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N-acetylcysteine Effects on Inflammatory Markers in Animal Models Exposed to Air Pollutants

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Abstract

Introduction: *Air pollution (AP) significantly contributes to morbidity and mortality worldwide. N-acetylcysteine (NAC) is a potent antioxidant with potential health benefits to people residing in poor air quality areas. Our study aimed to analyze animal studies conducted on rats and mice to assess the anti-inflammatory effects of NAC during exposure to air pollutants.*
Methods: *A systematic search on two large databases was performed. Nineteen studies were included in this review following a screening of duplicates and a full-text review.*
Results: *We found that NAC successfully ameliorates some pollution-associated inflammatory pathways.*
Discussion: *NAC is a potential therapeutic drug against inflammatory pathologies caused by AP, although its role in human models requires further studies.*

Introduction

With the increase in air pollution (AP), understanding its impacts on human health has been gaining interest in recent years. In 2019, the World Health Organization (WHO) informed that 99% of the global population was exposed to low-quality air (WHO, 2021). Moreover, AP is highly associated with morbidity and mortality, causing 4.2 million premature deaths every year worldwide (WHO, 2021).

Individuals exposed to poor air quality experience systemic inflammation, and their levels of inflammatory markers are markedly high. The presence of endothelial microparticles is associated with AP and elevated levels of inflammation (Pope et al., 2016). Furthermore, chronic exposure to AP leads to an increase in oxidative stress, causing inflammation (Hahad et al., 2020) as indicated by an increase in the levels of inflammatory markers, such as C-reactive protein (CRP), interleukin (IL)-6, and tumor necrosis factor-alpha (TNF-α) (Germolec et al., 2018).

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Antioxidants were suggested to combat the effects of AP on the lungs, such as oxidative stress (Kelly et al., 2003). Given that N-acetylcysteine (NAC), a mucolytic agent, has attracted interest because of its antioxidant properties to reduce reactive oxygen species (ROS) levels, thereby decreasing local inflammation (Raghu et al., 2021; Zhitkovich, 2019; Carlson et al., 2018). After deacetylation, NAC protects cells against ROS by acting as a cysteine donor, replenishing glutathione in functional cells, and reducing disulfide bonds in proteins (Aldini et al., 2018). This mechanism of action explains the high antioxidant activity of NAC, despite its weak direct action against oxidizing agents (Zhitkovich, 2019). However, these antioxidant effects have not been widely studied in human cell models. Most studies on NAC effects in AP-induced injury have been conducted in animal models. These preclinical trials have elucidated different mechanisms by which NAC may exert a therapeutic role in several conditions caused or aggravated by AP. Therefore, this review aims to summarize findings from studies reporting the antioxidant effects of NAC on inflammatory markers in animal models exposed to harmful air pollutants.

Materials and Methods

We used the Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for coping

reviews (PRISMA-ScR) guidelines. The MEDLINE-PubMed and Embase databases retrieved articles related to preclinical studies of the NAC effect on inflammatory biomarkers in animals exposed to air pollutants excluding other interventions. Free-text and Medical Subject Headings (MeSH) terms were used. Eligibility criteria included studies performed in rats or mice, using at least one mechanism of exposure to air pollution contaminants, evaluating at least one inflammatory marker, and using N-acetylcysteine as an intervention. In the Supplemental document, we have attached a detailed description of the inclusion and exclusion criteria and search strategy for which multiple versions of the terms "inflammation," "N-acetylcysteine," "rat OR mice," and "air pollution" were used.

The screening process for assessing the eligibility of an article to be included in the review was independently performed by two reviewers (MH and CR). In case of disagreement, additional reviewers participated (JD and DR) in the assessment process until a consensus was reached. Finally, 78 Embase and 21 PubMed titles were identified, of which 19 were included in the review. Figure 1 shows the flow diagram of the screening process. Bias risk was assessed using the SYRCLE Risk of Bias tool, an adapted version of the Cochrane Collaboration tool for animal studies (Higgins J, 2011).

Results

Nineteen preclinical animal studies were included in the minireview. These were generally performed to test the anti-inflammatory effect of NAC. The animal models were generated using different mice and rat strains (C57BL/6 and BALB). Most studies have evaluated interleukin expression, cell viability, and receptor expression inflammation biomarkers. The airways and respiratory-related tissues were the most investigated tissues. The details of these studies are presented in Table 1.

Omara et al. (2000) investigated the effects of EHC-93 urban particles (Ottawa dust) on immune functions (cell viability, lymphocyte blastogenesis stimulated by T-cell mitogen or B-cell mitogens, intracellular concentration, IL-2 production, and expression of receptors for transferrin and IL-2) of peripheral blood mononuclear cells and splenocytes from male rats and mice. The addition of NAC completely abolished the suppressive effect of Ottawa dust on mitogen-induced lymphocyte proliferation. Other studies have used diesel exhaust particles (DEP) to mimic AP exposure. Gowdy et al. (2010) evaluated the effects of diesel emission particle (DEP) exposure throughout an influenza infection in mice. Treatment with NAC (320 mg/kg, intraperitoneal) reduced glutathione in the lungs and decreased the number of polymorphonuclear cells earlier than in untreated mice. Furthermore, in mice exposed only to DEP but not influenza, NAC treatment reduced the production of IL-4. Li et al. (2007) exposed 12 mice to low-dose DEP, and following NAC treatment, a decrease in macrophages, neutrophils, and lymphocytes was noted in bronchoalveolar lavage (BAL) fluid. Li et al. (2009) showed that when BALB/c were exposed to prolonged low-dose DEP, a markedly increased number of eosinophils and mucous goblet cells and increased IL-5 and IL-13 expression were noted. However, following treatment with NAC, these effects were mitigated.

Reiprich et al. (2013) evaluated the effects of endotoxins before ovalbumin (OVA) sensitization on the development of allergic asthma in the offspring of exposed pregnant mice. The results suggest that NAC treatment before perinatal lipopolysaccharides exposure decreases airway inflammation, eosinophils count, OVA-specific immunoglobulin E levels, and T-helper (Th2) cytokine expression.

Hu et al. (2017) found that the exposure of mice to particulate matter less than 2.5 μm (PM2.5) increased the levels of IL-6, transforming growth factor (TGF)-β, TNF-α, and macrophage infiltration in mouse lung tissue in a dose-dependent manner. However, in the NAC treatment group, no considerable changes were observed in the proinflammatory biomarkers in the blood serum. Wang et al. (2017) showed that following PM2.5 exposure, BAL fluid obtained from mice showed increased IL-1β, IL-6, IL-8, cyclooxygenase (COX)-2, and matrix metalloproteinase (MMP)-9 expression levels. Moreover, NAC pretreatment was correlated with the downregulation of inflammatory markers. Steerenberg et al. (2004) used mice with natural resistance-associated macrophage protein activity, with NAC pre-treatment, deficient inducible nitric oxide synthase, and deficient IL-4 expression, which were co-exposed to OVA and Ottawa dust. This experiment showed no differences in histological inflammation or immunoglobulin formation. These conflicting results may arise from differences in follow-up and choice of the biological sample.

Ping et al. (2019) investigated the effect of NAC on Wistar rat models. Lung injury was induced in the rats by exposure to PM2.5 via inhalation. The ones exposed to particulate matter showed destruction of the typical lung tissue architecture, increased mucus secretion, and enhanced IL-6. In contrast, the NAC group showed lower lung tissue injury mediated by reduced activation of the mitogen-activated protein kinase (MAPK) pathway. This finding seemed to be directly related to the administered NAC dose. This relationship was also addressed by Lin et al.

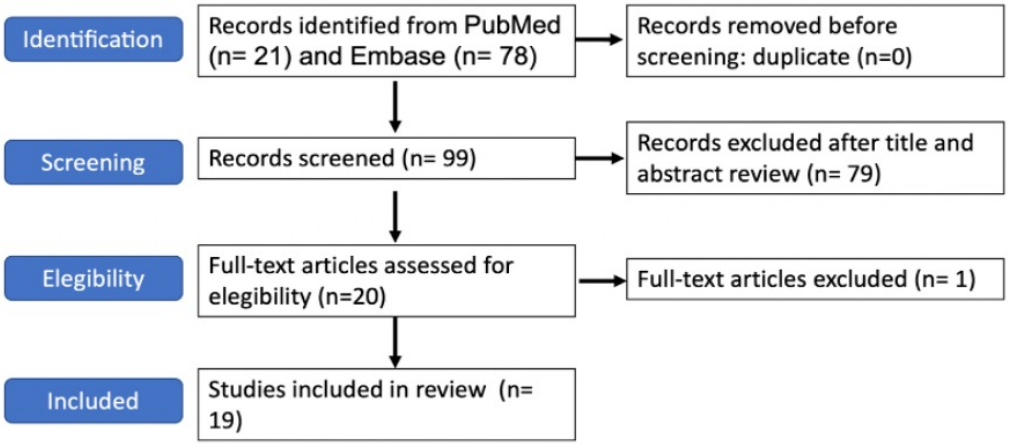

Figure 1: *Flow diagram of studies assessed for eligibility according to PRISMA criteria.*

Author (year)	Journal	Study design	Sample size	Follow up	Inflammatory marker	Type and via of pollution exposure	Biological sample
Omara et al. (2000)	Journal of toxicology and environmental health	Pre-clinical trial (*in vitro* model)	344 Male Fischer rats and C57Bl/6 mice	72 hours	Splenocytes blastogenesis, interleukin (IL)-2 levels, Ca^{2+} concentration, expression of IL-2 and transferrin receptors	EHC-93 urban particles (Ottawa dust)	Spleen
Steerenberg et al. (2004)	Inhalation toxicology	Preclinical trial (*in vivo* model)	8 mice specimens (BALB/cByJ.ico, BALB/cj, BALB/cIl4tm2Nnt, C57Bl/6, B6.129P2-Nos2tmLau, BALB/c/AnPt, and C.D2-Vil6)	42 days	Eosinophilic infiltrates, serum immunoglobulin (Ig) E and IgG	Ottawa dust. Intranasal exposure	Serum, bronchoalveolar lavage (BAL) fluid, lung tissue
Shima et al. (2006)	Toxicological Sciences	Preclinical trial	30 male BALB/c mice (six weeks old)	24 hours	Heme oxygenase-1 protein expression, inflammatory cell infiltration	Dichloromethane-soluble fraction from diesel exhaust particles (DEP) was fractionated into its n-hexane-soluble fraction and n-hexane-insoluble fraction.	Alveolar type II epithelial cell line, peritoneal lavage fluid
Li et al. (2007)	Experimental Lung Research	Preclinical trial (*in vivo* model)	12 female BALB/c and C57BL/6 mice (9 weeks old)	8 weeks	Macrophages, neutrophils, lymphocytes	Low-dose DEP. Inhalation	BAL fluid
Li et al. (2009)	Immunopharmacology and immunotoxicology	Preclinical trial	6 female mice per group of of BALB/c and C57BL/6 pregnant mice	24 weeks	Presence of goblet cells, cell differential counts, IL-1, tumor necrosis factor-alpha (TNF-α), IL-12, interferon(IFN)-γ, IL-4, IL-5, and IL-13 levels, IgE and IgG	DEP inhalation	Lung tissue, BAL fluid, serum
Gowdy et al. (2010)	Particle and fiber toxicology	Preclinical trial	19 female BALB/c mice	14 days	IL-4, IL-12, IL-13, IFN-γ	DEP inhalation	Lung tissue and BAL fluid
Reiprich et al. (2013)	Allergy	Preclinical trial	Offspring of pregnant BALB/c mice (sample size not mentioned)	6 weeks	Eosinophils, IgE, T-cell helper (Th) 2 cytokines	Mycotoxins and DEP	BAL fluid
Cui et al. (2015)	Cellular physiology and biochemistry	Preclinical trial	8 Wild-type (WT) male C57BL/6 mice	1 month	TNF-α and IL-1β	Fine particulate matter, intranasal instillation	Serum
Wang et al. (2015)	Inhalation toxicology	Preclinical trial	190 female C57BL/6J mice	28 days	IL-1β and IL-10	Sidestream cigaret smoke particulate matter	BAL fluid
Hu et al. (2017)	Environmental Toxicology and Pharmacology	Preclinical trial (*in vivo* model)	40 C57BL male mice	4 weeks	TNF-α, IL-6, and transforming growth factor (TGF)-β	Particulate matter equal or smaller than 2.5 micrometers ($PM_{2.5}$) inhalation from straw-burning	Lung tissue and BAL fluid
Wang et al. (2017)	Journal of Thoracic Disease	Preclinical trial (*in vivo and in vitro* model)	Male C57 mice (total sample size not mentioned)	2 days	IL-1β, IL-6, IL-8, cyclooxygenase 2, and matrix metalloproteinase-9	Inhalation of urban dust material SRM 1649b	Lung tissue and BAL fluid
Liu et al. (2018)	Particle and fiber toxicology	Preclinical trial (*in vivo and in vitro* model)	Male C57BL/6 WT mice and IL-6 knockout mice (sample size not mentioned)	14 days	Intercellular adhesion molecule-1 (ICAM-1) and IL-6	$PM_{2.5}$ intratracheal instillation	Lung tissue and plasma
Zhang et al. (2018)	Environmental Science and Pollution Research	Preclinical trial (*in vivo* model)	42 male Wistar rats	N/A	TNF-α, IL-6, TGF-β1, inducible nitric oxide synthase, and ICAM-1		
Dong et al. (2019)	Scientific Reports	Preclinical trial	48 male Balb/c mice aged 6–8 weeks	14 days	mRNA of IL-6, IL-18, TNF-α, TGF-β1, and reactive oxygen species HO-1		
Lee et al. (2019)	Molecular immunology	Preclinical trial (*in vivo* model)	Male BALB/c mice	72 hours	C-reactive protein, NLR family pyrin domain containing 3 (NLRP3) inflammasome, apoptosis-associated speck-like protein containing a CARD protein expression, IL-1β	$PM_{2.5}$ intratracheal instillation	Heart tissue
Ping et al. (2019)	Chinese medical sciences journal	Preclinical trial	48 male Wistar rats	4 weeks	IL-6	$PM_{2.5}$ intratracheal instillation	Serum, BAL fluid
Wang C. et al. (2020)	Biomedicine & pharmacotherapy	Preclinical trial (*in vivo* model)	24 male C57BL/6 mice	7 days	NLRP3, caspase-1, IL-1β, IL-18, IL-6 and TNF-α		
Wang J. et al. (2020)	Biochemical and biophysical research communications	Preclinical trial (*in vivo* model)	48 female AR rats	28 days	Th1/Th2-related serum cytokines	$PM_{2.5}$ intratracheal instillation	Lung tissue
Lin et al. (2022)	Ecotoxicology and environmental safety	Preclinical trial (*in vivo* model)	Male C57BL/6 mice (Sample size not mentioned)	7 days	Vascular endothelial growth factor, IL-6, and TNF-α	$PM_{2.5}$ intratracheal instillation	Lung tissue

Table 1: *Descriptive characteristics of all selected studies.*

(2022), who concluded that treatment with NAC via intratracheal spraying substantially attenuated the recruitment of neutrophils and Ly6C monocytes into the lung alveoli in PM2.5-exposed mice in a dose-dependent manner.

Similarly, Wang et al. (2020) investigated ROS-induced lung injury after exposure to PM2.5 in vivo. They found that NAC treatment could attenuate the accumulation of inflammatory cells, the thickening of the alveolar walls, and the degree of lung injury via the NOD-, LRR-, and pyrin domain-containing protein three pathways. Liu et al. (2018) also reviewed the effect of particulate matter on the respiratory system, finding that this exposure produced a marked increase in ROS and intercellular adhesion molecule-1 (ICAM-1) levels, which IL-6 mediated via the IL-6/AKT/STAT3/NF activation pathway. Furthermore, they found a reduction of these markers in cultures primed with NAC before exposure.

Zhang et al. (2018) found increased neutrophil counts and oxidative stress marker HO-1 levels in mouse lung tissues exposed to fine chalk dust. Moreover, TNF-α, IL-6, TGF-β1, inducible nitric oxide synthase, and (ICAM)-1 mRNA transcripts were elevated. On the other hand, NAC exposure in the same mouse population decreased the levels of inflammatory markers, which was probably mediated by decreased p38 levels and extracellular regulated proteinase (ERK) and MAPK signaling mechanisms. Finally, Dong et al. found a relationship between exposure to PM2.5 and cardiovascular morbidity and mortality. Using these data, they investigated the histopathological effects of various inflammatory markers in the heart tissues of mice intratracheally exposed to particulate matter. They reported an increase in ROS and intracellular free calcium levels. They also mentioned that NAC treatment with essential oils in mice markedly reduced these inflammatory markers. These findings suggest that NAC treatment could tackle other organs not directly exposed to inspired poor-quality air.

Discussion

Since the approval of NAC for use in humans in 1963 by the Food and Drug Administration, it has highly contributed to human research involving respiratory conditions such as asthma, pneumonia, tracheobronchitis, and cystic fibrosis (WHO, 2019; Hu et al., 2017). This can be attributed to its multiple mechanisms of action, including its antioxidant and anti-inflammatory effects mediated by a downregulation of ROS expression and its mucolytic capacity (Zhitkovich, 2019; Carlson et al., 2018). These characteristics have led to the evaluation of NAC, mainly in animal models, as a potential modulator agent for treating AP effects (Arias-Pérez et al., 2020).

The principal finding of this review is that NAC is efficacious in reducing, to some extent, the inflammatory effects of AP in animal models. Inflammation measurement was done generally through the levels of IL-6, TNF-α, and the presence of inflammatory cells. This beneficial effect has been tested in various respiratory conditions (e.g., influenza infection, emphysema, and lung fibrosis) and PM2.5-induced acute heart injury. In AP-induced lung inflammation, recent studies showed that NAC inhibits two critical mechanisms by which air pollutants induce an inflammatory response, the ROS-mediated activation of MAPK and nuclear factor kappa B (NF-kB) signaling pathways (Wang et al., 2017; Ping et al., 2019). Regarding the regulation of pro-inflammatory cytokines, most studies assessed the BAL fluid and lung tissue and found that NAC decreased the levels of IL-1β, IL-6, and TNF-α (Wang et al., 2015; Wang et al., 2017; Zhang et al., 2018; Ping et al., 2019; Wang C. et al., 2020; Wang J. et al., 2020). NAC was also beneficial in downregulating ICAM-1 expression (Liu et al., 2018). The anti-inflammatory effects of NAC measured by TGF-β, IL-6, and TNF-α not specified by pathways were seen in mice BAL fluid, even after four weeks, along with increased survival (Hu et al., 2017).

To our knowledge, this review is the first to summarize the current evidence on the association between NAC and inflammatory markers in animal models exposed to AP. The strengths of our study are as follows: 1) comprehensive search of Medline and Embase databases, with no language or publication date filters, and 2) low variability found in the inflammatory markers and biological samples used in the included studies. Although very promising, the animal model design, nature of the studies, overall poor reporting of methodological details, and the heterogeneity in the exposure type and intervention (dosage, presentation, route of administration) exhort cautionary conclusions. Furthermore, because inflammatory markers can have intercorrelated chain reactions, the individual effect of NAC can diverge among diseases with significant oxidant/inflammatory repercussions. Last, the preclinical literature on animal models does not allow for generalization to human populations but calls for studies to confirm the mechanisms presented here.

Conclusions

In summary, NAC is a potential therapeutic drug against inflammatory pathologies caused by AP, as shown in preclinical animal models. However, due to heterogeneity in inflammation surrogate biomarkers measurements, the diverse constituents present

in polluted air, and the degree of the AP impact that is attributed to respiratory and cardiovascular diseases, to discover whether NAC can produce considerable anti-inflammatory responses in humans, further studies are required, as justified by the compelling findings of our current review. Therefore, using other biomarkers such as CRP, ESR, free iron or homocysteine, and comparison with placebo in settings with AP are encouraged.

Author Contributions

Conceptualization, Maria Hernandez, Cristal Roman, Daniel Romero, Francisca Jaime, Pragati Sureka, Juan Delgado, Richard Vaca, Donghyun Ko.; methodology, Maria Hernandez.; software, Maria Hernandez.; validation, Maria Simon, Erica Stelmaszewski, Lucero Flores; formal analysis, Maria Hernandez, Cristal Roman, Daniel Romero, Francisca Jaime, Pragati Sureka, Juan Delgado, Richard Vaca, Donghyun Ko.; investigation, Daniel Romero, Cristal Roman, Maria Hernandez.; resources, Maria Hernandez, Cristal Roman, Daniel Romero, Francisca Jaime, Pragati Sureka, Juan Delgado, Richard Vaca, Donghyun Ko.; data curation, Maria Hernandez, Cristal Roman, Daniel Romero, Francisca Jaime, Pragati Sureka, Juan-Rene Delgado-Cornejo, Richard Vaca, Donghyun Ko.; writing—original draft preparation, Maria Hernandez, Cristal Roman, Daniel Romero, Francisca Jaime, Pragati Sureka, Juan Delgado, Richard Vaca, Donghyun Ko.; writing—review and editing, Maria Hernandez, Daniel Romero, Cristal Roman, Francisca Jaime, Lucero Flores, Erica Stelmaszewski.; visualization, Juan Delgado.; supervision, Maria Simon, Erica Stelmaszewski, Lucero Flores.; project administration, Maria Hernandez, Cristal Roman, Daniel Romero, Francisca Jaime, Pragati Sureka, Juan Delgado, Richard Vaca, Donghyun Ko.; funding acquisition, NA. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

Daniel Romero and Cristal Roman are employed at pharmaceutical companies. All other authors declare no conflict of interest.

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