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Research Article

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Nandina domestica Leaf Extracts Ameliorate Lung Injury by Decreasing Inflammatory Cytokine Levels and Neutrophil Accumulation in Mice Injected with Fine Dust

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Abstract

Exposure to dust particles can lead to respiratory problems, diseases, and even death. In this study, we examined the effects of Nandina domestica (ND) on bronchoalveolar lavage fluid (BALF) and lung of intra-nazal-trachea (INT) injected mice with fine dust mixture of coal, flay ash and diesel exhausted particle (CFD). ND significantly reduced the counts of neutrophils in BALF and histological changes in lung tissues including infiltration of inflammatory cells and goblet cell hyperplasia with lowering lung injury score and periodic acid Schiff (PAS) score. In addition, the elevated inflammatory cytokines such as interleukin (IL)-17 and tumor necrosis factor-α (TNF-α) in BALF, macrophage inflammatory protein-2 (MIP-2) and C-X-C motif chemokine 1 (CXCL-1) significantly decreased to a greater extent by ND treatment. Furthermore, ND significantly decreased levels of asymmetric dimethyl-arginine (ADMA) and symmetric dimethyl-arginine (SDMA) elevated by CFD in BALF. Therefore, these data suggest that ND ameliorated lung injury by suppressing inflammatory cytokines as well as by reducing the accumulation of neutrophils.

Keywords: *Nandina Domestica* (Nd), Fine Dust, Neutrophils, Inflammatory Cytokines, Lung Injury

Introduction

In China and neighboring regions, there is focusing health concern over the dust storm fine particulate matter (PM) generating in the Mongolian regions of China [1]. PM is a complex of various particles containing crustal material and bioaerosols. High levels of surrounding PM were also known to be related with elevated cardiopulmonary morbidity and mortality [2]. World Health Organization (WHO) report showed that exposure to dust fine PM caused unlucky deaths in 2012 worldwide, while the Global Burden of Disease Study (GBDS) indicated that exposure of dust fine PM was responsible for approximately 2.9 million deaths in 2013 [2].

Fine dust particles that penetrate the lungs can generate local damage as well as continuous systemic damage elsewhere in the person, including the kidney, lung and the brain [3, 4]. Fine dust particles that stay for a short time to days not only rise the incidence of respiratory diseases, but also generate other diseases, such as circulatory illness of the blood and skin respiration and in severe cases can result in death [3, 5]. The mechanisms leading to health problems induced by the permeation of fine dust particles is still not completely understood [6, 7].

Bronchoalveolar lavage (BAL) provides a significant diagnostic instrument that can promote the diagnosis of various extend lung diseases [8]. BAL can be used to examine inflammatory cell profiles and discovery pathogens [9]. BAL cytology and differential cell counts can even alternate with histology from the lung biopsy in rare lung diseases [10].

Nandina domestica is a plant possessing flower that come under the family Berberidaceae [11]. N. domestica has been known to be effective in the care of dermatophytic infections and solitary mastocytoma [12, 13]. In addition, the frits of N. domestica are reported to have anti-oxidant and anti-inflammatory efficacy [14]. N. domestica extracts possessing anti-oxidant property showed anti-inflammatory effect by regulating mitogen activated protein

kinases (MAPKs) signaling in lipopolysaccharide (LPS)-induced RAW264.7 cells [15]. However, effect of the leaf extracts of N. domestica in mouse model by fine dust particles has not been adequately reported. Therefore, we explored the effects of N. domestica leaf extracts on fine dust CFD (mixture of coal, flay ash and diesel exhausted particle)-induced inflammatory cytokine and neutrophil accumulation in INT(intra-nazal-trachea) injected mice.

Materials and Methods

Preparation of N Domestica

N. domestica leaves was obtained from surrounding garden at Kwangju Women's University (Gwangju, South Korea). N. domestica leaves were dried under shade and pulverized to obtain power. The 300 g powders were mixed with 70% ethanol 3.0 L and set at room temperature. After 3 days, filtration was conducted with 400 mesh filter paper. After centrifuging at 1,000 rpm for 15 min, a filtration was conducted with a whatman filter paper of No. 2.

Preparation of Fine Dust CFD

The fine dust CFD is mixture of coal, flay ash and diesel exhausted particle (DEP). The coal, fly ash and DEP solution made with DMSO at 5 mg/ml, 10 mg/ml and 5 mg/ml, respectively. The three mixture solution diluted with DMSO at 0.25 mg/ml, 0.5 mg/ml and 0.75 mg/ml. The fine dust CFD made with Alum (Aluminium Hydrocide Gel Adjuvant) at final concentration of 8%.

Animal Experiment

Seven-week old male balb/c mice were obtained from Central Lab Animal Inc (Seoul, South Korea). The mice were maintained in the animal care institution at Daejeon University. The animal experiments were conducted according to protocols approved by the Animal Care Committee of the Institute of Daejeon University, South Korea (No. DJUARB2019-021).

The male mice were divided into 4 groups, including a normal group (n = 5), dust CFD possessing the particles with a diameter of 10 micrometers (PM10 + D) vehicle group (n = 5), PM10 + D-dexamethasone (Dexa, positive control) group (n = 5), and PM10 + D-ND group (n = 5). To injection the fine dust CFD, the mice anesthetized with ketamine. After open the respiratory tract of mice by fixing with rubber band, the fine dust CFD was instilled two times by intra-nazal-trachea (INT) injection for 3 and 6 days to Balb/c mice. At one times, the fine dust CFD of 50 µl was instilled by intra-nazal injection and the another 50 µl was instilled by intra-trachea injection. ND suspended in 0.5% carboxymethyl cellulose (CMC) was treated by oral administration to the mice daily for 10 days (100 mg/kg). Dexa used as a positive control.

Preparation of Bronchoalveolar Lavage Fluid (BALF)

BALF was performed by cannulation of the trachea. After incision in the neck skin near the trachea using a scalpel, the catheter about 0.5 cm inserted into the trachea. The 1 ml syringe connected to the catheter and gently injected the 1 mL phosphate buffered saline (PBS) into the trachea. For neutrophils counts, in the collected BALF, BALF cells smears were prepared using Cytospin (Thermo Fisher Scientific) and were stained with Diff-Quik solution (Dade Diagnostics, Aguada, Puerto Rico) for differential counting on 400 cells to assess neutrophils. The neutrophils were calculated on cytospins of BALF using a Leica microscope under 200 X magnification. BALF was quickly centrifuged at 2000 rpm for 5 min. The gathered supernatant was keep at −70 °C until determination of cytokine levels by enzyme-linked immunosorbent assay (ELISA).

Measurement of Cytokine Levels in the BALF

Interleukin (IL)-17, tumor necrosis factor-α (TNF-α), macrophage inflammatory protein-2 (MIP-2), C-X-C motif chemokine 1 (CXCL-1), asymmetric dimethyl-arginine (ADMA) and symmetric dimethyl-arginine (SDMA) levels in BALF were quantified by ELISA using commercial kits (Thermo Fisher Scientific) according to the manufacturer's protocol.

Histological Analysis

After 10 days, mice were died for histological analysis. Lung tissue from mice was removed. After fixing with 10% neutral-buffered formalin, the lung tissue was dehydrated and embedded in paraffin. After cutting into 3-µm sections, the lung tissue was stained with hematoxylin and eosin (H&E) and periodic acid Schiff (PAS). Stained lung tissue sections were analyzed under a light microscope (Axio Imager M1; Carl Zeiss, Oberkochen, Germany). The degree of lung inflammation and goblet cell hyperplasia was scored on a subjective grade of 0 to 4 as previously reported [16]. The following features and the score showed in table 1. Five scores were analyzed for each slide and mean score was calculated.

Statistical Analysis

Results are recorded as means ± standard deviation. The data were analyzed by ANOVA and Duncan's multiple range tests. Significance was indicated at $p<0.05$, $p<0.01$ and $p<0.001$.

Table 1: Histological score scale on lung inflammation and goblet cell hyperplasia

Score scale	Lung inflammation	Goblet cell hyperplasia
0	Normal	<0.5% PAS positive cells
1	Few cells	<25% PAS positive cells
2	a ring of inflammatory cells 1 cell layer deep	25-50% PAS positive cells
3	a ring of inflammatory cells 2-4 cells deep	50-75% PAS positive cells
4	a ring of inflammatory cells of >4 cells deep	>75% PAS positive cells

Results

Effects of ND on Neutrophils in BALF

The total count of neutrophils in the BALF of mice with CFD (CTL group, 3.0 × 102) was significantly higher than normal group (Figure 1a and 1b). Administration with Dexa (5 mg/ml) was decreased count of neutrophils of fine dust CFD – induced mice (CTL group) showing count of neutrophils of 8.5 × 101. The count of neutrophils in the ND group were significantly lower than the CTL group with 4.5 × 101 (Figure 1a and 1b).

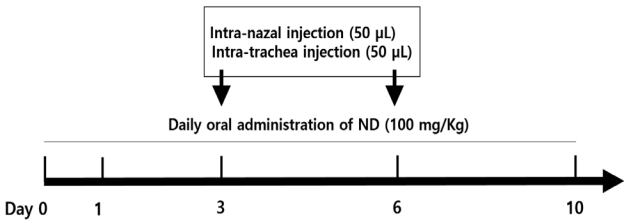

Figure 1: Experimental scheme of fine dust CFD-induced mice model. The fine dust CFD was instilled two times by intra-nazal-trachea (INT) injection for 3 and 6 days to Balb/c mice. At one times, the fine dust CFD of 50 µl was instilled by intra-nazal injection and the another 50 µl was instilled by intra-trachea injection. Nandina domestica Thunb extracts (ND) suspended in 0.5% carboxymethyl cellulose (CMC) was treated by oral administration to the mice daily for 10 days (100 mg/kg).

Effects of ND on Lung Inflammation and Goblet Cell Hyperplasia

To observe pathological changes in the lung tissues, H&E and PAS staining were conducted. The typical pathological features of lung injury were observed in CTL groups as compared to control groups by H&E and PAS staining. The CLT group showed eosinophilic infiltration in pulmonary vessels, alveolar ducts, and whole lung alveoli (Figure 2a). In contrast, the ND group mainly showed recovery in these characteristics (Figure 2a). PAS staining was markedly increased goblet cell hyperplasia in CTL group as compared to control group (Figure 2a). To evaluate the rigor of lung injury, the lung injury score and PAS score were calculated. As shown in Figure 2b and 2c, both the lung injury scores and PAS score were markedly attenuated by administration of ND. These results indicate that histopathological change in the lungs is induced depending on the mode of fine dust CFD challenge.

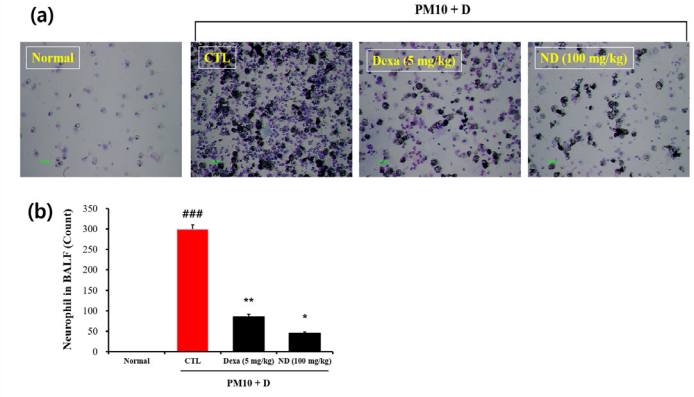

Figure 2: The effects of Nandina domestica Thunb extracts (ND) on neutrophils in bronchoalveolar lavage fluid (BALF) of fine dust CFD-induced mice. Diff-Quik™ (International Reagents, Kobe, Japan)-stained smears were used to identify the neutrophils profiles after cytospin preparation. The neutrophils counts were performed by examining 400 cells using a standard light microscope. Normal is normal control. CTL is fine dust CFD-treated control. Dexa is 5 mg/kg dexamethasone. ND is 100 mg/kg ND. Values are present as the means ± SEMs (n = 5). ### $p < 0.001$ compared with normal; * $p < 0.05$ and ** $p < 0.01$ compared with CTL. CFD is mixture of coal, flay ash and diesel exhausted particle with a diameter of 10 micrometers (PM10 + D).

Effects of ND on Inflammatory Cytokines Expression in BALF

Because cytokines are secreted in response to inflammation, the levels of these inflammatory cytokines (IL-17, TNF-α, MIP-2 and CXCL-1) were investigated in the BALF. From Figure 3, fine dust CFD induced-mice were increased significantly IL-17, TNF-α MIP-2 and CXCL-1 levels in BALF. ND are shown to significantly suppress the levels of IL-17, TNF-α MIP-2 and CXCL-1 (Figure 3).

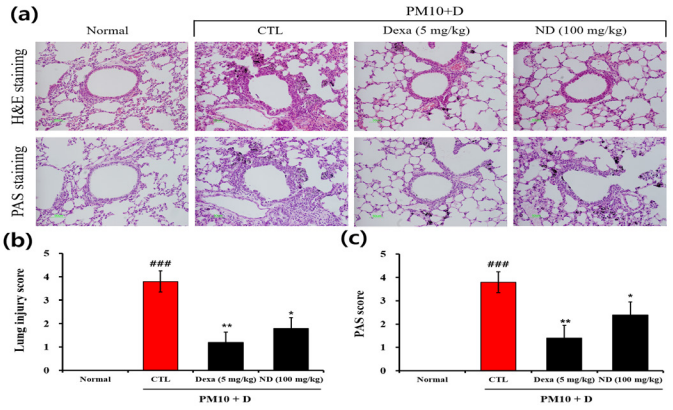

Figure 3: Infiltration of inflammatory and goblet cells in lung tissue from fine dust CFD challenged mice and ND treated mice. (a) Representative hematoxylin and eosin (H&E) and periodic acid Schiff (PAS) stained sections of lung; (b) Lung injury score by histological scoring of inflammatory cell infiltration; (c) PAS score by goblet cells. The lung tissue stained with H&E and PAS at 10 days after administration of ND. Normal is normal control. CTL is fine dust CFD-treated control. Dexa is 5 mg/kg dexamethasone. ND is 100 mg/kg ND. Values are present as the means ± SEMs (n = 5). ### $p < 0.001$ compared with normal; * $p < 0.05$ and ** $p < 0.01$ compared with CTL. CFD is mixture of coal, flay ash and diesel exhausted particle with a diameter of 10 micrometers (PM10 + D)

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Inhibitory Effects of ND on ADMA and SDMA Levels in BALF

To assess the effects of ND on ADMA and SDMA involved in the inflammation and endothelial dysfunction, ADMA and SDMA levels checked in BALF. As shown in Figure 4, both ADMA and SDMA levels were increased by the fine dust CFD in CTL group. Dexa treatment was decreased ADMA and SDMA levels. As observed in Figure 4, ND restored the increased ADMA and SDMA levels toward normal.

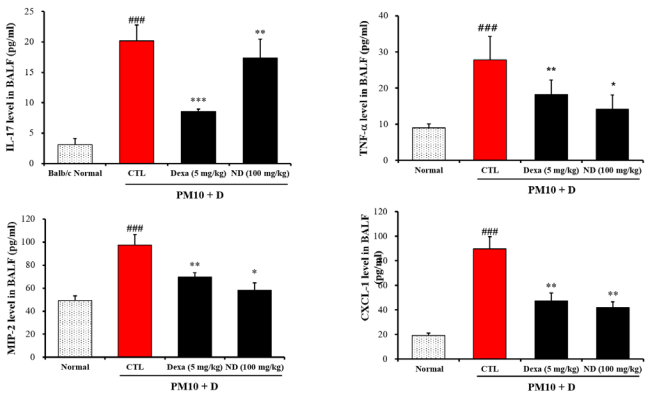

Figure 4: The effects of Nandina domestica Thunb extracts (ND) on levels of inflammatory cytokines (IL-17, TNF-α, MIP-2 and CXCL-1) in bronchoalveolar lavage fluid (BALF) of fine dust CFD-induced mice. The inflammatory cytokines levels in the BALF were evaluated by ELISA. Normal is normal control. CTL is fine dust CFD-treated control. Dexa is 5 mg/kg dexamethasone. ND is 100 mg/kg ND. IL-17 is interleukin 17. TNF-α is tumor necrosis factor-α. MIP-2 is macrophage inflammatory protein-2. CXCL-1 is C-X-C motif chemokine 1. Values are present as the means ± SEMs (n = 5). ### $p < 0.001$ compared with normal; * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ compared with CTL. CFD is mixture of coal, flay ash and diesel exhausted particle with a diameter of 10 micrometers (PM10 + D).

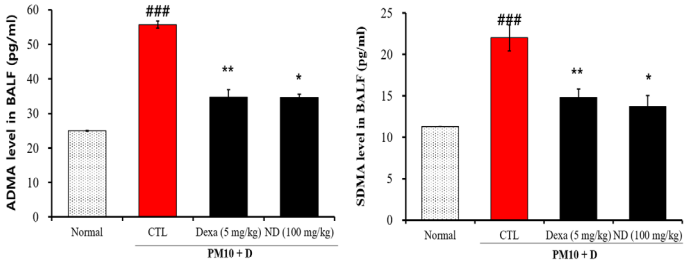

Figure 5: The effects of Nandina domestica Thunb extracts (ND) on levels of asymmetric dimethyl-arginine (ADMA) and symmetric dimethly-arginine (SDMA) in bronchoalveolar lavage fluid (BALF) of fine dust CFD-induced mice. Mice were exposed to CFD by intra-nasal trachea injection two times in 3 day intervals for 12 days. One time is 50 μl intra-nazal injection and the another 50 μl is intra-trachea injection. Nandina domestica Thunb extracts (ND) was treated by oral administration to the mice daily for 10 days (100 mg/kg). The ADMA and SDMA levels in BALF obtained from CFD mice by ELISA kit. Normal is normal control. CTL is fine dust CFD-treated control. Dexa is 5 mg/kg dexamethasone. ND is 100 mg/kg ND. Values are present as the means ± SEMs (n = 5). ### $p < 0.001$ compared with normal; * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ compared with CTL. CFD is mixture of coal, flay ash and diesel exhausted particle with a diameter of 10 micrometers (PM10 + D).

Discussion

Various ingredients of fine dust can penetrate the trachea and pulmonary system by inhalation and immediately affect the epithelium of the human respiratory systems [17]. With recurrent inhalation of these potentially hazardous materials, peoples are at risk for respiratory diseases such as chronic obstructive pulmonary disease (COPD), and hypersensitivity pneumonitis (HP) [18]. In this study, we examined whether ND could improve lung injury such as neutrophils accumulation in BALF, lung inflammation and goblet cell hyperplasia. ND decreased effectively neutrophils counts, lung inflammation and goblet cell hyperplasia. Furthermore, the lung injury scores and PAS score were markedly attenuated by administration of ND.

Airway epithelial cells work as physical barrier in the pulmonary systems, and also play important roles in the immune response against fine dust and produce pro-inflammatory cytokines such as IL-6 and IL-8 in response to fine dust particles [19]. IL-17 is naturally elevated in reparatory disease and TNF-α is found to be potently involved in many aspects related to airway pathology in reparatory disease [20, 21]. MIP2 which is activated by IL-17 [22]. In addition to these, CXCL-1 which attracts neutrophils to the sites of airway inflammation [23]. We observed levels of these inflammatory cytokines in BALF, primarily the IL-17, TNF-α, MIP-2 and CXCL-1 in BALF. ND decreased levels of these inflammatory cytokines (IL-17, TNF-α, MIP-2 and CXCL-1) in BALF.

Many studies have reported the effects of either ultrafine particulate matters, coal fly ash, or coal fly dust in various murine and rodent models of pulmonary ailments such as asthma and airway inflammation [24]. Walters et al. reported that PMs induced an increase in the amount of immune cells in BALF and lung cells and increased the levels of pro-inflammatory cytokines in lung [25]. Takano et al. reported that the inhalation of diesel exhaust particles generated allergen-related eosinophil recruitment and airway hyper-responsiveness in mice [26]. Similar to these studies, we found that exposure of mice to CFD resulted in elevated levels of ADMA and SDMA in BALF, they were positively inhibited by ND.

Conclusions

ND was decreased the counts of neutrophils in BALF and histological changes in lung tissues including infiltration of inflammatory cells and goblet cell hyperplasia due to downstream secretion of inflammatory cytokines such as IL-17, TNF-α, MIP-2 and CXCL-1. Furthermore, ND was decreased levels of ADMA and SDMA elevated by CFD. Therefore, we demonstrated the anti-inflammatory/anti-lung injury effects of ND

Conflict of interest

The authors have declared no conflict of interest.

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