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Mitochondrial ROS and NLRP3 Inflammasome in Acute Ozone-induced Murine Model of Airway Inflammation and Bronchial Hyperresponsiveness

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Abstract:

Oxidative stress is a key mechanism underlying ozone-induced lung injury. Mitochondria can release mitochondrial reactive oxidative species (mtROS), which may lead to the activation of NLRP3 inflammasome. The goal of this study was to examine the roles of mtROS and NLRP3 inflammasome in acute ozone-induced airway inflammation and bronchial hyperresponsiveness (BHR). C57/BL6 mice (n=8/group) were intraperitoneally treated with vehicle (phosphate buffered saline, PBS) or Mito-TEMPO (mtROS inhibitor, 20mg/Kg), or orally treated with VX-765 (caspse-1 inhibitor, 100mg/Kg) one hour before the ozone exposure (2.5ppm, 3 hour). Compared to PBS-treated ozone-exposed mice, MitoTEMPO reduced the level of total malondialdehyde in bronchoalveolar lavage (BAL) fluid and increased the expression of mitochondrial complexes II and IV in the lung 24-hour after single ozone exposure. VX-765 inhibited ozone-induced BHR, BAL total cells including neutrophils and eosinophils and BAL inflammatory cytokines including IL-1α, IL-1β, KC and IL-6. Both MitoTEMPO and VX-765 reduced ozone-induced mtROS and inhibited caspase-1 activity in lung tissue whilst VX-765 further inhibited DRP1 and MFF expression, increased MFN2 expression and down-regulated caspase-1 expression in lung tissue. These results indicate that acute ozone exposure induces mitochondrial dysfunction and NLRP3 inflammasome activation, while the latter has a critical role in the pathogenesis of ozone induced airway inflammation and BHR.

Key words: mitochondrial ROS, NLRP3 inflammasome, ozone, airway inflammation, bronchial hyperresponsiveness

Introduction

Ozone is a secondary atmospheric pollutant produced through photochemical reactions involving sunlight and precursor pollutants, including volatile organic
compounds (VOCs), nitrogen oxides (NO\textsubscript{X}) and carbon monoxide (CO), which are released by a wide variety of stationary and mobile sources [1]. Exposure to high ambient ozone has been linked to increased incidence of asthma [2], and increased emergency visits and hospitalizations of patients with asthma and chronic obstructive pulmonary disease (COPD) [3,4]. Experimental studies have demonstrated that high levels of ozone exposure can induce airway inflammation and bronchial hyperresponsiveness (BHR) in both humans [5,6] and mice [7,8]. The mechanism is not fully elucidated, however, as a potent oxidizing gas, ozone can induce oxidative stress, which is likely a key mechanism underlying ozone induced airway inflammation and BHR.

Mitochondria are double membrane bound organelles that exist in all eukaryotic organisms with multiple functions. The morphology of mitochondria is regulated by fission and fusion process. The former requires the recruitment of dynamin-related protein 1 (DRP1) from the cytosol to receptors including mitochondrial fission protein 1 (FIS1), mitochondrial fission factor (MFF), and mitochondrial elongation factors 1 and 2 (MID51 and MID49), while the latter is mediated by mitofusin 1 and 2 (MFN1/2) at the outer mitochondrial membrane and by optic atrophy 1 (OPA1) at the inner mitochondrial membrane [9]. The functions of mitochondria include generation of adenosine triphosphate (ATP) and mitochondrial reactive oxygen species (mtROS), which are produced by the oxidative phosphorylation (OXPHOS) enzyme complexes located in the inner mitochondrial membrane [10]. Under inflammatory conditions, mtROS can initiate downstream inflammatory pathways [11].

Nucleotide binding domain leucine-rich repeat-containing receptor (NLR) families are an important component of signaling by intracellular pattern recognition receptors in the innate immune system, of which the NLRP3 inflammasome is the best...
characterized member and acts as a potent inducer of inflammation in lung diseases including asthma and COPD. The NLRP3 inflammasome contains a sensor protein, NLRP3, an adaptor protein, apoptosis-associated speck-like protein containing caspase-recruitment domain (ASC), and pro-caspase-1 [12]. NLRP3 inflammasome can be activated by a variety of exogenous and endogenous agonists, including bacterial toxins, extracellular ATP and mtROS [13,14]. Stimulation of the NLRP3 inflammasome leads to caspase-1 activation and the subsequent production of proinflammatory cytokines such as IL-1\(\beta\) and IL-18 [12].

We hypothesized that the mtROS-NLRP3 inflammasome nexus is an important pathway in ozone-induced airway inflammation and BHR. Therefore, the goal of this study was to examine the roles of mtROS and NLRP3 inflammasome in acute ozone-induced airway inflammation and BHR using selective inhibitors.

**Materials and Methods**

**Mice and ozone exposure**

Pathogen-free, 8-10 week old male C57/BL6 mice (Shanghai Super -- B&K Laboratory Animal Corp. Ltd, Shanghai, China) were housed in specific-pathogen-free (SPF) conditions under a constant temperature (20°C) and relative humidity (40%-60%) with food and water supplied ad libitum. The experimental procedures involving animals and their care were approved by the Laboratory Animal Ethics Committee of the institute.

Mice were exposed to ozone produced from a generator (Model 300, AB Aqua Medic GmbH, Bissendorf, Germany), mixed with air for 3 hours at a concentration 2.5 parts per million (ppm) in a sealed perspex container in the morning. The ozone concentration was continuously monitored and maintained using an ozone switch.
OS-4, EcoSensors Division, KWJ Engineering, Inc., Newark, NJ, USA). At the same
time, control animals were exposed to filtered air only.

Mice (eight mice in each group) were administered intraperitoneally with vehicle
(phosphate buffered saline, PBS), MitoTEMPO (mtROS inhibitor, 20mg/kg dissolved
in PBS) or orally with VX-765 (caspase-1 inhibitor, 100mg/kg dissolved in distilled
water containing 0.5% sodium salt of caboxy methylelulose and 0.1% Tween-80)
one hour prior to ozone exposure.

Measurement of bronchial responsiveness
Twenty-four hours after exposure, mice were anesthetized with an intraperitoneal
injection of anesthetic solution containing ketamine and xylazine and then transferred
to a plethysmograph for measurement of resistance and compliance (EMMS, Hants,
UK), and airway responsiveness. Mice were ventilated with Mini ventilator at a rate
of 160 breaths/min and at a tidal volume of 220μl, and were monitored with a
pneumotachograph connected to a transducer. Transpulmonary pressure was assessed
via an esophageal catheter. Instantaneous calculation of pulmonary resistance (R\textsubscript{L})
was obtained. Increasing concentrations of acetylcholine (ACh) (Sigma-Aldrich, St
Louis,MS, USA, 4-256 mg/ml) were administered with an Aeroneb® Lab Micropump
Nebulizer (EMMS), and R\textsubscript{L} was recorded for a 3-min period following each
concentration. R\textsubscript{L} after each concentration was expressed as percentage change from
baseline R\textsubscript{L} measured following nebulized PBS. The concentration of ACh required to
increase R\textsubscript{L} by 200% from baseline was calculated (PC\textsubscript{200}) and –log PC\textsubscript{200} was taken
as a measure of airway responsiveness.

Bronchoalveolar lavage fluid and cell counting
Following terminal anesthesia with pentobarbitone 24-hour after ozone exposure, mice were lavaged with two 0.8 ml aliquots of PBS via a 1mm diameter endotracheal tube, and bronchoalveolar lavage (BAL) fluid was retrieved. The retrieved lavage aliquots were pooled and centrifuged at 4°C, 250g, for 10 min, from which supernatant was collected and stored at -80°C. The cell pellet from the BAL fluid was re-suspended in PBS and counted using a hemocytometer. Total cell counts and differential cell counts from cytospin preparations stained using the Diff-Quick method (Gentaur, Kampenhout, Belgium) were determined under an optical microscope (Olympus BH2, Olympus Optical Company Ltd., Tokyo, Japan). At least 500 cells were counted per mouse and identified as macrophages, neutrophils, lymphocytes and eosinophils according to standard morphology under x400 magnification.

**Malondialdehyde (MDA) and cytokine measurement in BAL fluid**

Concentrations of total MDA were measured using high-performance liquid chromatography (HPLC) system with fluorescent detection. An alkaline hydrolysis step described as follows was added before the reaction with TBA: 20 µl aliquot of sample was added 40 µl of 1N NaOH and incubated at 60°C for 30 mins in a shaking water bath. After incubation, 40 µl of 1N HCl was added into the mixture and then 500 µl phosphoric acid (440 mM) and 100 µl thiobarbituric acid (TBA, 42 mM) were added. The mixture was incubated at 80°C for one hour, a 20 µl aliquot of this final solution was injected into the HPLC system with fluorescence detector set at 532 nm for the excitation wavelength and 553 nm for the emission wavelength. A Nova-Pak C$_{18}$ column (Waters, Milford, MA, USA) was used with a mobile phase that was composed of 40% methanol and 60% water containing 50mM KH$_2$PO$_4$ (pH=6.8) at a
flow rate of 0.8 ml/min. The detection limit, extraction recovery and analytical precision of this method were 1.8 nM, 75.9%, and 2.2% (measured as RSD from 8 replicate injections), respectively.

Levels of chemokine (C-X-C motif) ligand 1 (CXCL1, KC), tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6) and IL-1β in BAL fluid were measured using ELISA DuoSets according to manufacturers' instructions (R&D Systems, Minneapolis, MN, USA).

**Lung mitochondrial ROS analysis and caspase-1 activity**

Mitochondria were extracted from fresh lung tissues using a Mitochondria Isolation Kit for Tissue (Beyotime, Haimen, Jiangsu, China) with protease inhibitors (Merck Millipore, Darmstadt, Germany) using a Dounce tissue grinder following the manufacturer’s instructions, and then quantified by BCA analysis (Thermo Fisher Scientific). Equal amounts of mitochondrial extract were incubated with 5µM MitoSOX™ Red (Invitrogen) for 10 minutes at 37°C and protected from the light. Red fluorescence was measured at 510/580nm using a Flexstation®2 fluorescence reader (Molecular Devices, San Jose, CA, USA).

The lung homogenates were extracted using RIPA buffer (Beyotime) with protease inhibitors (Merck Millipore). The levels of caspase-1 activity in mouse lung tissue were detected using a commercial assay kit (Beyotime) and measured at 405 nm by a microplate reader following the manufacturer’s instructions.

**Western blot analysis**

Equal amounts of protein from mitochondrial and whole lung extracts were separated by SDS-PAGE (Beyotime) and transferred to nitrocellulose membranes by electrophoresis. The membranes were incubated in blocking solution at room
temperature for 1h and then incubated with primary antibodies against total OXPHOS antibody cocktail (Abcam, Cambridge, MA, USA), voltage-dependent anion channel (VDAC, Abcam), DRP1 (Cell Signaling Technologies - CST, Beverly, MA, USA), MFF (CST), MFN2 (CST), OPA1 (CST), GAPDH (CST), NLRP3 (CST), Caspase-1 (Abcam) overnight at 4°C. A horseradish peroxidase-conjugated anti-rabbit secondary antibody (Cell Signaling Technology) was used, and chemiluminescent detection reagent (Merck Millipore) was used for detection. VDAC and tubulin were respectively used as loading controls. The density was quantitated using a densitometer.

**Statistical analysis**

All results were expressed as mean ± S.E.M. Two-way analysis of variance was performed for comparisons of % change in $R_L$ between individual groups. One-way analysis of variance (ANOVA) with Bonferroni post hoc test (equal variance) or Dunnett T3 post-hoc test (unequal variance) was performed for comparison between multiple groups. P<0.05 was considered significant.

**Results:**

**Airway Responsiveness**

There were no significant differences in the baseline lung resistance values following PBS challenge in the four experimental groups. Ozone-exposed mice demonstrated a leftward shift of the concentration-response curve (Figure 1(A)), indicating an increase in airway responsiveness to ACh compared to air-exposed mice (-logPC_{200}: 1.54±0.05 vs 2.11±0.15; P<.05, Figure 1(B)). VX-765 inhibited ozone-induced airway responsiveness to ACh (-logP_{200}: 2.02±0.12 vs 1.54±0.05, P<.05; Figure 1(B)), while MitoTEMPO showed no effect on airway responsiveness to ACh.
**BAL fluid cells and cytokine levels**

Ozone exposure increased total BAL cell counts including that of neutrophils and eosinophils compared with air-exposed mice ($P<.001$, $P<.001$ and $P<.05$ respectively, Figure 2(A)). Treatment with VX-765, not MitoTEMPO, decreased the numbers of total cells, neutrophils and eosinophils (all $P<.05$, Figure 2(A)). Ozone exposure increased BAL concentrations of IL-1α, IL-1β, KC and IL-6 in comparison with air-exposed mice ($P<.001$, $P<.001$, $P<.001$ and $P<.05$ respectively, Figure 2(B)-(E)). VX-765 treatment significantly decreased the concentrations of IL-1α, IL-1β, KC and IL-6 ($P<.01$, $P<.001$, $P<.001$ and $P<.01$ respectively, Figure 2(B)-2(E)). MitoTEMPO treatment significantly reduced the level of KC ($P<.001$) (Figure 2(D)).

**Oxidative stress in lung**

Ozone exposure enhanced total MDA levels in BAL and mtROS levels in lung tissue compared to control mice (both $P<.001$, Figure 3(A) & 3(B)). MitoTEMPO treatment reduced total MDA and mitochondrial ROS ($P<.05$ and $P<.01$ respectively, Figure 3(A) & 3(B)) whilst VX-765 significantly reduced mtROS ($P<.001$, Figure 3(B)).

**Expression of mitochondrial OXPHOS in lung tissue**

There was decreased expression of mitochondrial OXPHOS complexes II, III and IV in ozone-exposed mice compared with control mice ($P<.05$, $P<.01$ and $P<.001$ respectively, Figure 4(A), 4(C), 4(D) & 4(E)). MitoTEMPO increased the expression of mitochondrial complex II and IV in the lung of ozone-exposed mice ($P<.05$ and $P<.05$ respectively, Figure 4(A), 4(C) & 4(E)). VX-765 had no effect on the expression of OXPHOS complex proteins.
Mitochondrial related protein expression

The expression of DRP1 and MFF was increased in ozone-exposed mice compared to control mice (\(P<.01\) and \(P<.05\) respectively, Figure 5(A) & 5(B)). VX765 treatment inhibited the expression of DRP1 and MFF (\(P<.01\) and \(P<.05\) respectively, Figure 5(A) & 5(B)). The expression of OPA1 was unchanged and MFN2 was decreased in ozone-exposed mice compared to control mice (\(P<.01\), Figure 5(C) & 5(D)) and VX765 treatment increased MFN2 expression (\(P<.01\), Figure 5(D)).

Expression of NLRP3&caspase-1 and caspase-1 activity in lung tissue

The expression of NLRP3 was unchanged by ozone exposure when compared with control mice. Neither MitoTEMPO nor VX-765 affected NLRP3 expression (Figure 6(A)). However, the expression and activity of caspase-1 was increased in ozone-exposed mice when compared with control mice (\(P<.01\) and \(P<.05\) respectively, Figure 6(B) & 6(C)). VX-765 significantly inhibited the expression of caspase-1 in the lung tissue (both \(P<.01\), Figure 6(B)), furthermore, both VX-765 and MitoTEMPO reduced the activity of caspase-1 (\(P<.01\), Figure 6(C)).

Discussion

The present study demonstrated that in an acute ozone exposure model, VX-765 (caspase-1 inhibitor) inhibited airway inflammation and BHR, which was associated with suppression of ozone-induced BAL cell counts and cytokine levels, suppression of ozone-induced mitochondrial dysfunction and increased caspase-1 activity and expression in lung by VX-765. MitoTEMPO (mtROS inhibitor) decreased BAL levels of MDA and increased the expression of mitochondrial OXPHOS in the lung. These
data suggest that ozone exposure induces airway inflammation and BHR at least in part through activating the NLRP3 inflammasome in lung tissue.

The present research is consistent with previous studies which show that a single ozone exposure causes airway inflammation [7,8]. Ozone exposure increased the numbers of total cells, neutrophils and eosinophils, and elevated levels of IL-1α, IL-1β, KC and IL-6 in BAL fluid. Both IL-1α and IL-1β belong to IL-1 family and play an important role in modulating innate and adaptive immune response. In vitro, ozone exposure (0.1ppm, 1-hour) stimulated the secretion of IL-1α and IL-1β from rat alveolar macrophages [15] and in vivo, ozone exposure (0.3 ppm, 72-hour) increased IL-1α and IL-1β mRNA levels in lung tissue [16]. The production of IL-1α and IL-1β requires caspase-1 activation within the NLRP3 inflammasome complex to enable conversion from their inactive precursor forms [17]. KC and IL-6 are partially dependent on IL-1β as both cytokines are reduced in an IL-1α/β double knock-out mice [18]. The present study showed that VX-765 reduced the presence of BAL cells and cytokines and indicated that the NLRP3 inflammasome is directly related to inflammation in this acute ozone-challenge model. MitoTEMPO reduced ozone-induced BAL KC expression and only had a trend towards suppression of inflammatory cell infiltration.

BHR is a characterized effect of ozone exposure and this occurrence of excessive bronchoconstriction in response to a variety of inhaled stimuli is an important characteristic of both asthma and COPD [19]. Eosinophils can release a number of different mediators with the capacity to cause BHR in asthma [20], whereas lung neutrophilia is strongly associated with BHR in COPD probably as a result of their production of ROS and pro-inflammatory cytokines [21]. In the present study, NLRP3 inhibition attenuated ozone-induced BHR and inhibited BAL neutrophils and
eosinophils. In contrast, MitoTEMPO, an inhibitor of mtROS, did not ameliorate ozone-induced BHR or reduce inflammatory cells in BAL fluid. This result is different from a previous study, in which Necrox-5, the other mitochondrial ROS inhibitor, reduced BHR in an ovabulmin induced asthma model [22]. The data may indicate BAL inflammation, not mtROS, is associated with BHR.

Oxidative stress is an important mechanism implicated in the pathogenesis of both asthma and COPD and results from increases in free radicals and other reactive oxygen/nitrogen species and/or decreases in endogenous antioxidant defenses [23,24]. The exogenous sources of oxidative stress include cigarette smoking, environmental pollution such as particulate matter and ozone [22,23]. The endogenous sources of oxidative stress mainly come from inflammatory cells and epithelial cells via intracellular enzymes and organelles such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, nitric oxide synthase, peroxidase and mitochondria [23,24]. Ozone exposure can directly lead to the development of oxidized intermediates, which are either formed in the epithelial lining fluid or on cell surface proteins and lipids [25]. Consistent with previous reports [7], we confirmed that a single ozone exposure increased MDA levels in BAL fluid. MDA is a reliable marker of oxidative damage via lipid peroxidation [26] and this up-regulation seen with ozone was prevented by treatment with MitoTEMPO.

Mitochondria are sensitive to even mild oxidative stress [27], and thus they represent a potential target for ozone. Ozone causes alterations in mitochondrial respiratory chain enzymes [28]. A low concentration (0.5ppm, 8h/day) of ozone for 1 or 5 days induced oxidant stress and mitochondrial DNA damage (mtDNA) in lung and aortic tissues of C57/BL6 mice [29]. In contrast, a high concentration (3ppm, 3h, twice a week) of ozone for 1 or 6 weeks led to mitochondrial dysfunction represented by
decreased mitochondrial membrane potential (ΔΨm), increased mitochondrial oxidative stress, and reduced mitochondrial complex I, III, and V expression in lung of C57/BL6 mice [30]. In the present study, we found that mtROS was increased in lung tissue 24-hour after a single ozone exposure. Both MitoTEMPO and VX-765 reduced ozone-induced mitochondrial ROS and caspase-1 activity in lung. This indicates that cross-talk occurs between mitochondrial function and NLRP3 inflammasome activation [31] although a linear pathway relationship is unlikely. The effect of the acute ozone exposure on the expression of mitochondrial OXPHOS complex proteins II, III and IV was different from what have previously reported in chronic ozone-exposed mice [29, 32], which may reflect temporal differences.

Mitochondria are regulated through cycles of fusion and fission, which influence mitochondrial mixing, mitochondrial DNA integrity, mitochondrial respiration and energy metabolism. Upon exposure to stress (inflammation, environment insults), mitochondria undergo fission and display in fragmented morphology. Cigarette smoke extract (CSE) induced mitochondrial fragmentation and damaged their morphology by increased expression of DRP1 and decreased MFN2 in human airway smooth muscle cells [33]. Similarly, CSE enhanced DRP1 and MFF and decreased OPA1 and MFN2 in airway epithelial cell lines (A549 cells and Beas-2b cells) [34]. Acute PM$_{2.5}$ intranasal instillation increased DRP1 and MFF and reduced OPA1 and MFN1 in lung tissue in C57/BL6 mice [35]. The present study showed that acute ozone exposure resulted in elevated DRP1 and MFF and reduced MFN2 in lung tissue, while VX765 treatment inhibited the expression of DRP1 and MFF. The imbalance of mitochondrial fission and fusion proteins may contribute to mitochondrial dysfunction, such as decreased ATP synthesis and increased ROS production, leading to cell damage, apoptosis, inflammation and ultimately cell death.
Ozone-induced oxidant stress modifies several known cell signaling mechanisms such as the innate immune signaling pathways, which lead to the up-regulation of antioxidant genes and the enhanced release of damage-associated molecular pattern molecules (DAMPs) [36]. There is much evidence to indicate the involvement of NLRP3 inflammasome in the pathogenesis of airway diseases [37,38]. Consistent with our previous study in chronic ozone-exposed animals [39], we showed here that the NLRP3 inflammasome was activated by single ozone exposure evidenced by increased IL-1α and IL-1β concentration in BAL fluid and increased caspase-1 activity and expression in lung tissue. We initially hypothesized that mtROS acts as a trigger of NLRP3 activation, but the effects of VX-765 and mitoTEMPO are not consistent with this linear model and we suggest that these pathways are activated in parallel although cross-talk does occur between the two.

There are several limitations in the study which could be addressed in the future studies. First, MitoTEMPO was failed in inhibiting airway inflammation and BHR in the present study. Considering mitochondria are the major source of endogenous ROS and mitochondrial dysfunction plays an important role in COPD, new compounds preventing or reversing mitochondrial dysfunction can be investigated in vivo or in vitro. Second, more in vivo cell lines studies can be conducted to further examine the effect and mechanism of mitochondrial dysfunction-NLRP3 pathway in inflammation. Third, to elucidate the role of NLRP3 inflammasome in ozone-exposed model, NLRP3−/− mouse can be used in later studies.

In summary, this study suggests that acute ozone exposure induces mitochondrial dysfunction and NLRP3 inflammasome activation. NLRP3 inflammasome, can be activated by non-mtROS mechanism, has a critical role in the pathogenesis of ozone
induced airway inflammation and BHR, providing a novel therapeutic strategy for treating ozone-induced airway disorders.

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**Disclosure statement**

The authors report no conflict of interest.

**Data availability statement**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Figure captions

**Figure 1.** Effects of MitoTEMPO and VX765 on acute ozone exposure induced mean percentage increase in lung resistance (RL) to increasing concentrations of
acetylcholine (ACh) (A) and −loPC_{200} (B). *P<.05, **P<.01 compared to air-exposed mice; ***P<.001 compared to VX-765-treated ozone-exposed mice.

**Figure 2.** Effects of MitoTEMPO and VX765 on acute ozone exposure induced airway inflammation. (A). Effects of MitoTEMPO and VX765 on acute ozone exposure induced increased cell numbers in bronchoalveolar lavage (BAL) fluid, including total cells (TOTAL), macrophages (MAC), lymphocytes (LYM), neutrophils (NEU) and eosinophils (EOS). Each bar represents the mean ± S.E.M. (B)-(E). Effects of MitoTEMPO and VX765 on acute ozone exposure induced increased concentrations of cytokines in BAL fluid, including IL-1α (B), IL-1β (C), KC (D) and IL-6 (E). *P<.05, **P<.01, ***P<.001.

**Figure 3.** Effects of MitoTEMPO and VX765 on acute ozone exposure induced increases in malonaldehyde in BAL fluid (A) and mitochondrial ROS (B) in lung tissue. *P<.05, **P<.01, ***P<.001.

**Figure 4.** Representative Western blot images of mitochondrial OXPHOS and VDAC in lung tissue (A), (B)-(F). Effects of MitoTEMPO and VX765 on the protein expression of OXPHOS complex V (B), IV (C), III (D), II (E) and I (F) in lung tissue after acute ozone exposure. *P<.05, **P<.01.

**Figure 5.** Effects of MitoTEMPO and VX765 on the protein expression of mitochondrial fission/fusion-related proteins, including DRP1 (A), MFF (B), OPA1 (C) and MFN2 (D), in lung tissue after acute ozone exposure. Each panel shows representative Western blot. *P<.05, **P<.01.
Figure6. Effects of MitoTEMPO and VX765 on the protein expression of NLRP3 (A) and caspase-1 (B), and the activity of caspase-1 (C) in lung tissue after acute ozone exposure. Each panel shows representative Western blot. *$P<.05$, **$P<.01$. 