N-acetyl cysteine prevents ambient fine particulate matter-potentiated atherosclerosis via inhibition of reactive oxygen species-induced oxidized low density lipoprotein elevation and decreased circulating endothelial progenitor cell

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Abstract. Ambient fine particulate matter (PM) serves an important role in the development of cardiovascular disease, including atherosclerosis. Antioxidant N-acetyl cysteine (NAC) has protective effects in the cardiovascular system. However, it is unknown if NAC prevents PM-potentiated atherosclerosis in hyperlipidemia. Low-density lipoprotein (LDL) receptor knockout mice were pretreated with 1 mg/ml NAC in drinking water for 1 week and continued to receive NAC, high-fat diet and intranasal instillation of PM for 1 week or 6 months. Blood plasma was collected for lipid profile, oxidized (ox-)LDL, blood reactive oxygen species (ROS) and inflammatory cytokine (TNF-α, IL-1β and IL-6) measurement. Blood cells were harvested for endothelial progenitor cell (EPC) population and intracellular ROS analysis. Murine aorta was isolated for atherosclerotic plaque ratio calculation. NAC treatment maintained circulating EPC level and significantly decreased blood ox-LDL and ROS, inflammatory cytokines, mononuclear and EPC intracellular ROS levels as well as aortic plaque ratio. NAC prevented PM-potentiated atherosclerosis by inhibiting plasma ROS-induced ox-LDL elevation, mononuclear cell and EPC intracellular ROS-induced circulating EPC reduction and inflammatory cytokine production.

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Introduction

Ambient fine particulate matter (PM) is associated with cardiovascular disease (CVD), including coronary artery disease (CAD), heart failure and hypertension (1). However, the underlying mechanism is not well studied. Different types of CVD may be caused by duration of PM exposure; acute PM exposure increases myocardial infarction (2), stroke (3) and other acute cardiovascular events (4), while chronic exposure contributes to development of hypertension, diabetes and other cardiometabolic conditions (4). Following six months exposure of PM with diameter ≤2.5 μm, atherosclerosis development in apolipoprotein E knockout mice significantly increases and is accompanied by vasomotor tone alteration and vascular inflammation (5). However, the detailed mechanisms

underlying PM-potentiated atherosclerosis in hyperlipidemia is not well studied.

Endothelial dysfunction or injury is a key factor that contributes to the development of atherosclerosis and CAD (6,7). Bone marrow (BM)-derived endothelial progenitor cells (EPCs) serve a key role in vascular reendothelialization, angiogenesis and promotion of neointima formation following vascular injury (8-11). In addition, EPC number and function are significantly decreased in patients with CAD and hyperlipidemia (12,13). However, the exact mechanism underlying decreased EPC levels in patients with hyperlipidemia remains unknown. Oxidized low-density lipoprotein (ox-LDL) is a key component in hyperlipidemia and serves an important role in development of atherosclerosis, primarily via oxidative stress (14). Our previous study indicated that ox-LDL has a similar effect to chronic hyperlipidemia on BM and blood EPC populations (15). However, the effect of PM exposure on ox-LDL and EPC population in hyperlipidemia is still unknown.

Reactive oxygen species (ROS) are small molecules, such as hydrogen peroxide (H_2O_2), that regulate tissue oxidative stress (16). ROS-induced oxidative stress also regulates BM stem cell (BMSC) and BMSC-derived progenitor cell self-renewal, proliferation, mobilization, homing, migration, differentiation, apoptosis and senescence (17,18). Rate of ROS generation in peripheral blood monocytes is increased in hyperlipidemic patients, along with elevated plasma ox-LDL levels, which increases intracellular ROS formation in cultured endothelial cells (19,20). Our previous data showed that PM exposure also increases EPC intracellular ROS production in wild-type (WT) mice (21). To the best of our knowledge, however, ROS production following short- and long-term PM exposure in mice with hyperlipidemia has not been evaluated.

N-acetyl cysteine (NAC) is an antioxidant widely used to investigate CVD (22). Our previous data indicated that NAC prevents atherosclerosis by maintaining EPC population, decreasing both intracellular and extracellular ROS production in hyperlipidemic mice and preventing LDL oxidation in WT mice (15,23). NAC also protects EPCs from apoptosis and decreases levels of inflammatory cytokines following PM exposure in WT mice (21). To the best of our knowledge, however, the effects of NAC on development of atherosclerosis in hyperlipidemic mice with acute and chronic PM exposure has not been investigated. The present study amid to determine the protective effect and underlying mechanism of NAC on atherosclerosis in mice with hyperlipidemia following acute and chronic PM exposure.

Materials and methods

Animal model. All animal experiments were performed in accordance with the Guidelines of the Animal Care Committee of Shandong Provincial Hospital affiliated to Shandong First Medical University, Jinan, China. The experimental protocols for the present study were reviewed and approved by the Animal Care Committee of Shandong First Medical University (approval no. 2021-228). A total of 48 4-6-week-old male homozygous C57BL/6J LDL receptor knockout (LDLR KO) mice were obtained from Shanghai Model Organisms Center, Inc. and randomized into 6 groups (n=8/group) as follows: Normal diet

(ND); high-fat diet (HFD); HFD + NAC; PM + ND; PM + HFD and PM + HFD + NAC. In addition to *ad libitum* access to food and water, all mice were kept at room temperature with 40-60% humidity and a 12/12-h light/dark cycle. Following 1 week acclimation, mice were challenged with HFD (17% anhydrous milk fat; 0.2% cholesterol) to induce hyperlipidemia for 1 week or 6 months, as previously described (23). PM (cat. no. NIST2786; mean particle diameter, <4 μm) was purchased from MilliporeSigma. PM was dispersed in solution by ultrasonication in endotoxin-free water for 30 min at a concentration of 0.5 μg/μl as previously described (24,25). Hyperlipidemic mice were treated with 10 μg PM three times/week for 1 week or 6 months via intranasal instillation, as previously described (26). Endotoxin-free water was used as control. There are multiple routes for PM to invade the body following inhalation. The primary route is via the respiratory system; other routes include olfactory epithelium and digestive (swallowing sputum with PM) (27). Intranasal instillation was used to mimic natural PM exposure. To evaluate the protective effect of NAC on hyperlipidemic mice with acute and chronic PM exposure, animals were pre-treated with NAC (1 mg/ml in drinking water) for 1 week and continued NAC treatment for 1 week or 6 months, as previously described (23). Mice fed ND were used as control. Although animal health and behavior were monitored every 7 days, 6 mice in the 6-month PM group died before the end of experiment; this may have been due to advanced atherosclerosis development.

Following 1-week or 6-month PM, HFD and NAC treatment, 3.0 and 1.5% isoflurane was used to induce and maintain anesthesia, respectively. A total of 300-500 μl murine blood was collected via cardiac puncture. Then, carbon dioxide (50-70% of chamber volume/min) was used to euthanize the animals. Death was confirmed by cardiac and respiratory arrest or fixed and dilated pupils. Murine aorta was isolated after confirming the death of mice.

Blood lipid, plasma ROS and inflammatory cytokine measurement and atherosclerotic plaque ratio calculation. At the end of the experiment, murine blood was centrifuged at 300 g and room temperature for 20 min to collect plasma for lipid profile, ox-LDL, plasma ROS and inflammatory cytokine analysis. A total of 40 μl plasma was applied in the lipid profile test cassette (Cholestech LDX; cat. no. 10-989; Thermo Fisher Scientific, Inc.) for measuring total cholesterol (TC), triglyceride (TG), LDL, high-density lipoprotein (HDL), non-HDL cholesterol (28) and the ratio of TC/HDL by using Alere Cholestech LDX System. Murine plasma ox-LDL was measured with Ox-LDL ELISA kit (cat. no. CSB-E07933m; Cusabio Technology LLC) according to the manufacturer's instruction. Murine plasma was collected to detect blood ROS levels by using *In Vitro* ROS/Reactive Nitrogen Species (RNS) Assay (cat. no. STA-347; Cell Biolabs, Inc.) and Pierce™ Quantitative Peroxide Assay kit (lipid-compatible formulation; cat. no. 23285; Thermo Fisher Scientific, Inc.) following the manufacturer's instructions. Following incubation of plasma with reagent at room temperature for 30 min, samples were transferred to a 48-well plate for analysis using a microplate reader (Multiskan™ FC Microplate Photometer) at the wavelength of 595 nm to measure the optical density (OD). Plasma levels of inflammatory cytokines TNF-α (cat. no. 430915), IL-1β (cat. no. 432615) and IL-6 (cat. no. 431315) were

measured using ELISA kits (BioLegend, Inc.) according to the manufacturer's instructions. Murine aorta was dissected and directly stained with oil red (MilliporeSigma) for plaque formation measurement at room temperature for 5 min. The ratio of plaque area to total inner surface of aorta was calculated as previously described (29).

Mononuclear cell and EPC intracellular ROS detection and EPC measurement. An endothelial cell marker combined with a stem cell marker CD34$^+$/CD133$^+$ was used to identify EPC as previously described (30). Murine blood cells were harvested after sacrifice to detect EPC and mononuclear cell intracellular ROS formation via flow cytometry by using ROS Detection Reagents-FITC (cat. no. D399; Invitrogen; Thermo Fisher Scientific, Inc.) as previously described (31). After removing red blood cells (RBCs) using 1X RBC lysis buffer (cat. no. 00433357; Thermo Fisher Scientific), a total of 50,000 cells in each sample were incubated with 5 μg/ml ROS Detection Reagents-FITC for 10 min at 37˚C. BD™ LSRII (BD Biosciences) at a wavelength of 525 nm was used to calculate the positively fluorescent cells for intracellular ROS detection. For EPC measurement, cells were incubated with anti-mouse CD34-AF700 (cat. no. 560518; BD Biosciences) and CD133-PE (cat. no. 12-1331-82; eBioscience; Thermo Fisher Scientific, Inc.) as previously described (21), then incubated with 5 μg/ml ROS Detection Reagents-FITC for 10 min at 37˚C. Labeled cells were washed twice with PBS and suspended in warm PBS for analysis by flow cytometry on a BD™ LSRII system (BD Biosciences). All antibodies were diluted 1:100.

Statistical analysis. All data are presented as the mean ± SD (5-8 repeats) and were analyzed using one- or two-way ANOVA (PRISM Version 5.0.; GraphPad Software, Inc.) followed by post hoc conservative Bonferroni's test to minimize type I error. Normal distribution of data was tested using Shapiro-Wilk W-test; equal variance was tested using F-test. When the null hypothesis of normality and/or equal variance was rejected, non-parametric Mann-Whitney U-test was used. Two-tailed P<0.05 was considered to indicate a statistically significant difference.

Results

NAC prevents PM-potentiated atherosclerosis in hyperlipidemia. To test the effect of NAC on atherosclerosis development in hyperlipidemic mice following PM exposure, LDLR KO mice were pre-treated with NAC for 1 week, and continued with NAC, HFD and PM exposure for 6 months. Atherosclerotic plaque formation was increased up to 1.3 fold in PM + HFD compared with HFD mice (Fig. 1). NAC significantly decreased plaque formation in PM + HFD + NAC (0.43±0.20%) and HFD + NAC (0.42±0.22%) compared with PM + HFD (0.80±0.29%) and HFD (0.62±0.21%) (Fig. 1). There was no plaque formation in any 1 week treatment (data not shown). These results indicated that NAC prevented PM-potentiated atherosclerosis formation.

NAC decreases blood ox-LDL levels following chronic PM exposure in hyperlipidemic mice. The blood lipid levels,
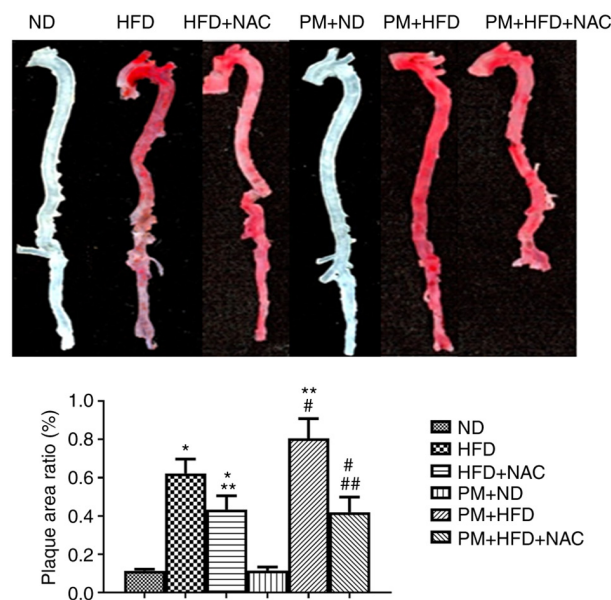

Figure 1. Mouse aortic atherosclerotic formation. Aorta was dissected and stained with oil red for atherosclerotic plaque analysis in LDLR KO mice after HFD with or without PM exposure for 6 months (magnification, x5). A significant amount of atherosclerotic plaque was identified in hyperlipidemic LDLR KO mice with PM exposure (PM + HFD). NAC extensively decreased atherosclerotic plaque area in PM + HFD + NAC) and (HFD + NAC) mice. n=5-8. *P<0.001 vs. ND; **P<0.001 vs. HFD; #P<0.001 vs. PM+ND; ##P<0.001 vs. PM + HFD. ND, normal diet; HFD, high-fat diet; NAC, N-acetyl cysteine; PM, ambient fine particulate matter; LDLR, low-density lipoprotein receptor; KO, knockout.

including TC, TG and LDL, were significantly increased in LDLR KO mice treated with HFD for 1 week or 6 months with or without PM exposure compared with ND mice (Table I). NAC had no effect on blood lipid profile in hyperlipidemic mice with or without PM exposure (Table I). Blood ox-LDL levels raised to 2.0±0.5 nmol/ml in LDLR KO 6-month PM + ND mice compared with 1-week PM + ND mice (0.44±0.10 nmol/ml; Fig. 2). Furthermore, blood ox-LDL level significantly increased to 14.00±2.00 nmol/ml in 6-month PM + HFD mice compared with 6-month HFD mice (10.02±2.21 nmol/ml; Fig. 2). NAC treatment significantly decreased murine plasma ox-LDL levels in LDLR KO mice following 6 month HFD treatment with (PM + HFD + NAC, 6.03±0.21 nmol/ml) or without PM (HFD + NAC, 5.80±3.00 nmol/ml) compared with 6-month PM + HFD (14.01±2.00 nmol/ml) or HFD groups (10.02±2.21 nmol/ml; Fig. 2). Blood ox-LDL levels were not elevated in any 1 week group compared with ND (Fig. 2). These data suggested that NAC prevented atherosclerosis in association with reducing PM-induced elevation of ox-LDL level in hyperlipidemia.

NAC maintains EPC levels following acute and chronic PM exposure in mice with hyperlipidemia. PM significantly decreased levels of circulating CD34$^+$/CD133$^+$ cell in LDLR KO mice fed ND for 1 week (0.044±0.003%) and 6 months (0.010±0.003%) and further decreased the cell population in PM + HFD mice (1 week, 0.02±0.01%; 6 months, 0.006±0.003%; Fig. 3). NAC treatment effectively reversed the effects of PM and hyperlipidemia on EPC (PM + HFD + NAC) in both 1 week (0.05±0.01%) and 6 month

Table I. Lipid profile of LDL receptor knockout mice.

A, 1 week

Lipid	ND	HFD	HFD + NAC	PM + ND	PM + HFD	PM + HFD + NAC
TC, mg/dl	228.60±25.66	1162.80±148.13[a]	1132.80±123.22[a]	230.60±27.88	1252.80±233.23[b]	1255.60±114.34[b]
HDL, mg/dl	88.00±9.64	82.80±6.87	85.40±7.54	90.00±10.24	85.50±7.07	82.50±6.34
TG, mg/dl	148.40±35.47	673.20±123.82[a]	632.80±87.32[a]	150.80±36.62	658.30±130.94[b]	642.50±90.54[b]
LDL, mg/dl	140.00±0.74	920.60±119.92[a]	983.80±108.50[a]	139.00±1.55	898.40±152.72[b]	884.20±110.30[b]
Non-HDL, mg/dl	146.40±24.25	1074.80±136.13[a]	1054.80±103.22[a]	148.30±26.72	1088.80±140.24[b]	1059.20±112.32[b]
TC/HDL	2.61±0.32	14.00±0.74[a]	13.20±0.85[a]	2.56±0.52	14.65±0.24[b]	15.22±0.62[b]

B, 6 months

Lipid	ND	HFD	HFD + NAC	PM + ND	PM + HFD	PM + HFD + NAC
TC, mg/dl	228.70±29.02	1704.0±228.70[a]	1728.00±234.80[a]	232.50±28.77	1789.00±242.30[b]	1732.00±243.80[b]
HDL, mg/dl	76.00±9.80	74.00±24.40	92.00±21.30	78.00±10.10	78.00±25.20	95.00±32.40
TG, mg/dl	103.70±35.00	597.50±217.30[a]	545.50±197.20[a]	105.50±42.00	566.40±220.40[b]	567.40±188.40[b]
LDL, mg/dl	132.00±23.10	1583.00±100.50[a]	1576.00±112.20[a]	140.00±34.20	1602.00±103.20[b]	1610.00±122.20[b]
Non-HDL, mg/dl	119.30±83.30	1700.00±90.50[a]	1720.00±100.60[a]	122.50±90.50	1689.00±89.50[b]	1656.00±112.40[b]
TC/HDL	2.80±0.40	23.00±5.40[a]	18.78±7.20[a]	2.98±0.70	22.90±4.40[b]	18.23±6.90[b]

Data are presented as the mean ± standard deviation (n=5-8). [a]P<0.01 vs. ND; [b]P<0.01 vs. PM + ND. HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglyceride; TC, total cholesterol; ND, normal diet; HFD, high-fat diet; NAC, N-acetyl cysteine; PM, ambient fine particulate matter.
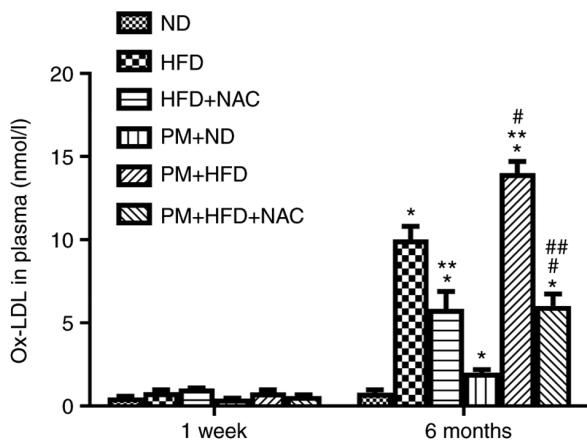

Figure 2. NAC significantly decreases plasma ox-LDL levels in hyperlipidemic mice with PM exposure. Murine plasma ox-LDL as noticeably increased in 6 month HFD LDLR KO mice and further elevated in 6 month PM + HFD mice compared with 6 month ND and PM + ND respectively. NAC effectively decreased plasma ox-LDL levels in HFD mice with or without PM exposure. n=5-8. *P<0.01 vs. ND; **P<0.01 vs. HFD; #P<0.01 vs. PM + ND; ##P<0.01 vs. PM + HFD. ND, normal diet; HFD, high-fat diet; NAC, N-acetyl cysteine; PM, ambient fine particulate matter; ox-LDL, oxidized low-density lipoprotein; LDLR, low-density lipoprotein receptor; KO, knockout.
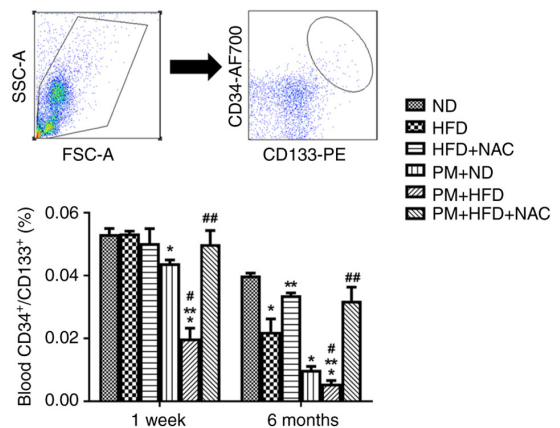

Figure 3. NAC maintains circulating EPC population in mice with hyperlipidemia following PM exposure. Circulating cells were isolated from LDLR KO mice fed HFD with or without PM exposure. Following red blood cell lysis, murine circulating CD34+/CD133+ cell population was analyzed by flow cytometry. Circulating EPC levels were significantly decreased in both 1-week and 6-month LDLR KO PM + HFD mice compared with HFD mice. NAC effectively maintained the EPC population. One week PM + ND exposure noticeably decreased EPC population while 1 week HFD had no effect on EPC compared with ND. n=5-8. *P<0.01 vs. ND; **P<0.01 vs. HFD; #P<0.01 vs. PM + ND; ##P<0.01 vs. PM + HFD. ND, normal diet; HFD, high-fat diet; NAC, N-acetyl cysteine; PM, ambient fine particulate matter; LDLR, low-density lipoprotein receptor; KO, knockout; EPC, endothelial progenitor cell.

(0.03±0.01%) groups compared with 1-week PM + HFD (0.02±0.01%) and 6-month PM + HFD (0.0056±0.0029%) groups respectively. Of note, EPC levels were not altered in 1-week HFD mice (HFD, 0.050±0.002% vs. ND, 0.050±0.005%) and were significantly decreased in 6-month HFD mice (HFD, 0.02±0.01% vs. ND, 0.040±0.002%). These data demonstrated that NAC prevented PM-induced decrease of EPC in hyperlipidemia.
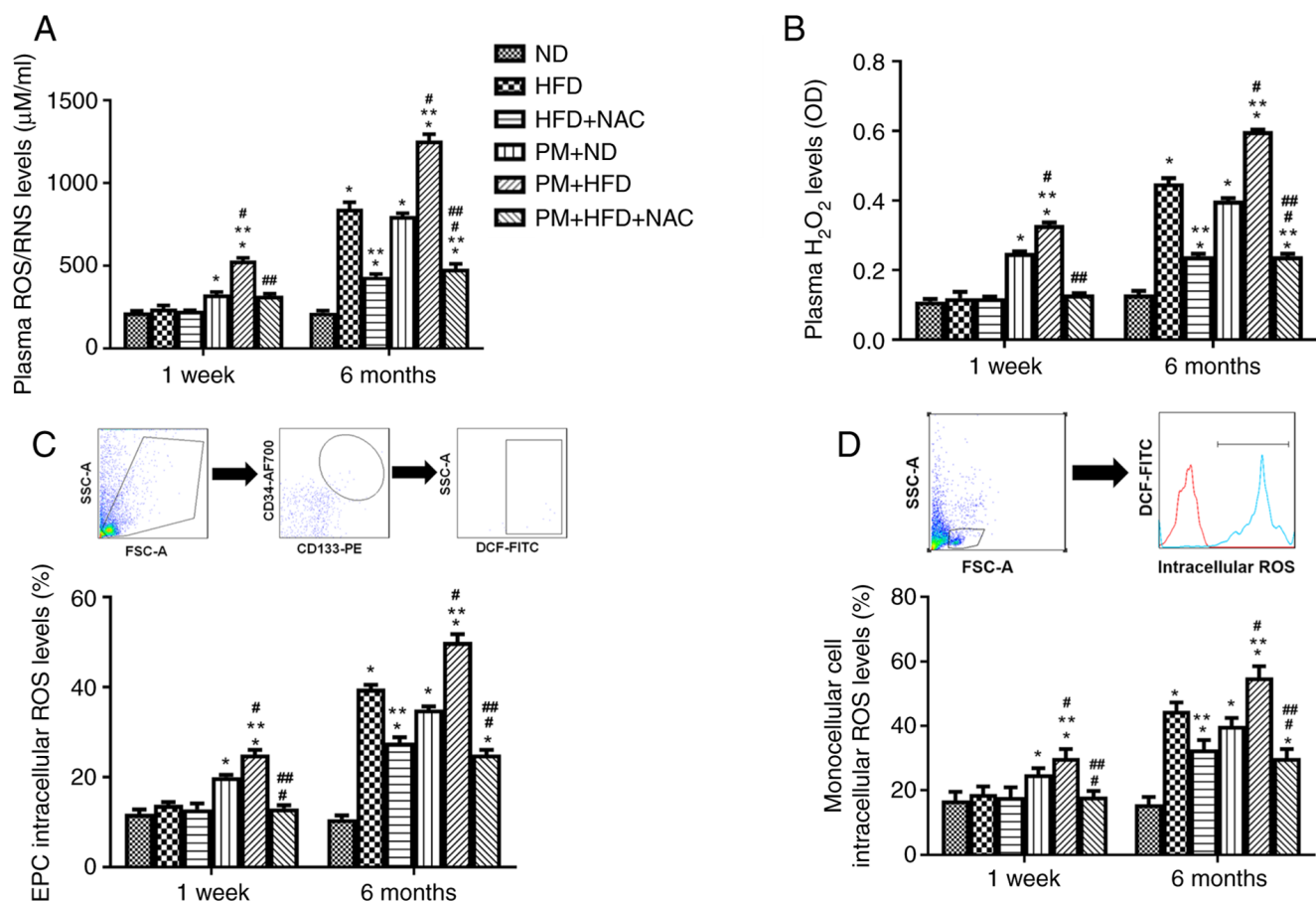
Figure 4. NAC blocks plasma ROS and both mononuclear and EPC intracellular ROS production in LDLR KO PM + HFD mice fed HFD. Plasma (A) ROS/RNS and (B) H$_2$O$_2$, (C) EPC and (D) mononuclear cell intracellular ROS production were significantly increased in 1 week and 6 month PM + HFD LDLR KO mice. Addition of NAC effectively decreased both plasma ROS and intracellular ROS production. n=5-8. *P<0.01 vs. ND; **P<0.01 vs. HFD; #P<0.01 vs. PM + ND; ##P<0.01 vs. PM + HFD. ND, normal diet; HFD, high-fat diet; NAC, N-acetyl cysteine; PM, ambient fine particulate matter; H$_2$O$_2$, hydrogen peroxide; OD, optical density; LDLR, low-density lipoprotein receptor; KO, knockout; EPC, endothelial progenitor cell; ROS, reactive oxygen species; RNS, reactive nitrogen species.

NAC blocks plasma, mononuclear cell and EPC intracellular ROS production in hyperlipidemic mice with acute and chronic PM exposure. Our previous study indicated that increased blood ROS production is associated with increased ox-LDL levels (23) and circulating EPCs are derived from mononuclear cells (32). To determine the mechanism underlying the protective effect of NAC on elevated plasma ox-LDL and decreased circulating EPC levels in mice with hyperlipidemia following acute and chronic PM exposure, plasma ROS, as well as circulating mononuclear cell and EPC intracellular ROS production, was measured in hyperlipidemic mice following 1-week and 6-month PM exposure with or without NAC. Total plasma ROS/RNS and H$_2$O$_2$ levels were significantly increased in 1-week PM + HFD mice (530±48 μM/ml; 0.33±0.02 OD) or 6 months (1,255±110 μM/ml; 0.60±0.01 OD) compared with 1 week HFD mice (239±57 μM/ml; 0.12±0.05 OD) or 6 month (845±107 μM/ml; 0.45±0.04 OD). NAC significantly blocked plasma ROS production in 1-week (PM + HFD + NAC, 318±35 μM/ml; 0.13±0.01 OD) or 6-month (PM + HFD + NAC, 480±88 μM/ml; 0.24±0.02 OD) PM and HFD treated mice compared with 1 week or 6-month PM + HFD mice (Fig. 4A and B). Similarly, mononuclear cell and EPC intracellular ROS production were significantly elevated up to 1.3-1.6-fold in LDLR KO 1-week or 6-month

PM + HFD mice compared with HFD mice (Fig. 4C and D). The increased EPC and mononuclear cell intracellular ROS production were effectively blocked by NAC in 1 week and 6 month PM + HFD + NAC mice (Fig. 4C and D). Of note, ROS production increased in 1 week and 6 month PM + ND mice and 6 month HFD mice compared with 1 week and 6 month ND mice. There was no ROS elevation in 1-week HFD compared with ND mice (Fig. 4A-D). These results indicated that NAC prevented atherosclerosis formation mainly through inhibition of ROS production in hyperlipidemic mice following PM exposure.

NAC effectively inhibits plasma inflammatory cytokine production in hyperlipidemic mice with acute and chronic PM exposure. Our previous study reported that NAC exerts powerful anti-inflammatory effects in WT mice with PM exposure (21). Therefore, the present study investigated whether PM inhibits production of inflammatory cytokines, including TNF-α, IL-1β and IL-6, in LDLR KO HFD mice. Inflammatory cytokine levels were significantly increased up to 1.3-2.0-fold in LDLR KO 1-week and 6-month PM + HFD mice compared with mice with either HFD or PM + ND (Fig. 5). NAC effectively prevented production of all three cytokines in PM + HFD mice (Fig. 5).
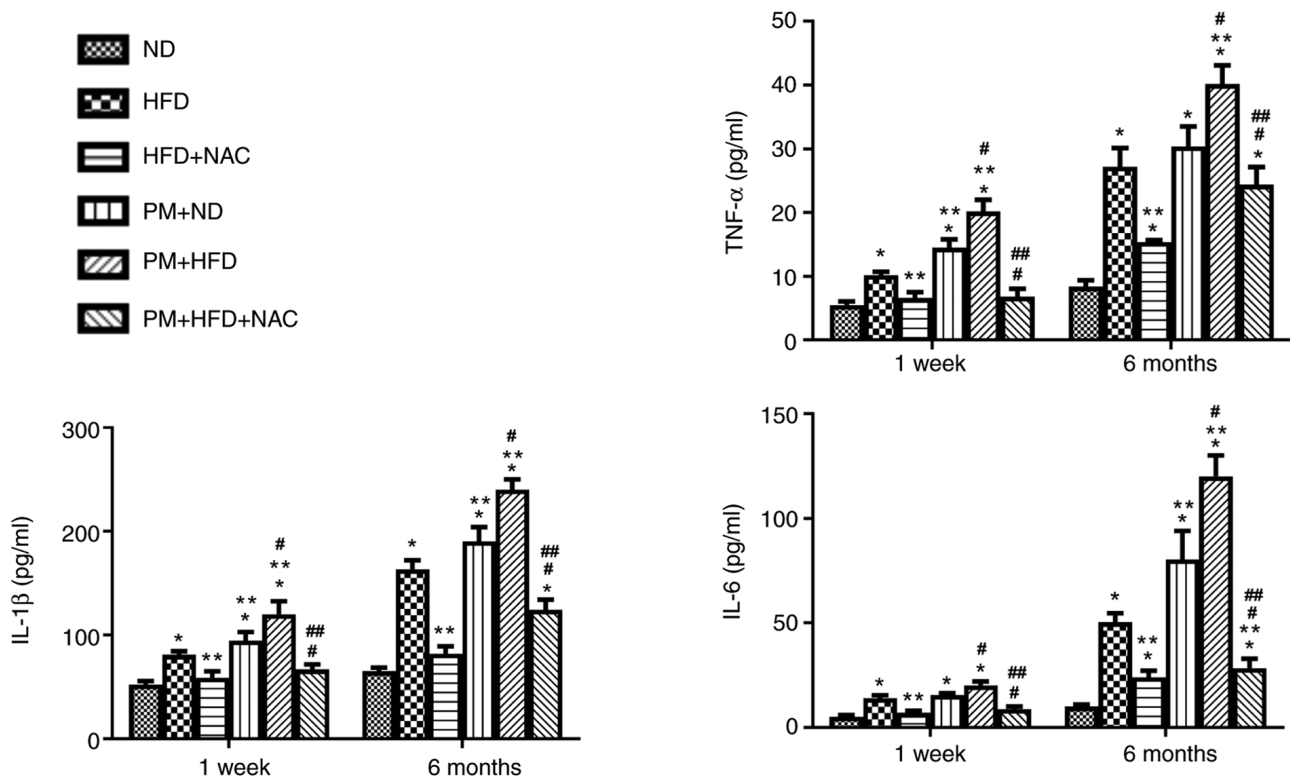
Figure 5. NAC inhibits plasma inflammatory cytokine production in LDLR KO PM + HFD mice. There was a significant increase in TNF-α, IL-1β and IL-6 in LDLR KO HFD mice with or without PM compared with PM+ND or ND mice. NAC effectively inhibited inflammatory cytokine production. Inflammatory cytokines were also increased in mice with 1 week and 6 month HFD or PM + ND compared with ND mice. n=5-8. $^*P<0.01$ vs. ND; $^{**}P<0.01$ vs. HFD; $^\#P<0.01$ vs. PM + ND; $^{\#\#}P<0.01$ vs. PM + HFD. ND, normal diet; HFD, high-fat diet; NAC, N-acetyl cysteine; PM, ambient fine particulate matter; LDLR, low-density lipoprotein receptor; KO, knockout.

Discussion

The present study demonstrated that PM exposure potentiated atherosclerosis formation by promoting ox-LDL levels and inflammatory cytokine production and decreasing circulating EPC levels in hyperlipidemic mice. The mechanisms primarily involved elevated ROS production. NAC effectively reversed both acute and chronic PM exposure-potentiated effects in LDLR KO HFD mice.

Cardiovascular risk factors, such as hyperlipidemia, diabetes and age are associated with incomplete revascularization and decreased re-reendothelialization following arterial injury in both humans and animals (33-35). Chronic hyperlipidemia exerts harmful effects on the cardiovascular system, including aggravation of atherosclerosis due to elevation of ox-LDL and TG levels and decreased HDL (36). In addition, short-term hyperlipidemia affects organs primarily via initiating inflammation (37-39). On the other hand, PM exposure is associated with atherosclerosis development in both human and animal studies (1,40). The mechanisms primarily involved in PM-potentiated atherosclerosis include early changes in vascular tone via elevated oxidative stress and inflammation, cholesterol modification and promotion of pro-thrombotic reactions (40). The present data indicated that acute and chronic PM exposure in hyperlipidemic mice further increased plasma ox-LDL and inflammatory cytokine levels and decreased circulating EPCs, which serve an important role in endothelial repair (1). The mechanisms primarily involve increased plasma ROS as well as EPC and mononuclear intracellular ROS

production. In addition, males have a higher risk for CVD than females; females are better protected than males against CVD due to undefined mechanisms (41). Our previous study demonstrated that CD34$^+$/CD133$^+$ EPC population was selectively decreased in male mice following PM exposure via elevated oxidative stress, while PM exposure had no effects on estrogen-independent EPC changes in female mice (42). PM exposure-induced decrease in EPC population in male mice may be due to decreased expression of pulmonary superoxide dismutase 1 (43). Therefore, the regulation of anti-atherosclerotic mechanisms in females requires further investigation. To the best of our knowledge, there is no way to determine the exact amount of PM in respiratory tissue following exposure; however, our previous study indicated that there is increased inflammation in murine pulmonary tissue following PM exposure compared with control (21).

NAC is an antioxidant with therapeutic value for decreasing endothelial dysfunction, inflammation, fibrosis, invasion, cartilage erosion, acetaminophen detoxification and prolong transplant life (44). In cardiovascular studies, NAC protects diabetic heart at risk of myocardial infarction and prevents ischemia- and non-ischemia-associated cardiac damage and ischemia-reperfusion-induced injury primarily via inhibition of oxidative stress in humans (22,45). NAC is also reported to prevent hypertrophy and fibrosis in β-MyHC-Q403 transgenic rabbits and cTnT-Q92 transgenic mice with hypertrophic cardiomyopathy via multiple thiol-responsive mechanisms (46) and inhibit platelet aggregation and reperfusion injury in both human and animals (47).

In accordance with our and other previous studies (23,48-50), the present study demonstrated that NAC had no effect on lipid profile in hyperlipidemia, whereas Korou *et al* (51) found NAC decreases serum LDL levels in hyperlipidemic mice. This difference may be due to different administration methods. Additionally, NAC decreases ox-LDL levels via inhibition of oxidative stress; to the best of our knowledge, however, the detailed mechanism has not previously been investigated (52-54). NAC prevents atherosclerosis formation via suppression of inflammation by inhibiting NF-κB-induced TNF-α production and blood homocysteine levels (55,56). NAC maintains EPC population, decrease conversion of LDL to ox-LDL *in vivo*, prevent atherosclerosis in humans and mice with hyperlipidemia and promote hind limb ischemia recovery in mice (15,23,30,57).

NAC exhibits protective effects against PM-associated damage in different organs (1). NAC could abolishes PM-induced suppression of lymphocyte function in rats and mice (58), inhibits pulmonary inflammation in mice following 5 h and 1 month PM exposure (21,59) and prevents PM-induced ROS production in human endothelial cells (60). To the best of our knowledge, however, the effect of NAC on air pollution-potentiated atherosclerosis has not been reported. Certain studies have reported that NAC prevents senescence of porcine coronary artery endothelial cells *in vitro* (61), attenuates carbon monoxide-induced ischemic heart failure (62), prevents cardiomyocyte apoptosis (63) and inhibits vascular smooth muscle proliferation (64) and heart arrhythmia (65) in rats following PM exposure. The mechanisms include inhibition of oxidative stress, deactivation of c-Jun N-terminal kinase, p38 MAP kinase and inhibition of inflammation via prevention of NF-κB transcription factor activity (44). NAC prevents apoptosis and promotes cell survival via extracellular signal-regulated kinase activation (44). Our previous studies indicated that NAC inhibits EPC apoptosis, promotes BMSC proliferation and inhibits inflammatory cytokine production via blocking ROS generation in mice following PM exposure for 1 month (21,66). The present data showed that NAC effectively attenuated PM-potentiated atherosclerosis formation via inhibition of plasma ROS-induced ox-LDL elevation and mononuclear cell and EPC intracellular ROS-induced decreases in circulating EPC levels. Of note, blood inflammatory cytokine levels in mice with hyperlipidemia following 1 week or 6 month PM exposure were also significantly decreased by NAC treatment.

Further studies are needed to determine the mechanism of NAC against PM-potentiated atherosclerosis including that underlying NAC-induced decrease in ox-LDL levels, inability of NAC to completely prevent atherosclerosis development and the cell signaling pathways involved in NAC suppression of inflammatory cytokine production.

In conclusion, the present study demonstrated that NAC effectively prevented PM-potentiated atherosclerosis via inhibition of plasma ROS-induced ox-LDL elevation and mononuclear cell and EPC intracellular ROS-induced decrease in circulating EPC levels, as well as suppression of inflammatory cytokine production.

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

YQC, XCM and LQC designed the experiments. YXX, HRB, YFJ, QWL, XWG and XQZ performed the experiments. KH, ZHS, QYZ, YC and HHS collected and analyzed the data. YQC and YXX wrote the manuscript. All authors have read and approved the final manuscript. YQC, XCM and YXX confirm the authenticity of all the raw data.

Ethics approval and consent to participate

All animal procedures were performed in accordance with the Guidelines of the Animal Care Committee of the Shandong Provincial Hospital affiliated to Shandong First Medical University (Jinan, China). The Animal Care Committee of Shandong Provincial Hospital affiliated to Shandong First Medical University (Jinan, China) approved the experimental protocols (approval no. 2021-228).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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