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N-acetylcysteine as a therapeutic approach to post-COVID-19 pulmonary fibrosis adjunctive treatment

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Abstract. – OBJECTIVE: Growing interest is directed to the outcomes of COVID-19 in survivors, both in the convalescent period and in the long-term, which are responsible for morbidity and quality of life deterioration. This article aims to describe the mechanisms supporting the possible use of NAC as an adjuvant treatment for post-COVID-19 pulmonary fibrosis.

MATERIALS AND METHODS: A search was performed in PubMed/MEDLINE.

RESULTS: Interstitial changes have been observed in the CT scan of COVID-19 pneumonia. In patients with respiratory outcomes in the post-COVID-19 stage, glutathione (GSH) deficiency was found and interpreted as a reaction to the inflammatory cascade caused by the viral infection, while the pathophysiological process of pulmonary fibrosis involves numerous cytokines, such as TGF-β, TNF-α, IL-1, PDGF and VEGF. NAC has a good tolerability profile, is easily administered orally and inexpensively, and has antioxidant and anti-inflammatory effects that may target the pathophysiologic mechanisms involved in pulmonary fibrosis. It may revert GSH deficiency, exerts direct and indirect antioxidant activity, anti-inflammatory activity and improves immune T-cell response.

CONCLUSIONS: The mechanism of action of NAC suggests a role in the treatment of pulmonary fibrosis induced by COVID-19.

Key Words:
COVID-19, NAC, Pulmonary fibrosis, Oxidative stress, Inflammation.

Introduction

Growing interest is directed to the outcomes of COVID-19 in survivors, both in the convalescent period and in the long term. A large population worldwide is addressing post-COVID-19 morbidity and quality of life deterioration. Serious pulmonary complications have been observed in the convalescent stage of pneumonia due to the SARS-COV-2 infection. Han et al[1] showed that fibrotic-like changes were observed in 35% of survivors of severe COVID-19 pneumonia at 6-month follow-up chest CT[1]. Aul et al[2] selected patients with definite fibrotic change on CT, and 9.3% of the study population had post-COVID-19 pulmonary fibrosis[2]. Although the prevalence, impact and future behavior of COVID-related fibrosis are not yet fully clarified, prompt identification strategies and effective therapeutic interventions for COVID-related fibrosis should be investigated. Meanwhile, slowing or mitigating the process of pulmonary interstitial fibrosis, improving the pulmonary function of patients with severe pneumonia, and improving patients' quality of life should be the aims of treatment for patients in the convalescent stage of COVID-19[3]. Indeed, it is to be acknowledged that current guidelines do not provide evidence-based recommendations for the correct treatment and the management of pulmonary fibrosis after COVID-19[4].

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Among possible adjunctive or supportive treatments of serious COVID-19 deserving researchers' attention, N-acetylcysteine (NAC) seems promising. NAC is a precursor of glutathione (GSH), whose deficiency has been suggested to be a key pathophysiological factor of COVID-19[5]. Indeed, NAC may revert GSH deficiency and activate mechanisms mediated by GSH replenishment within cells[6-8]. It is provided with a high safety profile, as shown by years of use.

This article describes the mechanisms supporting the possible use of NAC as an adjuvant treatment for post-COVID-19 pulmonary fibrosis.

Materials and Methods

A search was performed in PubMed/MEDLINE without any limitations in terms of publication date. Appropriate combinations of pertinent keywords were searched: "NAC," "GSH", "viral infection," "COVID-19", "oxidative stress," "inflammation," and "pulmonary fibrosis", retrieving preclinical and clinical studies. Articles in English or with English summaries were considered. The authors evaluated all clinical and preclinical studies and included them when considered relevant to the query, based on their expertise, and when content introduced new information. Based on chosen articles, a narrative review was prepared.

Pathophysiology of Advanced COVID-19

COVID-19 is often marked, even in asymptomatic patients, by the development of pneumonia with no specific or diagnostic abnormalities of chest CT imaging; this condition has a rapid evolution from focal unilateral ground-glass opacities to diffuse bilateral lesions, with consolidations either co-existing or occurring with within 1-3 weeks[9]. Starting from the second week of the disease, a reticular pattern with bronchiolectasis and irregular interlobular or septal thickening progressively organizes and increases. At this stage, interstitial changes appear, suggesting the development of fibrosis[9]. As suggested by a retrospective cohort study[10] carried out in China, these events seem to involve a large proportion of COVID-19 patients. The authors found that more than one-third of patients surviving pneumonia had residual respiratory failure, with fibrotic changes in the lungs. Later studies found that interstitial changes seen on CT during the recovery phase may be due to residual inflammation or reperfusion edema. So, the proportion of patients with post-COVID-19 fibrosis would be lower than suspected but still represent a large population considering the incidence of SARS-CoV-2 infection worldwide[11]. The study by Konopka et al[12] was the first study that has focused on surgical lung biopsies in patients with persistent signs of interstitial lung abnormalities after recovery from acute COVID-19[12]. In this retrospective cohort, the most frequently encountered histologic finding was usual interstitial pneumonia (UIP). Patients with UIP were older than patients without UIP, and more likely to have a history of chronic lung disease prior to COVID-19.

A hyperinflammatory phase with a cytokine storm represents the advanced stage of COVID-19. It is linked to diffuse alveolar damage, which is a histological hallmark for the acute phase of acute respiratory distress syndrome (ARDS)[13,14]. In its later phase, ARDS is known to organize into interstitial fibrosis and alveolar hyperplasia[15,16]. In the presence of lung fibrosis, both injured cells and immune cells are responsible for the overexpression and the release of growth factors and cytokines. Within this inflammatory process, myofibroblasts are activated and induce the accumulation of extracellular matrix. Alveolar re-epithelization in the injured area is reduced while the normal lung construction is destroyed, and lung fibrosis is further promoted[16,17]. Pulmonary inflammations were detected in lung autopsies of deceased COVID-19 patients, showing neutrophil infiltration in pulmonary capillaries, extravasation into the alveolar spaces and neutrophilic mucositis[18]. Inflammatory events documented in coronavirus-induced conditions include activation of the NLRP3 (NOD-, LRR- and pyrin domain-containing protein 3) inflammasome by SARS coronavirus E protein apoptosis, induced by the 3C-like protease of SARS coronavirus[19,20].

In patients with respiratory outcomes in the post-COVID-19 stage, GSH deficiency was found and interpreted as a reaction to the inflammatory cascade caused by the viral infection[5]. Indeed, it is known that inflammation and subsequent tissue damage due to infection with respiratory viruses, including coronaviruses, follow changes in the redox homeostasis in infected cells[21]. In terms of clinical evidence, a decreased expression of the antioxidant enzyme SOD3 in the lungs of elderly patients was linked to the disease severity of COVID-19[22].

On the other end, the pathophysiological process of pulmonary fibrosis involves numerous

cytokines, such as TGF-β, TNF-α, IL-1, PDGF and VEGF[23]. The increased expression of these fibrogenic cytokines leads to fibroblast activation and vasoconstriction, which sustain the advent of pulmonary fibrosis. Accordingly, increased oxidation in the lower respiratory tract produces alveolar epithelium alterations and represents a step in the pathogenesis of pulmonary fibrosis. In addition to this increase in oxidation, in pulmonary fibrosis, there is a deficit of GSH, which is the largest component of the human lung antioxidant defense system[24].

Rationale for the Use of N-Acetylcysteine

As previously mentioned, NAC is a precursor molecule for GSH, a pivotal antioxidant system, and it may activate mechanisms mediated by GSH replenishment within cells (Figure 1)[6,7]. NAC is a sulfur-containing amino acid with a lytic activity on disulfide bonds, resulting in increased mucoproteins' viscosity. Due to its fluidifying action on mucous secretions in respiratory processes, it is used as a mucolytic and antioxidant agent in pulmonary complications of fibrosis[25]. In addition, NAC, as a precursor in the synthesis of GSH, reintegrates GSH levels and has a cytoprotective activity in the respiratory system against damage induced by oxidative stress[26,27]. Among its antioxidant effects, NAC scavenges reactive oxygen species (ROS), especially hypochlorous acid and hydroxyl radicals (•OH), and several reactive nitrogen species responsible for the oxidation of lipids, proteins, and DNA[7,25]. Therefore, NAC has a direct antioxidant effect within cells thanks to the thiol group, which scavenges radical and non-radical oxidants. In addition, it has an indirect antioxidant effect acting as a precursor of Cys (through a deacetylation reaction catalyzed by aminoacylase I) and consequently of GSH[27].

Furthermore, NAC has an extracellular antioxidant activity. It was demonstrated that NAC, at the therapeutic concentration range, greatly increases the plasma antioxidant activity. This effect is reached by regenerating albumin Cys34 by breaking thiol-disulfide bonds[27].

As the inflammatory cascade of pulmonary fibrosis is associated with overexpression of mucins (e.g.MUC5AC), it was suggested that this mechanism might be blocked by NAC[28].

NAC has been widely used in many conditions to restore or protect against GSH depletion and has a wide safety margin[29]. One well-known clinical role of NAC is its use as an antidote to acetaminophen poisoning; NAC acts by restoring the hepatic depleted pool of GSH[30,31]. NAC has an immunomodulatory effect in chronic inflammatory diseases as high doses increase GSH levels in peripheral blood T lymphocytes and disrupt mTOR activation[32]. Moreover, NAC is used in patients affected by chronic obstructive pulmonary diseases (COPD). It ameliorates COPD-induced

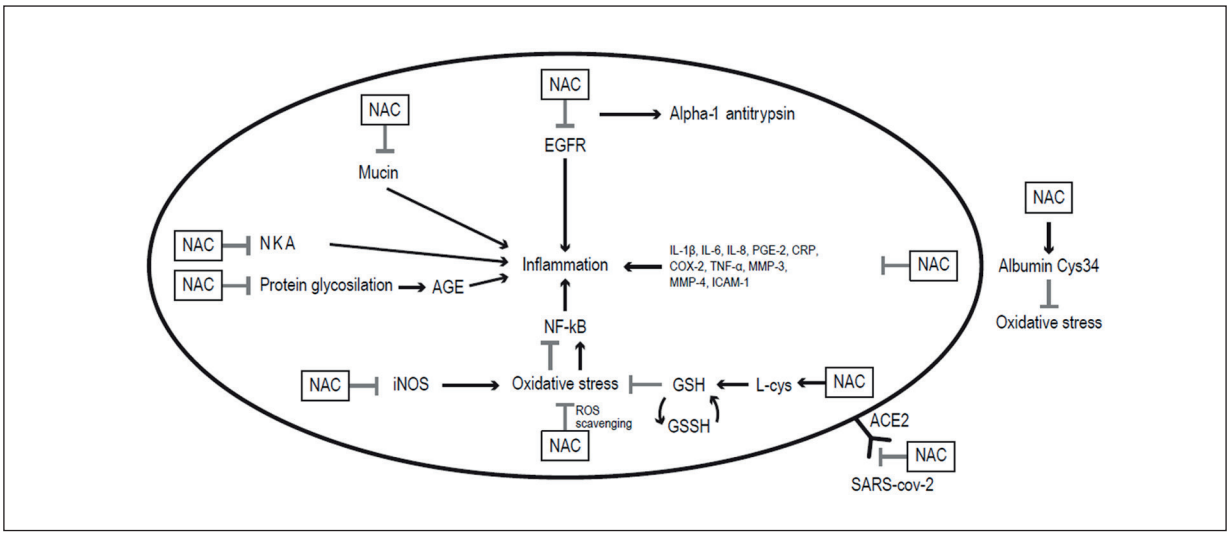

Figure 1. Major mechanisms responsible for the anti-inflammatory and antioxidant effects of NAC. Arrow = stimulation; T-shaped symbol = inhibition. GSH: reduced glutathione; GSSH: oxidized glutathione; Cys34: cysteine 34; IL: interleukin; CRP: C reactive protein; TNF: tumor necrosis factor; MMP: matrix metalloproteinase; ICAM-1: intercellular adhesion molecule-1; EGFR: epidermal growth factor receptor; iNOS: inducible nitric oxide synthase; AGE: advanced glycation end products. Source: De Flora 2020[6].

pulmonary fibrosis by promoting the immune response and inhibiting the epithelial-mesenchymal transformation process *via* the von Willebrand factor (VWF)/p38 MAPK axis[33]. Treatments with NAC allow more rapid improvement in the diffusing capacity of the lungs for carbon monoxide (DLCO) and exercise tolerance in patients survived from acute lung injury/acute respiratory distress syndrome (ALI/ARDS) caused by influenza A/H1N1[34]. Recently, it has been found[6] that NAC interferes with oxidative stress and biochemical pathways upregulating pro-inflammatory genes by inhibiting the activation of NF-κB. This mechanism has been demonstrated during infection by the influenza virus: the ROS-dependent activation of NF-κB (induced by endosomal Toll-like receptor 3/hemagglutinin) is inhibited by NAC, preventing the increased expression of pro-inflammatory cytokines[35].

Furthermore, NAC shows an anti-inflammatory activity independently from its antioxidant activity. As an example, NAC counteracts the release of neurokinin A (NKA). This effect inhibits neurogenic inflammation induced by lipopolysaccharide[36].

NAC, in addition, interacts with inflammation through the advanced glycation end products (AGE) pathway. Through endogenous glutathione and hydrogen sulfide synthesis, NAC attenuates methylglyoxal-induced protein glycation and additional protein glycosylation events in SARS-CoV-2 and other viral infections. Such compounds promote AGE formation, activating inflammatory cells by binding to RAGE[37,38].

NAC suppresses TNF-induced IκB kinases resulting in inhibition of NF-κB activation. The prevention of translocation of NFκB from the cytoplasm to the nucleus blocks the expression of pro-inflammatory cytokines and chemokines involved in the pathophysiology of several viral infections, including SARS-CoV-2[39,40].

The clinical relevance of these mechanisms of action was confirmed in a randomized, double-blind, placebo-controlled, prospective clinical trial performed in five ICUs on ARDS patients. The GSH pool of red blood cells was repleted by administering intravenous NAC (70 mg/kg body weight) every 8 hours for 10 days; this treatment was associated with a decreased number of days of acute lungs injury and increased cardiac index[41]. In another study, the administration of NAC (150 mg/kg on the first day, followed by 50 mg/kg for 3 days) to ARDS patients hospitalized in the ICU increased the extracellular total antioxidant power and total thiol molecules and improved clinical outcomes[42]. In stable COPD patients, 1,200 or 1,800 mg/day of NAC counteracted systemic oxidative stress induced by low flow oxygen administration, resulting in oxidized erythrocyte GSH, decreased thiol proteins and increased carbonyl proteins[43]. Finally, the role of NAC in the prevention of COPD exacerbations has been investigated by clinical trials[44,45].

At present, a beneficial effect of NAC in patients with pulmonary fibrosis has not been fully demonstrated by randomized clinical trials but seems to be expected in a subset of patients currently under investigation. The analysis of single-nucleotide polymorphisms has shown that patients with a specific genotype (*TOLLIP* rs3750920) have a better response to NAC than the general pulmonary fibrosis population. Therefore, patients with pulmonary fibrosis who may benefit from NAC are expected to be identified by genotyping and precision medicine[46].

Current Experience of NAC Use in COVID-19

Several clinical trials[47] are being carried out to evaluate the role of NAC in the treatment of COVID-19 and to evaluate the antioxidant activity in these patients, but none is focused on post-COVID-19 pneumonia. Despite some preliminary studies, up to date, there are no definitive data on the efficacy of NAC in post-COVID-19 patients, but some clinical experiences already published in the literature can be cited here[48-52]. A monocentric retrospective study on 1,083 patients hospitalized for COVID-19 pneumonia found that patients receiving NAC on admission and administered at a dosage of 300 mg intravenous TID, and switched to 600 mg per os BID once reached clinical stability had a shorter hospital stay. However, no impact of NAC on short- and long-term outcomes, including in-hospital mortality, ICU admission, impairment of lung diffusing capacity for carbon monoxide, and chest X-ray alterations at the 6-month follow-up, was present[48]. In a community-based study of 19,208 patients hospitalized with a diagnosis of COVID-19, the use of oral NAC 600 mg every 8 hours was associated with significantly lower mortality (OR 0.56; 95% CI: 0.47-0.67), despite these patients being older, more frequently male and with more comorbidities. On the contrary, there were no significant differences with the use of NAC on the mean duration of hospitalization, admission to the intensive care unit or use of invasive mechanical ven-

tilation[49]. In a prospective case-control study on 46 patients with confirmed COVID-19, therapy at a dose of 1200-1800 mg/day intravenous significantly improved SpO_2/FiO_2 compared to the controls in 10 days. In addition, the duration of hospitalization was shorter in patients receiving NAC (p=0.01)[50]. Zou and Li[51] reported that the usual treatment for pulmonary fibrosis in patients with COVID-19 in their center was based on glucocorticoids, NAC and pirfenidone. Most pulmonary lesions were absorbed after one week of treatment[51]. Patients with severe COVID-19 and weaned off from O_2 support received high-dose IV infusion of NAC and significant regression of ground-glass opacities, and pulmonary consolidation was seen on repeated CT[52].

Some authors reported[53-56] on the use of NAC in patients with COVID-19 in the early stages. As NAC was experienced based on its anti-inflammatory and antioxidant activities, which would also be involved in later stages of the disease.

In a descriptive cross-sectional study on 164 patients with confirmed COVID-19, it was observed that moderate-severe patients who received NAC with standard therapy had an average hospital stay duration of 12 days, a rate of discharge of 97%, an average duration of oxygen therapy of 8 days, and a limited transfer to critical care facilities, while only one patient died[53]. A randomized study on 46 subjects[54] evaluated NAC 1,200-1,500 mg/day efficacy as an adjunctive treatment of moderate COVID-19-associated pneumonia. A statistically significant increase in blood oxygen saturation and ventilatory function and a quicker reduction in the volume of lung damage were obtained in patients receiving NAC compared to the standard treatment group. A higher rate of C-reactive protein reduction and a shorter hospitalization duration was also obtained in the NAC group[54].

In a pilot study[55], 92 patients with mild-moderate COVID-19-associated ARDS were treated with standard-of-care treatment and placebo (n=45) or NAC IV 40 mg/kg/day for 3 consecutive days (n=47). Although no statistical significance level was reached, better outcomes in the NAC-treated group were obtained in the distribution of the clinical status at day 28, the proportion of patients who required invasive ventilator support (38.3% *vs*. 44.4%), the number of ventilator-free days (17.4 *vs*. 16.6) and median time of ICU and hospital stay. A retrospective study[56] on consecutive patients hospitalized with moderate or severe COVID-19 pneumonia compared standard of care with additional oral NAC 600 mg twice daily for 14 days. Treatment with oral NAC led to significantly lower progression rates to severe respiratory failure compared to the control group (p<0.01). Patients in the NAC group presented significantly lower 14-day and 28-day mortality than controls (p<0.001 and p<0.01, respectively). NAC improved the PaO_2/FiO_2 ratio over time and decreased the white blood cell, C-reactive protein, D-dimers and LDH levels. Contrary to these promising findings, a double-blind, randomized, placebo-controlled, single-center trial[57] on 135 patients with confirmed or suspected severe COVID-19, with oxyhemoglobin saturation <94% or respiratory rate >24 breaths/minute, failed to demonstrate the efficacy of NAC to reduce the need for mechanical ventilation. The authors suggested that the administration regimen and/or baseline disease severity may be confounding factors.

Discussion

While the COVID-19 pandemic is ongoing, the number of survivors of severe disease is rapidly increasing, and facing the disabling sequelae is becoming of utmost importance. Growing evidence indicates that a serious problem is linked to the progression of COVID-19 pneumonia towards pulmonary fibrosis, as shown by radiological and histological findings, with consequent permanent respiratory impairment. Hence, the rationale for the treatment of COVID-related fibrosis was investigated, and the need for antifibrotic therapies was addressed by researchers[3].

NAC has a good tolerability profile, is easily administered orally and inexpensively, and has antioxidant and anti-inflammatory effects that may target the pathophysiologic mechanisms involved in COVID-19 infection and in pulmonary fibrotic sequelae. It may revert GSH deficiency, exert direct and indirect antioxidant activity, anti-inflammatory activity, and improve immune T-cell response. Although no evidence is available on the use of NAC in patients with post-COVID-19 respiratory impairment, some clinical experiences in COVID-19 patients with the early disease were carried out based on a similar rationale and produced encouraging results[48-50,54-56].

The effect of NAC is currently studied by the phase III PRECISIONS trial (NCT04300920)[58] in a subset of patients with idiopathic pulmonary fibrosis, with polymorphism in *TOLLIP*

genes, who were found to have better outcomes in comparison with the whole patient sample. Despite the debatable results of the IFIGENIA, PANTHER-IPF and PANORAMA trials[59-61], a subsequent post hoc analysis of PANTHER-IPF revealed that a polymorphism in the gene encoding Toll-interacting protein (*TOLLIP*; rs3750920) modifies the treatment effect of NAC. Indeed, a benefit was found for patients with the *TOLLIP* TT genotype[58]. PRECISIONS will compare the effect of NAC plus standard of care with matched placebo plus standard of care, evaluating the time to a composite endpoint of relative decline in lung function. The study is estimated to be completed in 2025.

Conclusions

In conclusion, a precision-guided approach may support the effective and safe use of adjunctive NAC in idiopathic pulmonary fibrosis, while the mechanism of action of NAC suggests a role in the treatment of pulmonary fibrosis induced by COVID-19.

Conflict of Interest

JL Izquierdo reports personal fees from AstraZeneca, Bayer, Boehringer Ingelheim, Chiesi, GSK, Grifols, Menarini, Novartis, Orion, Pfizer, Sandoz, Teva and Zambon. S Avdeev reports personal fees from Boehringer Ingelheim, Chiesi, Astra Zeneca, Teva, Bayer, Sandoz, Pfizer, Roche, and ZAMBON. C Micheletto reports fees as a speaker in national and international Congresses received from Zambon, Chiesi, Novartis, Astrazeneca, GSK, Menarini, Sanofi. MC Pacheco reports fees as a speaker for AstraZeneca, Bayer, Boehringer Ingelheim, GSK, Novartis, Novamed and Pfizer. Robin A Rada Escobar reports participations in advisory board for AstraZeneca, Boheringer-Ingelheim, GSK, Novamed, Novartis, Pfizer. Activity as speaker in medical events for AstraZeneca, Boheringer - Ingelheim, Bayer, GSK, Novamed, Novartis, Pfizer. Honoraries from: AstraZeneca, Boheringer - Ingelheim, Bayer, GSK, Novamed, Novartis, Pfizer. Consultant: None. Employer: Hospital Military, Bogotá Colombia. Tobacco Industry relationship: None.

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Authors' Contribution

All authors contributed to conception and drafting of the article; all authors approved the manuscript for submission.

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