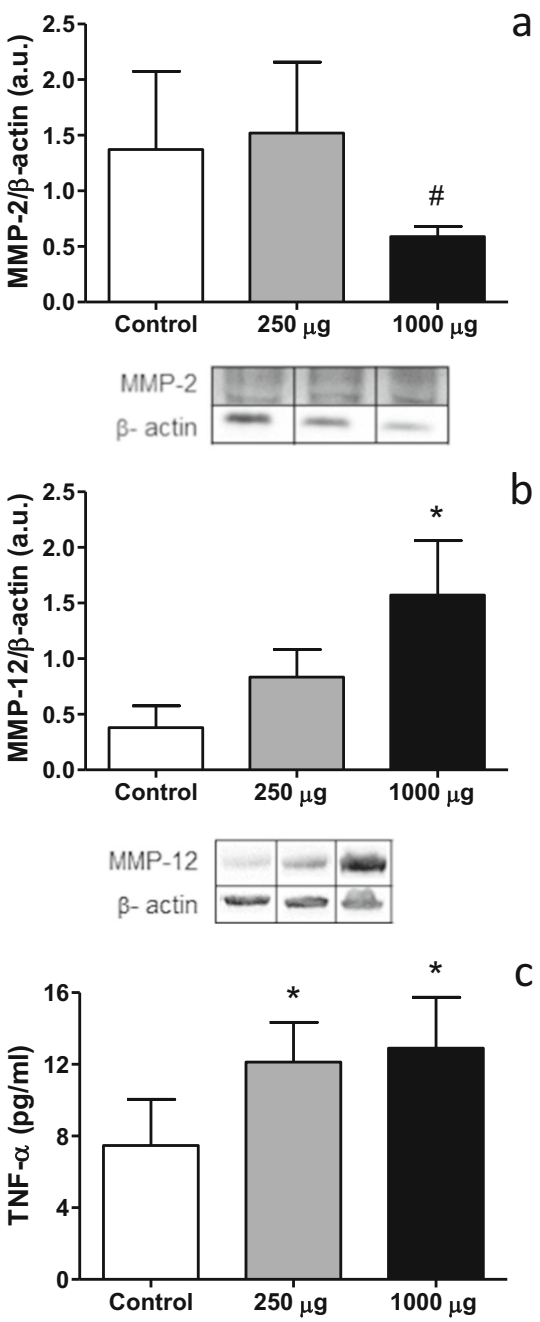

Fig. 3. Biochemical analyses of inflammatory responses of the control group and in those exposed for 5 days to diesel-biodiesel particulate matter produced by bus engines. **a** The gelatinolytic activity of MMP-2 through zymography in murine lungs. **b** Immunoblotting for MMP-12 in murine lungs. The β-actin was used as a loading control protein for immunoblotting. **c** TNF-α levels in the bronchoalveolar lavage fluid through ELISA. The densitometry is expressed as arbitrary units (a. u.) and β-actin was used as a loading control protein for all immunoblotting and zymography. Data ($n = 10$) are expressed as the mean ± SD. *$p < 0.05$ vs. the control group and # vs. the 250-μg group.

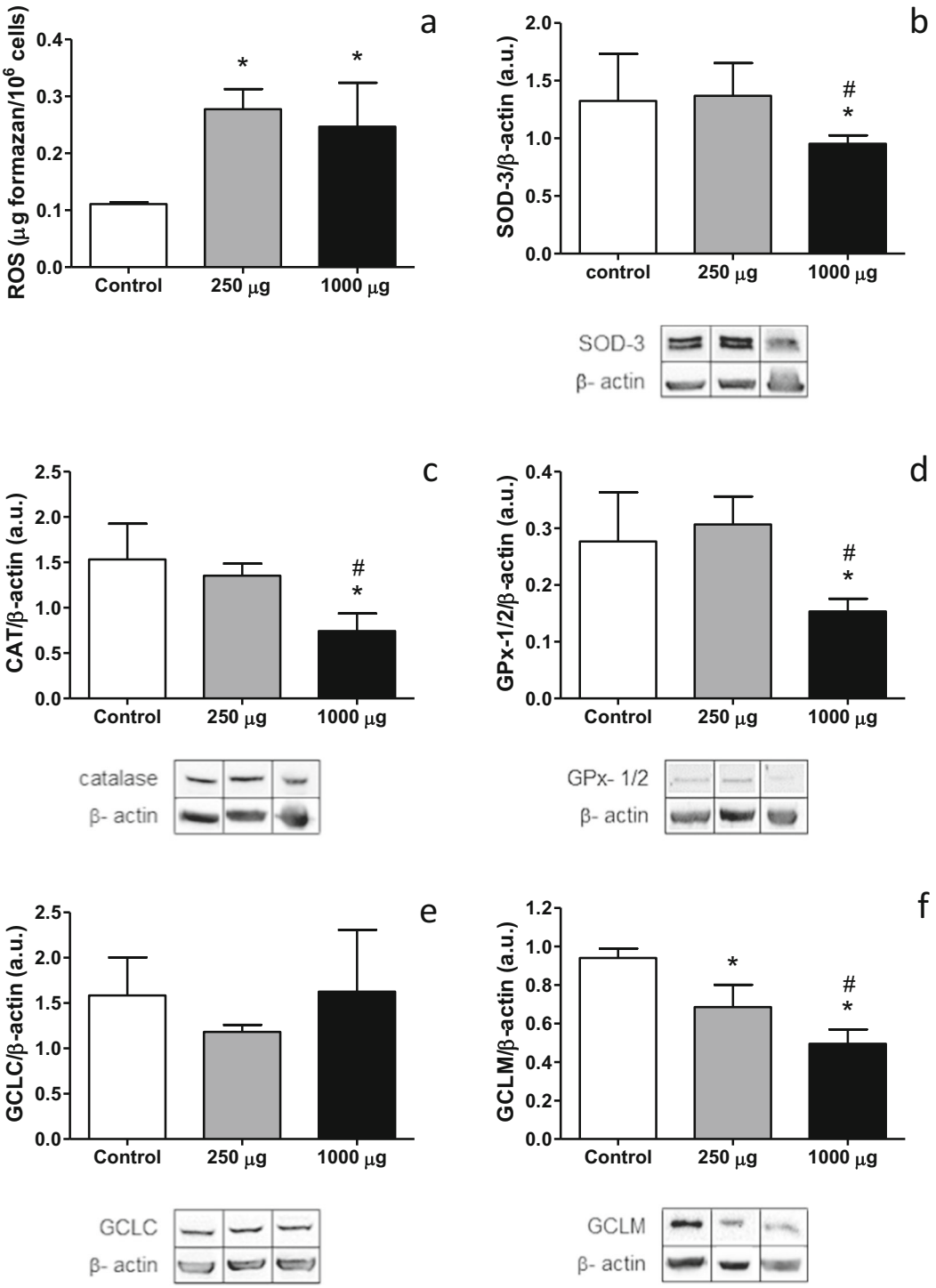

Fig. 4. Redox profile analyses in lungs from the control group and those exposed for 5 days to diesel-biodiesel particulate matter produced by bus engines. **a** The levels of ROS in bronchoalveolar lavage. **b** Immunoblotting for SOD-3 in murine lungs. **c** Immunoblotting for CAT in murine lungs. **d** Immunoblotting for GPx-1/2 in murine lungs. **e** Immunoblotting for GCLC in murine lungs. **f** Immunoblotting for GCLM in murine lungs. The densitometry is expressed as arbitrary units (a. u.) for all immunoblotting. β-actin was used as a loading control protein for all immunoblotting. Data ($n = 10$) are expressed as the mean ± SD. *$p < 0.05$ vs. the control group and # vs. the 250 group.

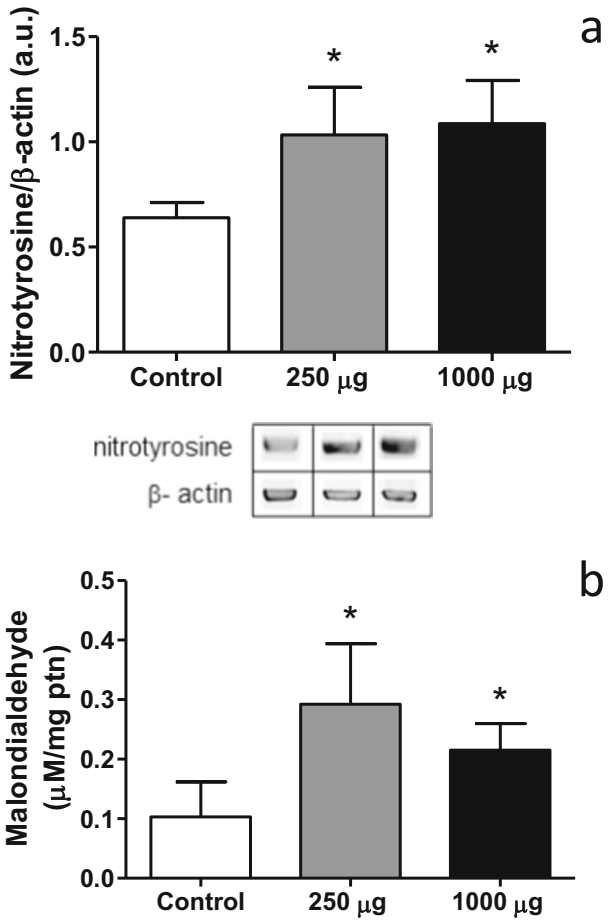

Fig. 5. Oxidative damage in the lungs of the control group and in those exposed for 5 days to diesel-biodiesel particulate matter produced by bus. **a** Immunoblotting for nitrotyrosine in murine lungs. **b** The levels of malondialdehyde in murine lungs. The densitometry is expressed as arbitrary units (a. u.) for all immunoblotting. β-actin was used as a loading control protein for all immunoblotting. Data ($n = 10$) are expressed as the mean ± SD. *$p < 0.05$ vs. control group and # vs. the 250-μg group.

respectively, in the lungs of the 250-μg and 1000-μg groups compared to the control group (Fig. 5b).

DB Particulate Matter Effects on Nrf2 and the p-NF-κB Pathway in Murine Lungs

All studied groups had Nrf2-positive bronchial epithelial cells after 5 days of intranasal instillation (Fig. 6a). Nonetheless, the immunostaining for Nrf2 was stronger in the 250-μg group compared to the control and 1000-μg groups after 5 days of intranasal instillation (Fig. 6b, c, respectively). To confirm this observation, the protein levels of Nrf2 were estimated in total lung lysate. The protein levels of Nrf2 were 2.34-fold greater in the 250-μg group than in the control group and 27% lower in the 1000-μg group than in the 250-μg group after 5 days (Fig. 6d). The protein levels of Keap-1 were 81% reduced in the 250-μg and 1000-μg groups compared to the control group after 5 days (Fig. 6e). The protein levels of HO-1 were 2-fold higher in the 250-μg group than in the control group; however, in the 1000-μg group, the protein levels of HO-1 were 81% lower compared to the control group and 40% compared to the 250-μg group (Fig. 6f). All studied groups also had bronchial epithelial cells positive for p-NF-kB 5 days after intranasal instillation (Fig. 7a–c). The protein levels of p-NF-kB were 1.29-fold higher in the 250-μg group than in the control group; however, in the 1000-μg group, the protein levels of p-NF-kB were 83% reduced compared to the control group and 64% reduced compared to the 250-μg group after 5 days of intranasal instillation (Fig. 7d).

DISCUSSION

The particulate matter derived from diesel combustion play an important role in air pollution, which increase the risk factors for pulmonary disease. In Brazil, one attempt to reduce the air pollution in large cities is the addition of 7% of biodiesel to diesel fuel. However, no study has assessed the pulmonary outcomes of DB combustion.

In this study, mice were treated with two doses (250 and 1000 μg) of DB particulate matter resulting from fuel burning by public busses in Rio de Janeiro City *via* intranasal instillation. The intranasal instillation method was chosen because it is a well-accepted model of exposure to particulate matter [17]. These doses were chosen to mimic the exposure of general population in Rio de Janeiro city. Since the traffic in Rio de Janeiro city is very busy with frequent congestion, the general population can be exposed to high levels of particulate matter daily.

Diesel combustion in vehicular engines may produce particulate matter with diameters of less than 2.5 μm, which deeply penetrate into the lung parenchyma [2, 3, 18]. In our study, DB particulate matter had an average diameter of 0.39 μm, suggesting that they were capable of penetrating murine lung parenchyma. We evaluated the DB particulate matter distribution in murine lungs and found both macro- and microscopically high numbers of black DB particulate matter. In addition, the elevated number of DB particulates in murine lungs may be related to the increase in the lung elastance and lung resistive pressure

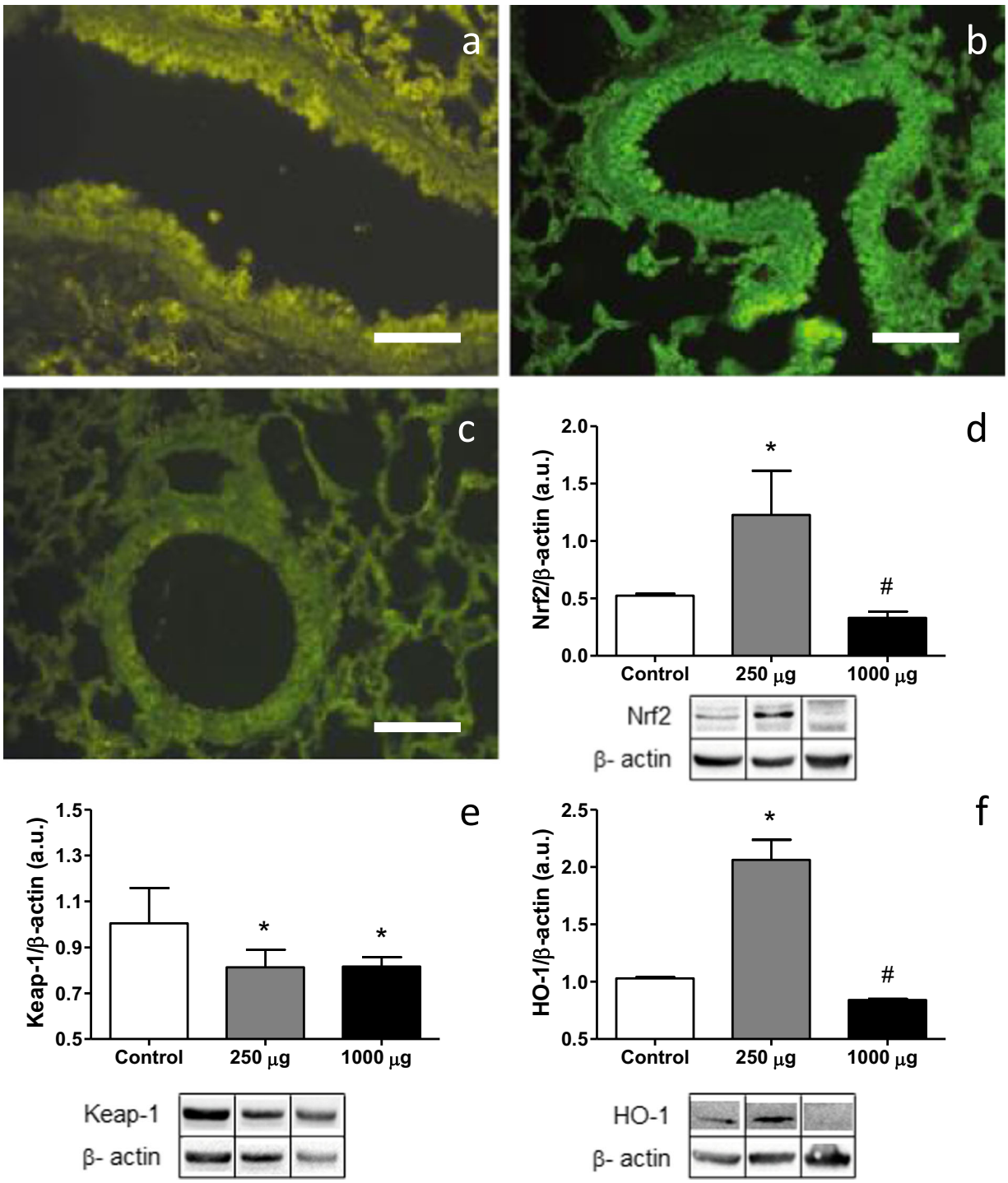

Fig. 6. Nrf2 pathway in the lungs of control group and in those exposed for 5 days to diesel-biodiesel particles produced by bus engines. **a** Immunofluorescence for Nrf2 in murine lungs from the control group. **b** Immunofluorescence for Nrf2 in murine lungs from the 250-μg group. **c** Immunofluorescence for Nrf2 in murine lungs from the 1000-μg group. **d** Immunoblotting against Nrf2 in murine lungs. **e** Immunoblotting against Keap-1 in murine lungs. **f** Immunoblotting for HO-1 in murine lungs. The densitometry is expressed as arbitrary units (a. u.) for all immunoblotting. β-actin was used as a loading control protein for all immunoblotting. Data ($n = 10$) are expressed as the mean ± SD. *$p < 0.05$ vs. the control group and # vs. the 250-μg group. Bar = 50 μm.

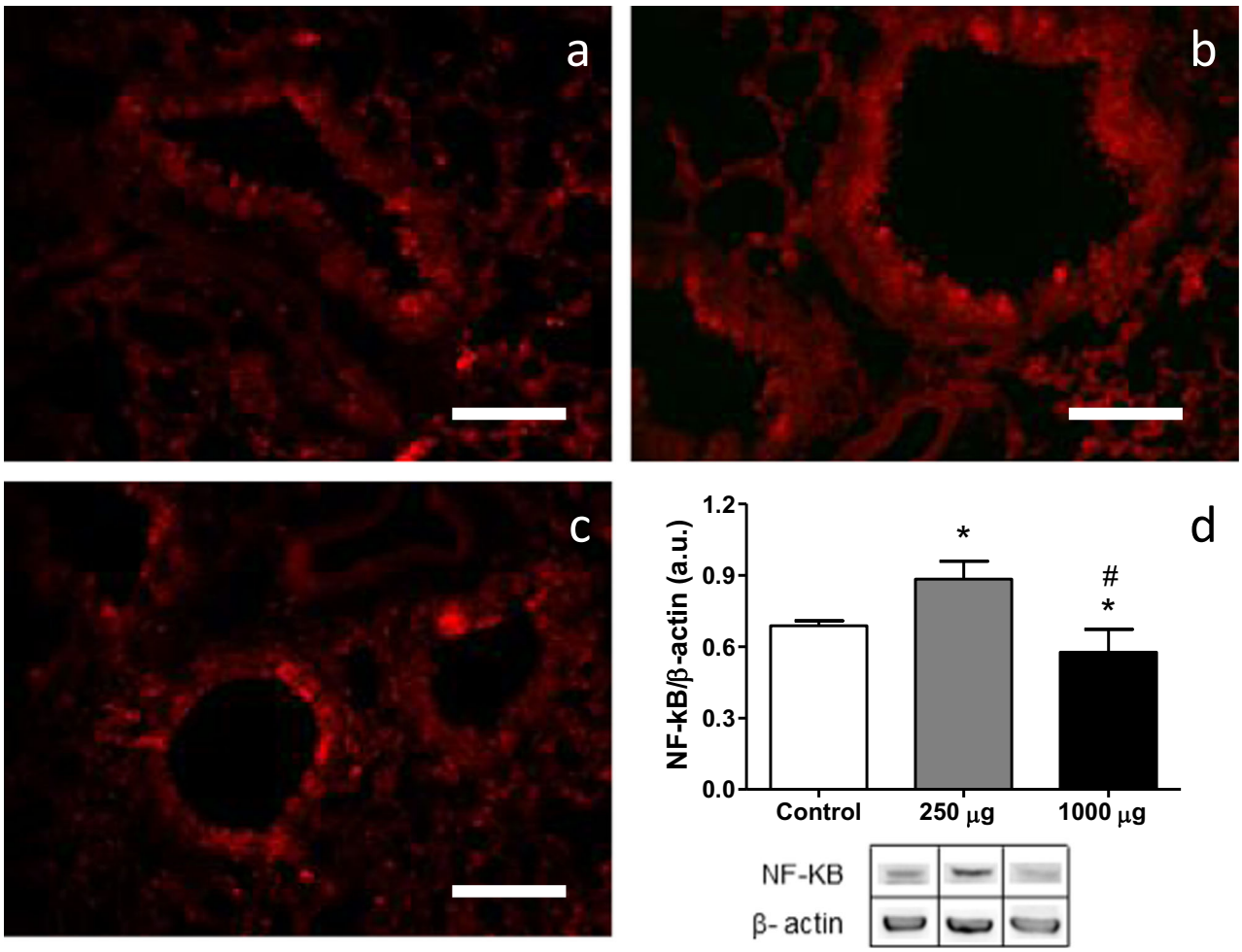

Fig. 7. p-NF-kB pathway in the lungs of control group and in those exposed for 5 days to diesel-biodiesel particulate matter produced by bus engines. **a** Immunofluorescence for p-NF-kB in murine lungs. **b** Immunofluorescence for p-NF-kB in murine lungs from the 250-μg group. **c** Immunofluorescence for p-NF-kB in murine lungs from the 1000-μg group. **d** Immunoblotting against p-NF-kB in murine lungs. The densitometry is expressed as arbitrary units (a. u.) for all immunoblotting. β-actin was used as a loading control protein for all immunoblotting. Data ($n = 10$) are expressed as the mean ± SD. *$p < 0.05$ vs. the control group and # vs. the 250-μg group. Bar = 50 μm.

observed in DB particulate matter-treated mice. In agreement, the intranasal instillation of traffic-derived and diesel particulate matter from São Paulo City increased the pulmonary static elastance and viscoelastic pressure in mice [17, 18]. Therefore, we suggest that the instillation of fine DB particulate matter might impair the alveolar integrity, contributing to lung mechanical instability.

Exposure to diesel particulate matter is associated with lung inflammation [19]. Mice exposed to diesel particulate matter for 7 days present an increased number of macrophages in their bronchoalveolar lavage fluid [7, 8]. TNF-α is a pro-inflammatory cytokine that is mainly derived from macrophages; the expression of TNF-α in young and aged mice lungs is increased 24 h after exposure to diesel particles [6, 20]. In the present study, the instillation of DB particulate matter increased the TNF-α levels in murine bronchoalveolar lavage fluid. Therefore, instillation of DB particulate matter may have triggered an acute inflammatory response in murine lungs due to the increased macrophage influx and TNF-α synthesis. MMPs constitute another important inflammatory factor. Surprisingly, we only observed an alteration in the levels of MMP-2 and MMP-12 in the 1000 group. p-NF-kB plays an important role in the inflammatory process by stimulating the gene expression of several pro-inflammatory cytokines, such as TNF-α [21]. The exposure of human bronchial epithelial cell culture to diesel exhaust particulate matter stimulates the nuclear translocation of p-NF-kB at 6 h after

treatment [22]. In our study, DB particulate matter increased the p-NF-kB expression. However, we probably did not observe p-NF-kB nuclear translocation due to the long period of DB particulate matter instillation. Therefore, these results suggest that p-NF-kB activation may be involved in the acute inflammatory response induced by DB particulate matter instillation.

The exposure to ultrafine petrol exhaust particulate matter or diesel exhaust particles in different concentrations increases the ROS levels in A549 cell and murine alveolar macrophage cultures at 1 h after exposure [9, 23]. In our study, exposure to DB particulate matter increased the ROS levels in murine lungs, demonstrating the capacity of these particles to promote ROS production. Moreover, we also observed lipid peroxidation in DB particulate matter-treated mice. In this context, exposure to ultrafine petrol exhaust particulate matter increases lipid peroxidation in A549 and RAW 264.7 cell cultures [23]. Our results demonstrate that instillation of DB particulate matter may increase ROS, leading to lipid peroxidation in murine lungs.

Endogenous antioxidants are essential to controlling ROS-induced oxidative damage. Surprisingly, DB particulate matter instillation in this study was not able to alter most of the antioxidant system (except in 1000 group). Nonetheless, exposure to diesel exhaust particulate matter increases SOD activity in rat lungs after 18 h of treatment [24]. On the other hand, rats exposed for 6 h to ultrafine particle, resulting from ethylene, oxygen, and argon combustion, do not have alterations in the lung catalase expression at 2, 24, and 48 h after aggression [25]. These observations demonstrate that the lung antioxidant system response to exposure to diesel particulate matter is still controversial and deserves further investigation.

The damage caused by ROS may be reversed by activation of the Nrf2 pathway. Keap-1 is a repressor protein that retains Nrf2 in the cell cytoplasm. In response to an oxidative or xenobiotic stress, the Keap-1 releases Nrf2, which is translocated to the nucleus [25]. In the nucleus, Nrf2 binds to antioxidant response elements, stimulating antioxidant expression, such as HO-1, SOD-1, GPx, and genes associated with glutathione pathway (GCLC and GCLM) [26]. Exposure to particulate matter (2.5 μm) induces Nrf2 nuclear translocation from 30 min to 1 h after treatment in A549 cell culture [27]. In the present study, the 250-μg group had increased Nfr2 and HO-1 expression with reduced Keap-1 expression. However, we did not observe Nrf2 nuclear translocation, which was probably due to the long duration of DB particulate matter instillation. Therefore, our data suggest that DB particulate matter-induced oxidative damage may have stimulated Nrf2 and HO-1 activation. In the 1000-μg, Nrf2 expression did not differ from control mice. In addition, the former group had elevated levels of ROS, MMP-12, and nitric oxide with reduced levels of MMP-2, catalase, GPx-1/2, and GCLM compared to the control and 250-μg group. These results suggest that the 1000-μg dose may have caused a more intense lung response, leading to the activation of other antioxidant pathways.

CONCLUSIONS

In conclusion, acute exposure to DB particulate matter was capable of penetrating pulmonary parenchyma, stimulating inflammatory response, and increasing ROS synthesis, which leads to oxidative damage. In addition, DB particulate matter-stimulated oxidative damage activated the Nrf2/HO-1 and p-NF-kB/TNF-α pathways.

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