PRINCIPLES AND PRACTICE OF CLINICAL RESEARCH
A Global Journal of Clinical Research

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

Maria Jose Hernandez Woodbine[1], Daniel Romero[1], Cristal Roman[1], Juan Rene Delgado Cornejo[1], Richard Vaca[1], Francisca Jaime[1], Donghyun Ko[1], Pragati Sureka[1], Erica Victoria Stelmaszewski[1], Maria Simon[1], Lucero Flores[1*]

[1]Principles and Practice of Clinical Research, Harvard T.H. Chan School of Public Health.

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).

*Corresponding author: luceximena@gmail.com
Daniel Romero, Cristal Roman, Juan Rene Delgado Cornejo, Richard Vaca, Francisca Jaime, Donghyun Ko, and Pragati Sureka have contributed equally to this work.
Received: November 3, 2022 **Accepted:** December 23, 2022
Published: July 10, 2023
Editor: Felipe Fregni **Reviewers:** Caio Kasai, Messiel Mendez, Celso Vespasiano, Jorge Sakon
Keywords: acetylcysteine, inflammation, air pollution
DOI: http://dx.doi.org/10.21801/ppcrj.2023.91.9

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

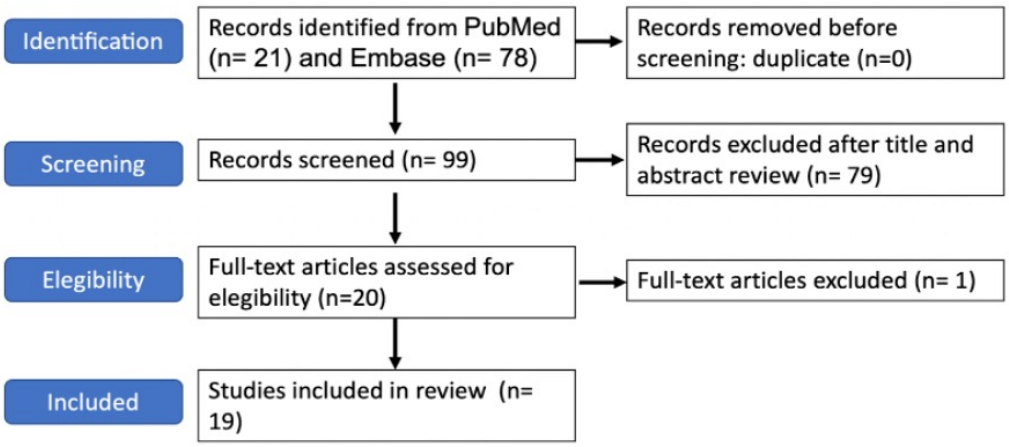

Figure 1: *Flow diagram of studies assessed for eligibility according to PRISMA criteria.*

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

First author (year)	Population and disease	Study Characteristics	Primary Outcome Measure (scale)	Experimental intervention (preparation, type, dose, and duration)	Control intervention (type, dose, and duration)	Main outcome results	Adverse effects (curcumin vs control)	Author's conclusions
Agarwal et al. (2011)	n=50 f=41, m=9 mean age 38.44 ± 12.7 years. Laparoscopic cholecystectomy	RCT, two arms, single-blinded, single center	Post-operative pain (VAS)	Curcumin 500mg 4x/day 3 weeks	Matching Placebo 500 mg 4x/day 3 weeks	VAS change CR 5±5.204; PL 30 ±13; p=0.000	No	CR improves postoperative pain
Al-Askar et al, (2022)	n=91 f=54, m=37 CR 58.4 ± 7.3; PL 57.2 ± 5.2 years Surgical periodontal therapy	RCT, two arms, single-blinded, single center	Post-operative pain (NRS)	Curcumin 400 mg 3x/day 3 days	Mefenamic acid 500 mg 3x/day 3 days	NRS No statistically significant difference	NS	Compared with MA, curcumin is ineffective for pain and discomfort management after SPT.
Asadi et al. (2019)	n=80 age 30-60 years old Non-insulin-dependent Diabetes Mellitus	RCT, double-blinded, placebo- controlled, single center	Sensorimotor (TCNS)	Nano-curcumin capsules 80 mg 1x/day 8 weeks	Placebo 1x/day 8 weeks	Neuropathy score (TCNS) CR −2.07(2.1); PL −0.60(1.5) p=0.3	2 cases of GI side effects	Improvement and reduction of severity of DSPN
Brinkhaus et al. (2005)	n=106 age 48±12 years IBS	RCT, triple arms, double-blinded, parallel, placebo -controlled, single center	IBS-related pain (VAS)	Curcuma xanthorrhiza 60 mg 3x/day 18 weeks	Placebo 3x/day or Fumitory 500mg 3x/day 18 weeks	VAS change CR +2.0±9.5; PL −0.3±9.9; Fumitory −0.9±11.5; p=0.81	No significant difference between arms regarding tolerability	No significant differences between groups
Gomes et al. (2021)	n=24 64±11.22 OA	RCT, triple arm, parallel, open-label, group trial, single center	VAS and WOMAC	Curcuma longa 500 mg 2x/day, 30 days	Miconia Albicans 500mg 2x/day Ibuprofen 1200mg/day	VAS reduction day 0: 7.25; day 30: 3.88; p=0.002	NS	Herbal medicine can interfere in the pain and function of patients with knee osteoarthritis
Hesami et al. (2021)	n=128 women CR: 22.11±2.09; PL: 23.19± 1.99; CR+ Mefenamic acid: 22.37 ± 2.41 Mefenamic acid: 23.01 ± 3.02 Healthy women	RCT, double-blinded, 2X2 factorial design	Dysmenorrhea (VAS)	Curcumin 500 mg Curcumin+ Mefenamic acid 500/250 mg Mefanic acid 250 mg 1x/day 5 days	Placebo 500mg 1x/day 5 days	VAS change CR 7.14±0.63 to 5.67±0.8; CR+ Mefenamic acid 7.35±0.75 to 4.86±0.1; Mefenamic acid 7.8±0.92 to 6.14±0.19 p=0.0392	NS	CR decrease pain, combination is more effective
Kia et al. (2021)	n=50 age 55.96±1.10 Post-radiation with/without head/ neck cancer	RCT, double-blinded, placebo-controlled, single center	Oral mucositis (NRS)	Nano-micelle curcumin capsules, 80 mg 2x/day 7 weeks	Placebo 2x/day 7 weeks	NRS difference CR 5.28 ± 0.75; PL 6.16 ± 2.13 p≤ 0.001	NS	CR is effective in preventing radiotherapy- induced OM and decrease severity in pain compared to placebo
Kia et al. (2020)	n=57 CR 51.86±9.94; PL 53.67±8.90 years OLP	RCT, two arms, double-blinded, parallel, placebo-controlled, single center	OLP related pain (VAS)	Nano-curcumin capsule 80 mg 1x/day 4 weeks	Prednisolone 10 mg 1x/day 4 weeks	VAS change CR 2.69±2.89; PL 2.33±2.03 p≤ 0.001	NS	No significant differences between groups
Kuptniratsaikul et al. (2009)	n=107, CR 61.4±8.7; PL 60.0±8.4 years OA	RCT, two-arms single center	Knee pain on walking and on stairs (numerical rating scale)	Curcuma extracts 500mg 4x/day 6 weeks	Ibuprofen 400 mg 2x/day 6 weeks	Pain on level walking CR 2.7±2.6; IB 2.0±2.3; p=0.2 Pain on stairs CR 2.5±2.2; IB 2.5±2.60; p=0.92	No significant difference between arms, both GI tract mild AE	CR noninferior to IB in efficacy and safety for the treatment of knee OA
Kuptniratsaikul et al. (2014)	n=367 CR 60.3±6.8; IB 60.9±6.9 years OA	RCT, two-arms, double-blinded, active controlled, multicenter	Knee pain (WOMAC pain subscale)	Ethanolic extracts of turmeric - curcuminoids 75- 85% 1500 mg 1x/day 4 weeks	Ibuprofen 1200 mg/day 4 weeks	WOMAC pain CR 3.17±1.98; IB 3.25±2.11; p=0.018	Abdominal distention significantly higher in IB arm	CR noninferior to IB regarding pain control and functionality, w/ fewer GI side-effects for CR
Lopresti et al. (2022)	n=101 CR 59.59±0.92; PL 57.92±0.88 years OA	RCT, two-arms, double-blind, placebo- controlled, single-center	Knee pain (KOOS pain subscale)	Curcuminoids extract - curcuminoids 50% (Curcugen®) 500mg 2x/day 8 weeks	Placebo 2x/day 8 weeks	KOOS pain change CR 11.98 (7.38-16.59); PL 5.52 (0.75-10.28); p=0.009	No significant difference between arms	CR effective for pain compared to PL, but no changes in functionality scores
Madhu et al. (2013)	n=120 PL 56.77±9.98; CR 56.63±10.58 years OA	RCT, single-blind, parallel, placebo- controlled, single-center	Knee pain (VAS)	NR-INF-02 (extract from Curcuma longa Turmacin™) 500mg 2x/day 42 days	Placebo 400 mg 2x/day 42 days	VAS change CR 19.48±17.84; PL 46.03±20.84; p < 0.01	6.6% reported AE, all of them being dyspepsia	CR showed a statistically significant decrease in knee OA pain
Maulina et al. (2018)	n = 90 f=46, m=44 age 18-40 years Post-surgical removal of molars	RCT, double-blinded, parallel, single center	Oral pain (NRS)	500 mg amoxicillin + 200 mg curcumin capsule, 3x/day 24 hours	500 mg amoxicillin + 500 mg mefenamic acid 3x/day 24 hours	NRS reduction: 1st CR -2.31, PL -1.49; 2nd CR -4.11, PL -2.98; 3rd CR -5.87, PL -4.53; p=0.001	NS	Intervention group significantly less pain as control group
Nicol et al. (2015)	n=19 age 18–39 years Healthy men	RCT, double-blind randomized-controlled unilateral crossover trial, single center	Muscle Soreness (VAS)	Curcumin 2.5g 2x/day for 2.5 days prior to exercise, then 5 capsules 2x/day for 2.5 days after exercise	Placebo 2x/day 2.5 days prior to exercise, then 5 capsules 2x/day for 2.5 days after exercise	Single-leg Post-exercise baseline 0.9 ± 1.0; 24 h-baseline −0.5 ± 1.0; 48 h-baseline −0.8 ± 1.2; 24h post exercise −1.4 ± 1.0; 48 h-post-exercise −1.7 ± 1.0	No	CR supplementation prior to and following heavy eccentric exercise in healthy men lowered subsequent pain
Panda et al. (2018)	n=50 age 40-75 years OA	RCT, double- blinded, placebo-controlled, single center	Knee pain (WOMAC total score)	Curcuma extracts (Curene®) 500mg 1x/day	Placebo 1x/day	WOMAC pain CR -19.44±3.74; PL -6.6±3.66; p<0.05	No	Therapeutic efficacy and safety of CR over PL in the management of symptoms of OA
Raj et al. (2020)	n=90 Knee joint pain	RCT, triple arm, double-blind, placebo- controlled, single center	Knee pain (VAS)	Turmacin 1g 2x/day or 0.5g 2x/day 12 weeks	Placebo 2x/day 12 weeks	Pain scores CR 1g compared to PL -1.33±2.25; p=0.004; CR 0.5g vs PL -1.47±1.97; p<0.001	Heartburn in 4/90 (¾ in the PL group and ¼ in CR 1 g group)	CR (0.5 and 1 g) effective when compared to PL in increasing the pain threshold and knee ROM in healthy participants
Shep et al. (2019)	n=149 CR 53.09±4.17; DI 72.14±3.76 years OA	RCT, two-arms, open-labeled, active controlled, single center	Knee pain (VAS)	Curcumin BCM-95® - curcuminoids 95% 500mg 3x/day 28 days	Diclofenac sodium 50mg 2x/day 28 days	VAS change CR -5.93±0.99; p<0.01; DI -5.61±0.88; p<0.01	Fewer GI side effects CR group	CR similar to DI regarding pain relief, but better safety profile
Singhal et al. (2021)	n=144 f=107, m=37 CR 53.1 (10.9); PA: 50.8 (9.9) OA	RCT, two arms, single center, non-inferiority	Knee pain (WOMAC pain)	turmeric extract 500mg 2x/day 6 weeks	Paracetamol 650 mg 3x/day 6 weeks	WOMAC pain p=0.00004	5.48% (restlessness (4.11%), tingling sensation (1.37%))	CR noninferior to paracetamol in improving the physical function and alleviating pain and stiffness of OA
Thanawala et al. (2021)	n = 106 CR = 53 PL = 53 OA	RCT, double- blinded, placebo-controlled, multicenter	Knee pain (VAS)	WDTE60N (water-dispersible turmeric extract): 250mg/day, 90 days	Placebo capsules	VAS reduction CR -1.5±0.7; PL -0.6±0.8; p=0.0001	NS	CR capable of alleviating chronic knee pain.
Wang et al. (2020)	n=70 f=18, m=52 mean age 61.5 years SD CL 8.5 SD PL 8.8 OA	RCT, two arms, single center	Knee pain (VAS)	Curcuma extract 2 x 500-mg capsules per day, 12 weeks	Placebo 2x/day 12 weeks	VAS change CL -23 (-29.8 to -17.7) CR -14.6 (-20.8 to -8.5) p=0.039	No	Curcuma was more effective than placebo for knee pain but did not affect knee effusion-synovitis.

RCT, randomized controlled trial; OA, osteoarthritis; CR, curcumin; DI, diclofenac sodium; PA, paracetamol; IB, ibuprofen; KOOS, knee injury and osteoarthritis outcome score; VAS, visual analog scale; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index; GI, gastro-intestinal; NRS, numeric rating scale, NS not stated, ORL oral lichen planus, RA, Rheumatoid Arthritis; IBS, Irritable bowel syndrome; AE, Adverse effect; m, male; f, female

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

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. (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.

Funding

This research received no external funding.

Acknowledgments

We wish to acknowledge all the professors and staff of the Principles and Practice of Clinical Research Course 2022.

Conflicts of Interest

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

References

- Aldini, G., Altomare, A., Baron, G., Vistoli, G., Carini, M., Borsani, L., & Sergio, F. (2018). N-Acetylcysteine as an antioxidant and disulphide breaking agent: the reasons why. Free radical research, 52(7), 751-762.
- Arias-Pérez, R. D., Taborda, N. A., Gómez, D. M., Narvaez, J. F., Porras, J., & Hernandez, J. C. (2020). Inflammatory effects of particulate matter air pollution. Environmental science and pollution research international, 27(34), 42390–42404. https://doi.org/10.1007/s11356-020-10574-w
- Carlson, T. L., Lock, J. Y., & Carrier, R. L. (2018). Engineering the mucus barrier. Annual review of biomedical engineering, 20, 197.
- Cui, Y., Xie, X., Jia, F., He, J., Li, Z., Fu, M., Hao, H., Liu, Y., Liu, J. Z., Cowan, P. J., Zhu, H., Sun, Q., & Liu, Z. (2015). Ambient fine particulate

matter induces apoptosis of endothelial progenitor cells through reactive oxygen species formation. Cellular physiology and biochemistry: international journal of experimental cellular physiology, biochemistry, and pharmacology, 35(1), 353–363. https://doi.org/10.1159/00036970

- Dong, L., Sun, W., Li, F., Shi, M., Meng, X., Wang, C., ... & Song, L. (2019). The harmful effects of acute PM2. 5 exposure to the heart and a novel preventive and therapeutic function of CEOs. Scientific reports, 9(1), 1-12.
- Germolec, D. R., Shipkowski, K. A., Frawley, R. P., & Evans, E. (2018). Markers of inflammation. Immunotoxicity Testing, 57-79.
- Gowdy, K. M., Krantz, Q. T., King, C., Boykin, E., Jaspers, I., Linak, W. P., & Gilmour, M. I. (2010). Role of oxidative stress on diesel-enhanced influenza infection in mice. Particle and fibre toxicology, 7, 34. https://doi.org/10.1186/1743-8977-7-34
- Hahad, O., Lelieveld, J., Birklein, F., Lieb, K., Daiber, A., & Münzel, T. (2020). Ambient air pollution increases the risk of cerebrovascular and neuropsychiatric disorders through induction of inflammation and oxidative stress. International journal of molecular sciences, 21(12), 4306.
- Higgins, J. P., Altman, D. G., Gøtzsche, P. C., Jüni, P., Moher, D., Oxman, A. D., ... & Sterne, J. A. (2011). The Cochrane Collaboration's tool for assessing risk of bias in randomized trials. Bmj, 343.
- Hu, Y., Wang, L. S., Li, Y., Li, Q. H., Li, C. L., Chen, J. M., ... & Li, H. P. (2017). Effects of particulate matter from straw burning on lung fibrosis in mice. Environmental Toxicology and Pharmacology, 56, 249-258.
- Kelly, F. J., Dunster, C., & Mudway, I. (2003). Air pollution and the elderly: oxidant/antioxidant issues worth consideration. European Respiratory Journal, 21(40 suppl), 70s-75s. item Aldini, G., Altomare, A., Baron, G., Vistoli, G., Carini, M., Borsani, L., & Sergio, F. (2018). N-Acetylcysteine as an antioxidant and disulphide breaking agent: the reasons why. Free radical research, 52(7), 751-762.
- Lee, C. C., Cheng, Y. W., & Kang, J. J. (2005). Motorcycle exhaust particles induce IL-8 production through NF-kappaB activation in human airway epithelial cells. Journal of toxicology and environmental health. Part A, 68(17-18), 1537–1555. https://doi.org/10.1080/15287390590967496
- Li, Y. J., Kawada, T., Matsumoto, A., Azuma, A., Kudoh, S., Takizawa, H., & Sugawara, I. (2007). Airway inflammatory responses to oxidative stress induced by low-dose diesel exhaust particle exposure differ between mouse strains. Experimental Lung Research, 33(5), 227-244.
- Li, Y. J., Takizawa, H., Azuma, A., Kohyama, T., Yamauchi, Y., Kawada, T., ... & Sugawara, I. (2009). The effects of oxidative stress induced by prolonged low-dose diesel exhaust particle exposure on the generation of allergic airway inflammation differ between BALB/c and C57BL/6 mice. Immunopharmacology and immunotoxicology, 31(2), 230-237.
- Lin, C. M., Huang, T. H., Chi, M. C., Guo, S. E., Lee, C. W., Hwang, S. L., & Shi, C. S. (2022). N-acetylcysteine alleviates fine particulate matter (PM2. 5)-induced lung injury by attenuation of ROS-mediated recruitment of neutrophils and Ly6Chigh monocytes and lung inflammation. Ecotoxicology and Environmental Safety, 239, 113632.
- Liu, C. W., Lee, T. L., Chen, Y. C., Liang, C. J., Wang, S. H., Lue, J. H., ... & Chen, Y. L. (2018). PM2. 5-induced oxidative stress increases intercellular adhesion molecule-1 expression in lung epithelial cells through the IL-6/AKT/STAT3/NF-κB-dependent pathway. Particle and fibre toxicology, 15(1), 1-16.
- Omara F.O, Michel Fournier, Renaud Vincent, Barry R. Blakley (2000) Suppression Of Rat And Mouse Lymphocyte Function By Urban Air Particulates (Ottawa Dust) Is Reversed By N-Acetylcysteine, Journal of Toxicology and Environmental Health, Part A: Current Issues, 59:2, 67-85, DOI:10.1080/009841000156989. http://dx.doi.org/10.1080/009841000156989
- Ping, F., Cao, Q., Lin, H., & Han, S. (2019). Antagonistic Effects of N-acetylcysteine on Mitogen-activated Protein Kinase Pathway Activation, Oxidative Stress and Inflammatory Responses in Rats with PM2. 5 Induced Lung Injuries. Chinese Medical Sciences Journal, 34(4), 270-276.
- Pope III, C. A., Bhatnagar, A., McCracken, J. P., Abplanalp, W., Conklin, D. J., & O'Toole, T. (2016). Exposure to fine particulate air pollution is associated with endothelial injury and systemic inflammation. Circulation research, 119(11), 1204-1214.
- Raghu, G., Berk, M., Campochiaro, P. A., Jaeschke, H., Marenzi, G., Richeldi, L., ... & Calverley, P. M. (2021). The multifaceted therapeutic role of N-Acetylcysteine (NAC) in disorders characterized by oxidative stress. Current Neuropharmacology, 19(8), 1202.
- Reiprich, M., Rudzok, S., Schütze, N., Simon, J. C., Lehmann, I., Trump, S., & Polte, T. (2013). Inhibition of endotoxin-induced perinatal asthma protection by pollutants in an experimental

mouse model. Allergy, 68(4), 481-489.
- Steerenberg, P. A., Withagen, C. E. T., Dalen, W. J., Dormans, J. A. M. A., & Loveren, H. (2004). Adjuvant activity of ambient particulate matter in macrophage activity-suppressed, N-acetylcysteine-treated, iNOS-and IL-4-deficient mice. Inhalation toxicology, 16(13), 835-843.
- Wang, C., Meng, X., Meng, M., Shi, M., Sun, W., Li, X., Zhang, X., Liu, R., Fu, Y., & Song, L. (2020). Oxidative stress activates the TRPM2-Ca2+-NLRP3 axis to promote PM2.5-induced lung injury of mice. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie, 130, 110481.
- Wang, J., Huang, J., Wang, L., Chen, C., Yang, D., Jin, M., ... & Song, Y. (2017). Urban particulate matter triggers lung inflammation via the ROS-MAPK-NF-κB signaling pathway. Journal of thoracic disease, 9(11), 4398.
- Wang, J., Guo, Z., Zhang, R., Han, Z., Huang, Y., Deng, C., ... & Zhuang, G. (2020). Effects of N-acetylcysteine on oxidative stress and inflammation reactions in a rat model of allergic rhinitis after PM2. 5 exposure. Biochemical and Biophysical Research Communications, 533(3), 275-281.
- Wang, L. S., Hu, Y., Li, C. L., Li, Y., Wei, Y. R., Yin, Z. F., ... & Li, H. P. (2015). N-acetylcysteine attenuates cigaret smoke-induced pulmonary exacerbation in a mouse model of emphysema. Inhalation Toxicology, 27(14), 802-809.
- World Health Organization. (2022, April 4). Billions of people still breathe unhealthy air: new WHO data. Retrieved from https://www.who.int/news/item/04-04-2022-billions-of-people-still-breathe-unhealthy-air-new-who-data
- Zhang, Y., Yang, Z., Chen, Y., Li, R., Geng, H., Dong, W., ... & Dong, C. (2018). Fine chalk dust induces inflammatory response via p38 and ERK MAPK pathway in rat lung. Environmental Science and Pollution Research, 25(2), 1742-1751.
- Zhitkovich, A. (2019). N-acetylcysteine: antioxidant, aldehyde scavenger, and more. Chemical research in toxicology, 32(7), 1318-1319.