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REVIEW

Impact of N-Acetylcysteine on Mucus Hypersecretion in the Airways: A Systematic Review

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Abstract: Mucus clearance is crucial for airway protection, and its dysfunction leads to chronic obstructive pulmonary disease (COPD) characterized by mucus hypersecretion (MHS) and impaired clearance. MUC5AC and MUC5B mucin proteins are key components of airway mucus, with MUC5AC being particularly responsive to environmental stimuli, making it a potential COPD biomarker. N-acetylcysteine (NAC) is a mucolytic agent with known effects on mucus viscosity and clearance, but its precise mechanisms in COPD remain unclear. This systematic review evaluated the impact of NAC on MHS in the airways, reporting significant inhibitory effects on MUC5AC and MUC5B gene and protein expression, as well as a reduction in the number of goblet cells. NAC has demonstrated efficacy in vitro and in animal models of MHS, including COPD models, but data on human bronchial tissue are lacking. This systematic review suggests that NAC acts as a mucolytic and a mucoregulator, directly inhibiting mucus secretion and goblet cell hyperplasia. Given the critical role of MHS in COPD progression, exacerbations, and mortality, these findings highlight the potential of NAC as a targeted therapy for hypersecretion COPD phenotypes. However, further studies are needed to confirm the results of this systematic review, even in human bronchial tissue, to provide translatable evidence in clinical settings. Understanding the intimate mechanism of NAC *versus* MHS regulation may pave the way for more effective treatments targeting airway mucus dysfunction in COPD, ultimately improving patient outcomes and reducing morbidity and mortality associated with chronic mucus hypersecretion.

Keywords: COPD, mucus hypersecretion, N-acetylcysteine, systematic review

Introduction

The human respiratory system is characterized by a mucus clearance system that shields airway surfaces from the deleterious effects of inhaled substances.[1] This intricate system effectively captures deposited materials, preventing their accumulation and facilitating their subsequent elimination from the lungs.[1] Airway mucus acts as a native immune system of the lung to trap particulate matter (PM) and pathogens, allowing them to clear from the lung via coughing and ciliary transport.[1]

Mucus, characterized by adhesive and viscoelastic properties, plays a crucial role in lung and airway defense.[2,3] This gel layer traps and eliminates bacteria inhibits bacterial growth and biofilm formation, and protects against inhaled irritants and fluid loss. The mucus secretion clearance process serves as a vital defense mechanism for the lungs, entailing the trapping of pathogens and particles, followed by their removal through coordinated actions involving airflow and ciliary hairs.[4,5] Under normal conditions, mucus facilitates bacterial motility and the diffusion of quorum factors necessary for biofilm formation away from the airways.[6] Goblet, mucous, and serous cells secrete mucus, responding to signals, such as inflammatory stimuli.[7,8] Impaired mucus clearance leads to abnormalities in lung function. Therefore, precise regulation of airway mucin production is essential in managing chronic inflammatory airway diseases.

Mucus dysfunction, a central pathology in patients with chronic obstructive pulmonary disease (COPD), is characterized by excess mucus production, hypersecretion, and reduced clearance, leading to the formation of mucus plugs within the

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airways.[1,9,10] The altered mucus dynamics are fundamental to the pathophysiology of COPD, highlighting the relevance of addressing mucus-related abnormalities for a comprehensive understanding and targeted management of this chronic respiratory disorder.[1,9,10]

The mucin protein comprises oligosaccharide side chains, and the elongated glycoproteins linearly polymerized to form an entangled network-like secondary structure.[1] In sputum, a secondary polymer network comprises neutrophil-derived DNA and cell-wall-associated filamentous actin.[1] Two predominant gel-forming mucins, namely MUC5AC and MUC5B, play a central role in the airway mucus composition.[11] While MUC5AC plays a crucial role in various physiological functions, encompassing lubrication and hydration,[12,13] MUC5B facilitates mucociliary clearance.[11,14]

Notably, MUC5AC exhibits responsiveness to various environmental stresses or infectious agents, indicating its involvement in adaptive responses to external factors in the pulmonary microenvironment.[11,14] Previous investigations in chronic bronchitis indicated that sputum MUC5AC concentrations were relatively low in baseline healthy conditions.[1,14,15] However, these concentrations exhibited an altered increase in individuals with COPD compared with MUC5B concentrations.[11] Moreover, MUC5AC was identified as a more sensitive indicator of early airway damage induced by cigarette smoke, suggesting that MUC5AC may be a potential biomarker to identify smokers at risk of developing COPD.[11]

N-acetylcysteine (NAC) is a mucolytic agent with multifaceted effects, discovered in the early 1960s for chronic lung diseases.[16] It not only lyses sputum DNA and increases the thickness of airway surface liquid but also promotes airway clearance. Several possible mechanisms for the mucolytic activity of NAC have been proposed, such as the hydrolysis of disulfide bonds within mucin, disruption of oligomers, and reduction in mucin viscosity. Additionally, NAC can also inhibit mucus secretion, cellular hyperplasia, and MUC5AC expression.[17] Interestingly, NAC also has antioxidant and anti-inflammatory effects.[18] From a clinical point of view, NAC is effective in reducing the risk of COPD exacerbation.[19]

Chronic mucus hypersecretion (CMH) has a relevant role in the pathogenesis and progression of COPD, and there is growing interest in the current international recommendation for the management of CMH with mucolytic agents in patients with COPD.[20] However, despite the known benefits of NAC in clinical practice, there is a lack of systematic evaluation of its specific impact on CMH in COPD patients. A clear understanding of the efficacy of NAC in reducing mucus hypersecretion (MHS) remains to be established, which highlights the need for further research.

Therefore, in order to address this gap, the aim of this study was to perform a comprehensive systematic review to evaluate the impact of NAC against MHS in the airways.

Materials and Methods

Review Question

This systematic review aimed to evaluate the effect of NAC on MHS in the airways.

Search Strategy

This systematic review was performed in agreement with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMA-P).[21] The flow diagram is shown in Figure 1. This study met all the items recommended by the PRISMA-P checklist.[22]

As previously described,[23] the literature search strategy was developed using the PICO (problem, intervention, comparator, and outcome) framework. Specifically, the "problem" was stimulus-induced hypersecretion; the "exposure" involved NAC; the "comparator" included negative control; and the "outcomes" were MUC5AC, MUC5B, goblet cells, and mucus clearance.

A comprehensive literature search was performed to find original studies written in English investigating the effect of NAC on MHS in the airways. The search was conducted in the MEDLINE and SCOPUS databases to identify relevant studies available with no limit up to 8 November 2023.

The research terms were "MUC5AC", "N-acetylcysteine", "respiratory", "bronchi", "airways". The string used for the search in MEDLINE was as follows: "MUC5AC AND N-acetylcysteine AND (respiratory OR bronchi OR airways)"; the string used for the search in SCOPUS was as follows: "TITLE-ABS-KEY (MUC5AC AND n-acetylcysteine AND (respiratory OR bronchi OR airways))".

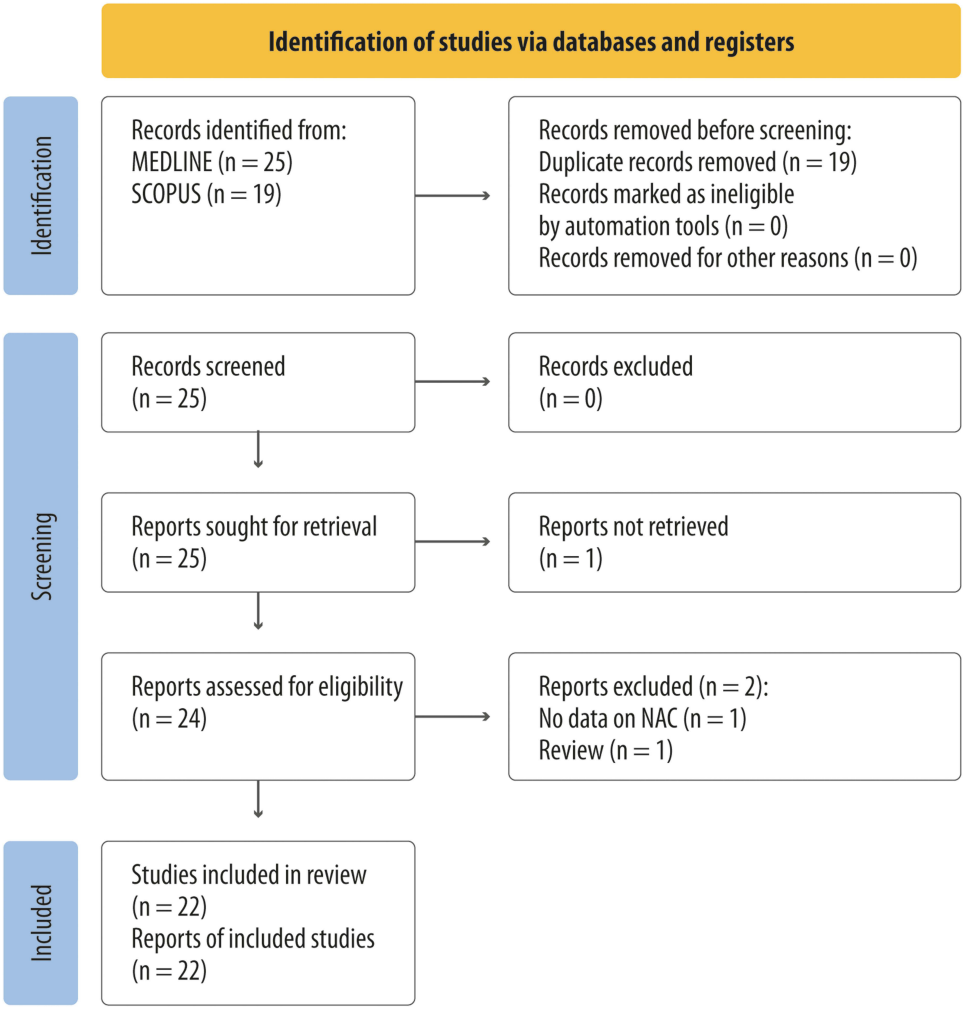

Figure 1 PRISMA 2020 flowchart for identifying clinical studies included in the qualitative and quantitative syntheses.
Abbreviation: NAC, N-acetylcysteine; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

The literature search results were uploaded to EPPI-Reviewer 4 (EPPI-Center Software. London, UK), a web-based software program for managing and analyzing data in literature reviews that facilitates collaboration among reviewers during the study selection process.

Study Selection

The study reported results concerning the efficacy of NAC on MHS in the airway. Two reviewers (L.C. and S.G.) independently checked the relevant studies identified in MEDLINE and SCOPUS. The studies were selected in accordance with the previously mentioned criteria, and any difference of opinion on eligibility was resolved by consensus.

Data Extraction

Data from included studies were extracted and controlled for study characteristics, models, NAC dose, subject characteristics, and outcomes.

Endpoint

The endpoint of this systematic review was to evaluate the efficacy of NAC on MHS in the airways.

Data Analysis

Data are reported as qualitative synthesis, with statistically significant results derived from original articles for $p<0.05$.

Results

Studies Characteristics

Of the 25 potentially relevant records screened in MEDLINE and SCOPUS, 22 original articles were deemed eligible for this study and published between 2003 and 2023. This systematic review included data from six in vivo studies performed on experimental animals[17,24–28] and 14 in vitro studies performed on cells and tissues.[5,13,29–40] Two studies were carried out both in vitro and in experimental animals.[41,42]

The included studies investigated models of COPD,[24,25] exudative pneumonia,[27] and asthma,[28] performed in experimental animals and models of viral infection,[5,29,30] mucus hypersecretion,[13,37,38] cigarette smoke exposure,[33,34] bacterial infection[32,41,42] and inflammatory models[35] performed in vitro. Oxidative stress and inflammation models were used in experimental animals and in vitro.[17,26,31,36,39,40] No study has reported data concerning the impact of NAC on CMH in human bronchial tissue.

The main characteristics of the studies included in this systematic review are reported in Table 1.

Table 1 Main Characteristics of the Studies Included in the Systematic Review

Study	Study Characteristics	Models	NAC (Dose, Timing, Route of Administration)	Analyzed Subjects or Cells	Subject Characteristics	Outcomes
Xu et al 2023[24]	Experimental animals	COPD model	54 mg/kg/d intragastrically	48 SD rats	Rat model of COPD via inhalant exposure to CS (3000±500 ppm for 40 minutes twice a day) and KP (0.1 mL, 6×10^8 CFU/mL once every 5 days) for 8 weeks	MUC5AC protein, MUC5B protein, goblet cells
Ma et al 2022[25]	Experimental animals	COPD model	54 mg/kg/d intragastrically	32 SD rats	Rat model of COPD via inhalant exposure to CS (3000±500 ppm for 40 minutes twice a day) and KP (0.1 mL, 6×10^8 CFU/mL once, every 5 days) for 8 weeks	MUC5AC protein, MUC5B protein, goblet cells
Chi et al 2022[29]	in vitro	Viral infection model	1 mM	HBECs	BEAS-2B and HEp-2 cells infected by RSV (MOI 0.5) for 24 hours	MUC5AC gene and MUC5AC protein
Wang et al 2022[27]	Experimental animals	Exudative pneumonia model	15 mg/kg/d IP injection	60 SPF Wistar rats	Rat model of exudative pneumonia via IP LPS (2 mg/kg) and IN KP (50 μL 1×10^7 CFU/mL) daily for 5 days	MUC5AC protein
Xiaoyan Xu et al 2021[13]	in vitro	Hypersecretion model	3 mM	HBECs	16HBE14o cells exposed to NE (100 ng/mL) for 30 minutes	MUC5AC protein

(*Continued*)

Table 1 (Continued).

Study	Study Characteristics	Models	NAC (Dose, Timing, Route of Administration)	Analyzed Subjects or Cells	Subject Characteristics	Outcomes
Albano et al 2020[31]	in vitro	Oxidative stress and inflammatory model	10 mM	HBECs	A549 and pHBECs exposed to PBDEs (0, 0.01, 0.1, 1 and 10 μM) for 24 hours	MUC5AC gene and MUC5AC protein; MUC5B gene and MUC5B protein
Zhang et al 2019[40]	in vitro	Oxidative stress model	3 mM IP injection	HBECs	16HBE cells exposed to TGF-β3 (PeproTech, 100–36E; 10 ng/mL) for 24 hours	MUC5AC protein
Ping et al 2019[26]	Experimental animals	Oxidative stress and inflammatory model	10 mL/kg, 125 mg/kg, 250 mg/kg, 500 mg/kg intragastrically	48 Wistar rats	Rat via inhalant exposure to PM2.5 (7.5 mg/kg) once a week for four-times	MUC5AC protein
Kim et al 2017[36]	in vitro	Oxidative stress and inflammatory model	1 mM	NHBECs	NHBECs exposed to $CLB_{2.0}$ (10 mg/mL) for 4 hours	MUC5A gene
Cao et al 2017[33]	in vitro	Cigarette smoke model	30 μM	NHBECs	NHBECs exposed to MSWSS (Marlboro Red (R60) or Marlboro Silver (S60)), 4 hours per day for 1–5 days	MUC5AC protein
Koizumi et al 2015[32]	in vitro	Bacterial infection model	20 mM	HBECs	NHBECs exposed to bacterial (*Streptococcus pneumoniae*) co-culture (10 μL) for 8 hours	MUC5AC protein
Kanai et al 2015[34]	in vitro	Cigarette smoke model	10 mM	HBECs	NCI-H292 and NHBECs exposed to CSE (100 μL) for 7–10 days	MUC5AC protein
Mata et al 2012[5]	in vitro	Viral infection model	0.1, 1 and 10 mM	HBECs	NHBECs infected by RSV (100 μL) for 2 hours	MUC5AC gene, goblet cells
Seagrave et al 2012[35]	in vitro	Inflammatory model	10, 30, 100 and 300 μM	Normal, human-derived tracheal/bronchial epithelial cells	EpiAirway stimulated with IL-13 (1 ng/mL) for 3 days	MUC5AC protein
Ho Choi et al 2011[37]	in vitro	Hypersecretion model	10 mM	Human lung carcinoma cell	A549 cells exposed to acrolein (30 nM) for 4 hours	MUC5AC gene and MUC5AC protein

(*Continued*)

Table 1 (Continued).

Study	Study Characteristics	Models	NAC (Dose, Timing, Route of Administration)	Analyzed Subjects or Cells	Subject Characteristics	Outcomes
Mata et al 2011[30]	in vitro	Viral infection model	0.1, 1 and 10 mM	HBECs	A549 cells infected with influenza (strains A and B) via RSV (0.3 MOI) for 1 hour	MUC5AC protein and MUC5AC gene
Sprenger et al 2011[41]	Experimental tissue and in vitro	Bacterial infection model	0.3, 3.0, 15 and 30 mg/mL	Surface epithelial cells and explanted airway mucosal tissue	Calu-3 cells and explanted human mucosa stimulated with either PA, LPS from alginate-producing PA (sPA-LPS), or non-alginate-producing PA (rPA-LPS) (200 ng/mL) for 24 hours	MUC5AC gene and mucin protein
Chiba et al 2011[38]	in vitro	Hypersecretion model	1.1 mM	Human pulmonary mucoepidermoid carcinoma cells and NHBECs	NCI-H292 and NHBECs stimulated with B[a]P (1 μM) for 24 or 48 hours	MUC5AC gene
Jang et al 2010[39]	in vitro	Oxidative stress model	1.5 and 10 mM IP	Human mucoepidermoid bronchiolar carcinoma cells	NCI-H292 cells exposed to SHP-1 siRNA (1 μg) for 24 hours	MUC5AC gene
Hauber et al 2007[42]	in vivo and in vitro	Bacterial infection model	0.3, 3.0 and 30 mM	in vivo: 16 male, 24 female explanted human airway mucosa and in vitro mucoepidermoid cells	in vivo: tissue samples stimulated with LPS (10 ng/mL) or PAM3 (50 and 200 nM) and in vitro Calu-3 cells stimulated with LPS (10, 50, and 200 ng/mL) or PAM3 (50 and 200 nM) for 24 hours	MUC5AC gene and mucin protein
Mata et al 2003[17]	Experimental animals	Oxidative stress model	3 mM/kg orally	SD rats	Rats exposed to a single dose of bleomycin (2.5 U/kg^{-1}) dissolved in saline (0.25 mL) for 1 week	MUC5AC gene
Blesa et al 2003[28]	Experimental animals	Asthma model	3 mM/kg orally	Brown Norway rats	Rats exposed to antigen ovalbumin (6.25–100 mg/kg body weight^{-1}) for 1 hour	MUC5AC gene

Abbreviations: B[a]P, benzopyrene; $CLB_{2.0}$, carboxyl latex beads 2 μM; CS, cigarette smoke; CSE, cigarette smoke extract; COPD, chronic obstructive pulmonary disease; CKS, changkil saponin; ECC-BYF III, effective-component compatibility of Bufei Yishen formula III; ER, exercise rehabilitation; HDM, house dust mite; HBECs, human bronchial epithelial cells; IN, intranasal; IP, intraperitoneal; KP, *Klebsiella pneumoniae*; LPS, lipopolysaccharides; MOI, multiplicity of infection; NAC, N-acetylcysteine; NE, neutrophil elastase; NHBECs, normal human bronchial epithelial cells; PA, *Pseudomonas aeruginosa*; PAM3, synthetic lipoprotein; PBDEs, polybrominated diphenyl ethers; PBS, Phosphate-buffered saline; pHBECs, primary human bronchial epithelial cells; PM2.5, particulate matter; rPA-LPS, rough *Pseudomonas aeruginosa* lipopolysaccharides; RSV, respiratory syncytial virus; SD, Sprague–Dawley; SHP-1, Src homology 2 domain-containing protein tyrosine phosphatase; siRNA, small interfering RNA; sPA-LPS, smooth *Pseudomonas aeruginosa* lipopolysaccharides; SPF, specific pathogen-free; TCID, tissue culture infection dose; TGF-β3, transforming growth factor-beta; MSWSS, mainstream whole smoke solutions.

Models of MHS

In vitro Studies

Viral Infection

The human laryngeal carcinoma cell line, HEp-2, and the transformed human bronchial epithelial cell (HBEC) line, BEAS-2B, were incubated in 500 µL Dulbecco′s Modified Eagle′s Medium (without penicillin-streptomycin solution and fetal bovine serum). Subsequently, both cell lines were infected with respiratory syncytial virus (RSV) at a multiplicity of infection (MOI) of 0.5 for 24 hours.[29] RSV infection markedly increased the mRNA expression of MUC5AC in BEAS-2B cells and induced a significant increase in MUC5AC production from the supernatant of BEAS-2B cells compared with the negative control group.[29]

Normal human bronchial epithelial cells (NHBECs) were exposed to 100 µL RSV for 2 hours.[5] This exposure resulted in increased goblet cells and elevated MUC5AC expression in NHBECs compared with the negative control group.[5]

The HBEC line A549, derived from lung alveolar cells, was infected with the influenza virus (strains A and B) and RSV at a MOI of 0.3 for a duration of 1 h. The level of MUC5AC expression increased significantly after virus infection compared with the negative control group.[30]

Chemicals

A549 human lung carcinoma cells were exposed to 30 nM acrolein for 4 hours in a serum-free medium.[37] Acrolein treatment is known to enhance MUC5AC mRNA and protein expression in the A549 cells compared with the negative control group.[37]

Cigarette Smoke Extract

NHBECs were exposed to mainstream whole smoke solutions (MSWSS) at concentrations ranging from 0.1% to 1.0% of R60 and S60, utilizing a smoking regimen with a puff volume of 35 mL, a puff interval of 60 seconds, a puff duration of 2.0 seconds, and 0% blocking of the ventilation filter. This exposure regimen was conducted for 4 hours/day throughout 1–5 days.[33] The results showed a significant increase in the production of reactive oxygen species (ROS) and the induction of MUC5AC secretion after a single treatment with MSWSS when compared with the negative control group.[33]

The human pulmonary mucoepidermoid carcinoma cell line (NCI-H292) was exposed to 100 µL cigarette smoke extract (CSE) and 10 µg/mL poly(I:C) for 7–10 days.[34] CSE stimulated MUC5AC production in airway epithelial cells when compared with the negative control group.[34]

Bacterial Infection

HBECs were examined with 10 µL bacteria (*Streptococcus pneumoniae*) for 8 hours.[32] Immunofluorescent staining of MUC5AC showed strong expression in the untreated cells; this expression was even stronger with bacterial co-incubation than in the negative control group.[32]

Mucus-producing Calu-3 cells and human mucosal tissue explanted from upper airway mucosa (sinus) specimens were stimulated with *Pseudomonas aeruginosa* (PA), lipopolysaccharide (LPS) from alginate-producing PA (smooth, sPA-LPS) and non-alginate-producing PA (rough, rPA-LPS) at a concentration of 200 ng/mL for 24 hours.[41] In Calu-3 cells, stimulation with PA, sPA-LPS, and rPA-LPS significantly ($p<0.05$) induced mucin protein and MUC5AC gene expression. This phenomenon was also observed in mucosal tissue explanted from upper human airways. Both PA and PA-LPS significantly ($p<0.05$) increased mucin protein and MUC5AC gene expression when compared with the negative control group.[41]

The explanted human airway mucosal tissue and Calu-3 cells were stimulated with 10 ng/mL LPS in tissue and 10, 50, and 200 ng/mL in cells, as well as with 50 and 200 nM PAM3 (a synthetic lipoprotein) for 24 hours.[42] Exposure to LPS significantly ($p<0.05$) increased MUC5AC mRNA expression and mucin protein expression in both Calu-3 cells and explanted human airway mucosal tissue.[42] Stimulation with the synthetic lipoprotein PAM3 caused a dose-dependent increase in the expression of mucin protein in Calu-3 cells.[42] In the tissue, PAM3 significantly ($p<0.05$) increased MUC5AC mRNA and mucin protein expression compared with the negative control group.[42]

Inflammation and Oxidative Stress

The immortalized HBEC line, 16HBE14o, was subsequently treated with 100 ng/mL neutrophil elastase (NE) for 30 minutes.[13] The results revealed that exposure to 100 ng/mL NE increased MUC5AC protein production in both the supernatant and cytoplasm compared with the negative control group.[13]

Primary human airway epithelial cells were stimulated with interleukin-13 (IL-13) at a concentration of 1 ng/mL for 3 days.[35] The expression of MUC5AC increased significantly ($p<0.05$) in response to IL-13 in the cells compared with the negative control group.[35]

NCI-H292 and NHBECs were stimulated with benzopyrene (B[a]P) at a concentration of 1 μM for 24 hours.[38] Exposure to B[a]P resulted in the induction of MUC5AC and significant upregulation in MUC5AC mRNA levels in NCI-H292 cells when compared with the negative control group.[38]

A549 epithelial cell line and primary HBECs cultured in the air–liquid interface were stimulated with polybrominated diphenyl ethers (PBDEs 47, 99, 209) at concentrations ranging from 0.01 to 1 μM for 24 hours.[31] The results showed that PBDEs (47, 99, and 209) significantly increased the secretion of MUC5AC and MUC5B in A549 cells when compared with the negative control group.[31]

Carboxyl Latex Beads $(CLB)_{2.0}$ is a component of PM that induces the secretion of multiple cytokines and chemokines that regulate airway inflammation.[36] NHBECs were treated with $CLB_{2.0}$ dose-dependently for 4 hours.[36] $CLB_{2.0}$ significantly ($p<0.05$) increased the gene expression of MUC5AC in the cells compared with the negative control group.[36]

16HBE cells were treated with transforming growth factor-beta (TGF-β3) at a concentration of 10 ng/mL for 24 hours.[40] The data revealed that stimulation of 16HBE cells with TGF-β3 is associated with an increase in mucus production.[40] Immunofluorescent staining of MUC5AC protein showed a significant ($p<0.05$) increase in 16HBE cells treated with TGF-β3 compared with the negative control group.[40]

Suppression of the Src homology 2-containing protein tyrosine phosphatase (SHP-1) is associated with the development of airway inflammation and increased levels of ROS.[39] NCI-H292 was exposed to (SHP-1) siRNA at a concentration of 1 μg for 24 hours.[39] SHP-1-suppressed H292 cells exhibited increased MUC5AC mRNA compared with control cells and the negative control group.[39]

Studies in Experimental Animals

COPD Model

A Sprague–Dawley (SD) rat model of COPD was induced via inhalation, exposing the rats to CS at a concentration of 3000±500 ppm for 40 minutes twice a day and *Klebsiella pneumoniae* (KP) administration at a volume of 0.1 mL, 6×108 colony forming units (CFU)/mL once every 5 days for 8 weeks.[24,25] Compared with the negative control, the COPD model resulted in increased goblet cells and elevated levels of MUC5AC and MUC5B.[24,25]

Asthma Models

Male brown Norway rats weighing 250–300 g were exposed to the antigen ovalbumin at doses of 6.25–100 mg/kg for 1 hour.[28] The observed effect included a marginal increase in MUC5AC expression in the antigen-exposed group of animals when compared with the negative control group.[28]

Exudative Pneumonia

Sixty specific pathogen-free Wistar male rats were selected for this study. The rat model of exudative pneumonia (EP) was induced by intraperitoneal injection of LPS at a dose of 2 mg/kg and bilateral nasal instillation of 50 μL 1×107 CFU/mL KP for 5 consecutive days.[27] In fluorescence microscopy results, a significant increase in MUC5AC expression was observed in the alveolar and bronchial epithelial cells of rat lung tissue infected with the bacteria compared with the negative control group.[27]

Oxidative Stress and Inflammation

C57BL/6J female mice, aged 6–8 weeks, were treated with 20 μg house dust mite and 1 mg aluminum hydroxide $Al(OH)_3$ through intraperitoneal injection for 7 days.[40] Lung tissues of house dust mite-treated mice exhibited a notable

increase in ROS generation, as detected by immunofluorescence staining.[40] The elevated ROS levels were accompanied by increased MUC5AC expression compared with the negative control group.[40]

Forty-eight male Wister rats were subjected to intratracheal instillation of 7.5mg/kg PM2.5 suspension mixed with ultrasonic oscillation, administered once a week for a total of four exposures.[26] Rats exposed to PM2.5 exhibited a significant increase in blue-stained mucus within bronchial epithelial cells compared with the negative control group.[26]

SD rats weighing 200–250 g were administered a single sublethal dose of 2.5 U/kg^{-1} bleomycin dissolved in 0.25 mL saline for 1 week.[17] Bleomycin-treated rats exhibited a significant ($p<0.05$) increase in mucin production and the presence of airway secretory cells when compared with the negative control group.[17]

Effect of NAC on MHS

In vitro Studies

Viral Infection

Lei Chi et al[29] investigated the impact of NAC at a concentration of 1 mm on MUC5AC expression in RSV-infected BEAS-2B cells. The results showed that NAC treatment significantly ($p<0.05$) reduced the gene and protein expression of MUC5AC from the BEAS-2B cell supernatant compared with the positive control group.[29]

In the study by Mata et al,[5] RSV cells were pretreated with NAC at concentrations of 0.1, 1 and 10 mm for 1 hour before infection. Their results indicated that NAC treatment significantly ($p<0.05$) reduced the gene expression level of MUC5AC and the number of goblet cells in NHBECs to normal levels compared with the positive group.[5]

Another study conducted by Mata et al[30] indicated that in A549 cells infected with RSV, influenza A and influenza B viruses, NAC significantly ($p<0.05$) reduced gene and protein expression of MUC5AC in a dose-dependent manner compared with the positive control group.

Chemicals

Based on the findings reported by Ho Choi et al,[37] 10 nM NAC significantly ($p<0.05$) reduced acrolein-induced gene and protein overexpression of MUC5AC in A549 human lung carcinoma cells compared with the positive control group.

Cse

In a study conducted by Cao et al,[33] it was observed that treatment with NAC at a concentration of 30 μM significantly ($p<0.05$) reduced the protein secretion of MUC5AC induced by both ISO and MSWSS compared with the positive control group.[33]

Kanai et al[34] reported that pretreatment with 10 mm NAC had a significant ($p<0.05$) effect in decreasing the level of protein overexpression of MUC5AC induced by poly(I:C) compared with the positive control group.[34]

Bacterial Infection

In a study conducted by Koizumi et al,[32] it was shown that HBECs treated with 20mM NAC significantly ($p<0.05$) reduced the overexpression of MUC5AC protein induced by bacterial infection compared with the positive control group.[32]

The results of the study by Sprenger et al[41] provided evidence that NAC, administered at concentrations of 0.3, 3.0, 15, and 30 mg/mL, significantly ($p<0.05$) decreased PA-induced MUC5AC gene expression at all concentrations in Calu-3 cells.[41] NAC also showed a dose-dependent reduction in both sPA-LPS- and rPA-LPS-induced MUC5AC gene expression, reaching statistical significance ($p<0.05$) at a concentration of 15 μg/mL compared with the positive control group.[41]

NAC also significantly ($p<0.05$) decreased sPA-LPS- and rPA-LPS-induced MUC5AC gene and mucin protein expression in explanted mucosa compared with the positive control group.[41]

In a study conducted by Hauber et al,[42] various doses of 0.3, 3.0 and 30 mm NAC inhibited the LPS-induced mucin protein expression and significantly ($p<0.05$) decreased MUC5AC gene and mucin protein expression in Calu-3 cells compared with the positive control group.[42] Additionally, NAC numerically inhibited PAM3-induced mucus protein expression, returning the epithelium to normal levels.[42]

Inflammation and Oxidative Stress

Xu et al presented compelling evidence demonstrating that pretreatment with 3 mmol/L NAC significantly ($p<0.05$) reduced the level of MUC5AC protein in 16HBE14o-cells exposed to NE ($p<0.01$).[13]

In a study conducted by Seagrave et al, cells were pretreated with varying concentrations of 10, 30, 100, and 300 μM NAC for 3, 8 and 24 hours.[35] The researchers reported a significant ($p<0.05$) decrease in the cellular content of MUC5AC protein in IL-13-treated cells compared with the positive control group.[35]

Chiba et al observed a positive effect of 1.10 mm NAC and a significant ($p<0.05$) reduction in B[a]P-induced MUC5AC gene levels when compared with the positive control group.[38]

Albano et al showed that pretreatment with 10 mm NAC restored the negative effects of PBDEs in epithelial cells.[31] Their study revealed a significant ($p<0.05$) inhibition and reduction in gene and protein expression of both MUC5AC and MUC5B compared with the positive control group.[31]

Kim et al reported a significant ($p<0.05$) reduction in the gene expression of MUC5AC with the use of NAC at a concentration of 1 mm compared with the positive control group.[36]

In a study conducted by Zhang et al, immunofluorescence staining revealed a significant ($p<0.05$) reduction in MUC5AC protein levels in 16HBE cells treated with 3 mmol/kg NAC ($p<0.01$) compared with the positive control group.[40]

Jang et al showed a positive effect of 1, 5 and 10 mm NAC ($p<0.05$) in inhibited MUC5AC gene stimulated by H_2O_2 in SHP-1-suppressed H292 cells compared with the positive control group.[39]

Studies in Experimental Animals

COPD

The results of the studies conducted by Xu et al[24] and Ma et al[25] indicated that NAC administered at a dose of 54 mg/kg/d significantly ($p<0.05$) reduced the levels of both MUC5AC protein and expression of MUC5B protein in an animal model of COPD. The group treated with NAC exhibited fewer goblet cells than the positive control group.[24,25]

Asthma

Blesa et al[28] presented evidence indicating that pretreatment with oral NAC at a dose of 3 mmol/kg significantly ($p<0.05$) reduced MUC5AC gene secretion in an animal model of asthma compared with the positive control group.

Exudative Pneumonia

Wang et al reported that NAC, administered at a dose of 15 mg/kg/d, significantly ($p<0.05$) reduced the MUC5AC protein expression in a rat model of exudative pneumonia compared with the positive control group.[27]

Oxidative Stress and Inflammatory

In the study by Zhang et al, NAC at a dose of 3 mmol/kg significantly ($p<0.05$) decreased MUC5AC protein expression in the airway epithelial cells of mice compared with the positive control group.[40]

Another investigation by Ping et al revealed that increasing doses of NAC (10, 125, 250, 500 mg/kg) yielded a significant and progressively more pronounced reduction ($p<0.05$) in MUC5AC protein secretion in lung tissue of rats compared with the positive control group.[26]

The results of the study conducted by Mata et al indicated that NAC treatment at a dose of 3 mmol/kg had a positive effect on reducing the level of MUC5AC protein ($p<0.05$) and improving pulmonary lesions in SD rats exposed to bleomycin compared with the positive control group.[17]

Discussion

In contrast to previous reviews, which have focused on general aspects of CMH in COPD,[43–47] this systematic review is the first to specifically evaluate the impact of NAC on MHS. By addressing this gap in the literature, our review provides a comprehensive evaluation of the available evidence on the efficacy of NAC in managing MHS in COPD patients. This adds critical insight to the current body of knowledge and offers a clearer understanding of the role of NAC in the treatment of CMH, which has been a growing focus in international recommendations.[20] Detailed information on the effect of NAC on MHS in the airways is reported in Table 2.

Table 2 Effect of N-Acetylcysteine on Mucus Hypersecretion in the Airways

Model Inducing MHS	*MUC5AC* Gene Expression	MUC5AC Protein Expression	*MUC5B* Gene Expression	MUC5B Protein Expression	Goblet Cells	Mucin Protein
RSV infection	↓[5,29,30]*	↓[29,30]*	NA	NA	↓[5]*	NA
Influenza A infection	↓[30]*	↓[30]*	NA	NA	NA	NA
Influenza B infection	↓[30]*	↓[30]*	NA	NA	NA	NA
Chemical	↓[37]*	↓[37]*	NA	NA	NA	NA
CSE	NA	↓[24,25,33,34]*	NA	↓[24,25]*	↓[24,25]*	NA
Bacterial infection	↓[41]*	↓[32]*	NA	NA	NA	NA
rPA-LPS	↓[41]*	NA	NA	NA	NA	NA
sPA-LPS	↓[41]*	NA	NA	NA	NA	↓[41]*
LPS	↓[42]*	↓[27]*	NA	NA	NA	↓[42]*
PAM3	↓[42]*	NA	NA	NA	NA	↓[42]†
Inflammation and oxidative stress	NA	↓[35]*	NA	NA	NA	NA
B[a]P	↓[38]*	NA	NA	NA	NA	NA
SHP-1	↓[39]*	NA	NA	NA	NA	NA
$CLB_{2.0}$	↓[36]*	NA	NA	NA	NA	NA
NE	NA	↓[13]*	NA	NA	NA	NA
PBDEs	↓[31]*	↓[31]*	↓[31]*	↓[31]*	NA	NA
TGF-β3	NA	↓[40]*	NA	NA	NA	NA
KP	NA	↓[24,25,27]*	NA	↓[24,25]*	↓[24,25]*	NA
Antigen ovalbumin	↓[28]*	NA	NA	NA	NA	NA
PM 2.5	NA	↓[26]*	NA	NA	NA	NA
Bleomycin	↓[17]*	NA	NA	NA	NA	NA

Notes: *Statistically significant vs positive control ($p<0.05$). †numerical change vs positive control.
Abbreviations: B[a]P, benzopyrene; $CLB_{2.0}$, carboxyl latex beads 2 μM; CSE, cigarette smoke extract; KP, *Klebsiella pneumoniae*; LPS, lipopolysaccharides; MHS, mucus hypersecretion; NE, neutrophil elastase; PAM3, synthetic lipoprotein; PBDEs, polybrominated diphenyl ethers; PM2.5, particulate matter; rPA-LPS, rough *Pseudomonas aeruginosa* lipopolysaccharides; RSV, respiratory syncytial virus; SHP-1, Src homology 2 domain-containing protein tyrosine phosphatase; sPA-LPS, smooth *Pseudomonas aeruginosa* lipopolysaccharides; TGF-β3, transforming growth factor-beta.

Evidence from this systematic review indicates that NAC significantly inhibited the gene and protein expression of MUC5AC and MUC5B in several models of airway MHS. NAC was also significantly effective in reducing the number of goblet cells. Interestingly, this evidence arises from several validated models of MHS carried out both in vitro and in vivo in experimental animals under specific stimuli, such as viral and bacterial infection, chemicals, CSE, and pro-inflammatory and pro-oxidant mediators. In this regard, NAC has been found effective in counteracting the MHS in several HBEC lines and bronchial tissue, specifically in rat models of asthma and COPD.

Indeed, the main mucolytic mechanism of action of NAC is related to the hydrolysis of disulfide bonds within mucin, disruption of oligomers, and reduction in mucin viscosity.[17] However, evidence from this qualitative synthesis suggests that NAC counteracts MHS directly at the level of bronchial airways and epithelial cells by inhibiting mucus secretion and preventing goblet cell hyperplasia.

Therefore, NAC should not only be considered as a mere mucolytic and antioxidant agent but should also be classified as a mucolytic and mucoregulator drug. This pivotal finding may have important translational relevance in treating patients with COPD characterized by a hypersecretion phenotype. Indeed, airway mucus accumulation contributes to sputum production, airflow obstruction, and exacerbations in muco-obstructive pulmonary disease, a condition known as CMH.[1,9,10]

CMH is the major contributor to the increased risk of morbidity and mortality within specific subsets of patients with COPD.[10,45] CMH is also a prevalent feature of several patients with COPD and represents an independent risk factor for disease progression.[10,45] Interestingly, CMH also emerges as a key treatable trait in the COPD spectrum.[48] Unexpectedly, mucus plugs completely occluding the airways are observed in 25% to 67% of computed tomography (CT) scans of individuals with COPD. Moreover, persistent mucus plugs are observed in up to 73% of COPD individuals after 5 years of observation, resulting in a substantial increase in the risk of adverse outcomes.[10,45] Of note, in the Copenhagen General Population Study, CMH may also be significantly reported in 20% of early patients with COPD, who are individuals under 50 years of age with >10 years of tobacco consumption with at least one of the following conditions: forced expiratory volume in 1 second/forced vital capacity below the lower limit of normal and/or chest tomography scan abnormalities and/or accelerated decline in forced expiratory volume in 1 second.[49] CMH is associated with a significantly increased likelihood of acute exacerbations, resulting in increased rates of hospitalization.[10,45] Moreover, CMH is also related to physical activity limitations, diminished quality of life, and a marked escalation in mortality risk.[10,45]

The pathogenesis of CMH in COPD is intricate, encompassing factors, such as inflammation, oxidative stress, proteinase balance and signal transduction pathways.[45] In chronic obstructive respiratory disorders, persistent and recurrent inflammatory stimuli induce substantial metaplasia of bronchial epithelial goblet cells, along with hypertrophy and hyperplasia of submucosal bronchial glands. This cascade of events culminates in an extensive increase in MHS.[45,50] These alterations result in airway obstruction, airflow obstruction, rapid decline in lung function, increased frequency of acute exacerbations, and a significant risk of death.[45,50]

Concluding, in this detrimental scenario, NAC may have a pivotal role in managing the hypersecretion of patients with COPD. However, further research is warranted to assess whether the qualitative evidence of this systematic review can also be confirmed at the level of human bronchial tissue, using adequate and validated human ex vivo models to elicit MHS, as recently described.[51] Additionally, a meta-analysis should be conducted to provide quantitative evidence on the effect of NAC against the protein and gene expression of MUC5AC (https://live.ersnet.org/programme/presentation/561861). Future studies should also explore the development of inhaled mucolytic compounds, given the promising proof-of-concept evidence.[52,53]

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