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Where are we with the use of N-acetylcysteine as a preventive and adjuvant treatment for COVID-19?

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Abstract. – OBJECTIVE: As N-acetylcysteine (NAC) is promising as a re-purposed drug for the adjunctive or supportive treatment of serious COVID-19, this article aimed to describe current evidence.

MATERIALS AND METHODS: A search was performed in PubMed/Medline for "NAC", "viral Infection", COVID-19", oxidative stress", "inflammation", retrieving preclinical and clinical studies.

RESULTS: NAC is a pleiotropic molecule with a dual antioxidant mechanism; it may neutralize free radicals and acts as a donor of cysteine, restoring the physiological pool of GSH. Serious COVID-19 patients have increased levels of reactive oxygen species (ROS) and free radicals and often present with glutathione depletion, which prompts a cytokine storm. NAC, which acts as a precursor of GSH inside cells, has been currently used in many conditions to restore or protect against GSH depletion and has a wide safety margin. In addition, NAC has anti-inflammatory activity independently of its antioxidant activity.

CONCLUSIONS: Clinical and experimental data suggest that NAC may act on the mechanisms leading to the prothrombotic state observed in severe COVID-19.

Key Words:
N-acetylcysteine, Viral infection, Oxidative stress, Glutathione, Adjunctive therapy.

Introduction

Many people who have been infected by the novel coronavirus SARS-CoV-2 (severe acute respiratory syndrome-coronavirus-2) undergo asymptomatic, mild or moderate coronavirus disease 2019 (COVID-19). However, 14% and 5% of patients developed the severe or critical disease, respectively, in the first year of the pandemic, and morbidity is still a problem mainly for unvaccinated people[1,2]. The morbidity and mortality linked to this viral infection promoted active research to understand pathogenic mechanisms, with the aim to identify effective drugs for disease prevention and treatment. COVID-19 causes multiorgan dysfunction, including acute respiratory distress syndrome (ARDS), pneumonia with pulmonary hemorrhage and hepatic, cardiac and renal injury, along with a prothrombotic state, which may be responsible for pulmonary embolism, myocardial infarction, disseminated intravascular coagulation, stroke and death[3]. Since the beginning of the pandemic, older people and subjects with comorbidities had higher serious illness and death rates. This observation suggested that age- and disease-related biological factors may increase the detrimental effect of environmental stress factors, such as infectious viral agents. Indeed, individual susceptibility to environmental insults could be related to impaired redox homeostasis and reduced response to oxidative stress, occurring due to viral infections[4,5].

Reactive oxygen species (ROS) and free radicals are produced in the body of patients with serious COVID-19, who often present with a cytokine storm, i.e., increased concentrations of pro-inflammatory cytokines, such as interleukin (IL)-6, IL-1 and tumor necrosis factor-α (TNF-α)[6]. An endogenous deficiency of glutathione (GSH) has been suggested as a key factor in this imbalance of redox homeostasis, leading to serious manifestations and death in COVID-19 patients[5]. Considering these findings, N-acetylcysteine (NAC) is promising as a re-purposed

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drug for the adjunctive or supportive treatment of serious COVID-19. NAC is a pleiotropic molecule with a dual antioxidant mechanism; it may neutralize free radicals due to its sulphidrilic group -SH and, in addition, acts as a donor of cysteine, restoring the physiological pool of GSH[7]. Such activities could reduce patients' disease progression to severe forms, hospitalization, in-hospital mortality, and post-discharge comorbidity.

This article presents available evidence on the possible role of NAC in the preventive or adjunctive treatment of COVID-19.

Materials and Methods

This article is a narrative review of published studies. A search was performed in PubMed/Medline for "NAC," "viral Infection," COVID-19", oxidative stress," and "inflammation," retrieving preclinical and clinical studies.

The Role of Oxidative Stress in SARS-CoV-2 and Other Viral Infections

Oxidative stress is a nonspecific pathological condition characterized by an imbalance between ROS production and detoxification due to increased ROS production and inadequate homeostatic systems' response[5]. It is known that infection with respiratory viruses, including coronaviruses, is linked with changes in redox homeostasis in infected cells, leading to inflammation and subsequent tissue damage[8]. In several virus-induced conditions, inflammatory events are documented, such as activation of the NLRP3 (NOD-, LRR- and pyrin domain-containing protein 3) inflammasome by severe acute respiratory syndrome coronavirus E protein[9], and apoptosis induced by the 3C-like protease of severe acute respiratory syndrome coronavirus[10]. Lung autopsies of deceased COVID-19 patients demonstrated neutrophil infiltration in pulmonary capillaries, extravasation into the alveolar spaces and neutrophilic mucositis[11]. Viral infections are associated with a decrease in antioxidant defenses, in addition to the increased release of ROS, as exposure to pro-oxidants usually leads to nuclear translocation of the master redox-sensitive transcription factor NRF2, which activates antioxidant defenses[12]. Indeed, respiratory viral infections have been associated with inhibiting the NRF2-mediated pathways and the nuclear factor k chain transcription in B cells (NF-κB) signaling activation, promoting inflammation and oxidative damage during these infections[12]. Furthermore, there is evidence of a link between decreased expression of the antioxidant enzyme superoxide dismutase 3 (SOD3) in the lungs of elderly patients with COVID-19 and disease severity[13].

In addition, excessive ROS production may affect membrane lipids, integrins and cytoplasmic proteins in various circulating cells. These effects may be critical for red blood cells (RBCs), which may become dysfunctional. ROS excess may upset the Fe^{2+}/Fe^{3+} balance and disturb iron homeostasis, for which iron must be kept in the Fe^{2+} state to bind oxygen. The protonation of superoxide ions associated with Fe^{3+} within the hemoglobin heme keeps the iron in its higher oxidation state and is incapable of binding oxygen, resulting in less efficient oxygen transport despite a high oxygen supply[14]. Finally, an increased NADPH oxidase 2 activation was found in patients with COVID-19[15], and high levels of inflammatory markers were seen by some authors[16,17].

Glutathione Depletion and Viral Infection

GSH is a tripeptide essential for body homeostasis, with high antioxidant activity. It has a crucial role in the defense against cell damage induced by ROS and in several metabolic pathways[5]. The maintenance of the highest (millimolar) concentrations of reduced GSH in most cell types is necessary for the control of various biological processes, such as detoxification of foreign and endogenous compounds, protein folding, regeneration of vitamins C and E, maintenance of mitochondrial function, antiviral defense, regulation of cellular proliferation, apoptosis and immune response[5]. GSH deficiency has been detected in many conditions associated with an increased risk of COVID-19 infection and/or poor disease outcomes, such as advanced age, comorbidity, male sex and smoking[5,18]. In addition, reduced GSH has been found in chronic respiratory conditions and subjects prone to infectious events[19]. In the presence of GSH deficiency, mechanisms, such as inflammation promotion, reduction of the innate immune system response, and lack of direct antiviral activity of glutathione itself, seem to cooperate to induce severe forms of viral infection disease[18]. When ROS production is increased due to the low activity of GSH, cytokine release

and neutrophil infiltration are promoted[14]. In critically ill COVID-19 patients, a high neutrophil to lymphocyte ratio was reported and was found to be related to hospital mortality[20]. In addition, activated neutrophil infiltration may be a mechanism of the diffuse microvascular thrombosis and capillary leak syndrome frequently observed in critically ill patients with COVID-19[21]. Thrombosis was reported in 56.3% of over 2,900 patients with severe COVID-19, and 34% of ICU admitted patients were found to have thrombotic complications, where 16.1% were reported with deep vein thrombosis and 12.6% with pulmonary embolism[22]. Increased ROS production may favor thrombosis and affect RBCs function by interaction with membrane lipids, integrins and cytoplasmic proteins, with oxidation of membrane polyunsaturated fatty acids. Biophysical and biomechanical changes in the RBC membrane may interfere with the diffusion of oxygen and carbon dioxide and the deformability of RBCs[21].

NAC, an analog and a precursor of reduced GSH, has been demonstrated to improve the structural conformational integrity and the transcytosis of α-1-antitrypsin (A1AT) via a glutathione-mediated mechanism. These effects result in improved A1AT cellular uptake and functions[23]. Indeed, growing evidence indicates that alpha1-proteinase inhibitors might counteract infection by SARS-CoV-2. A1AT has been considered a candidate for COVID-19 treatment because of its protective effects against coagulation, inflammation, and cell death[24]. Thus, it may be speculated that NAC may act synergistically with A1AT because of its protecting role of A1AT inactivation mediated by ROS[25].

NAC Activity on Pathologic Events Present in COVID-19

Glutathione Depletion and Oxidative Stress

Long-term treatment with NAC has been observed to obtain a significant attenuation of influenza-like episodes, provided that it did not prevent viral infection[26]. In a randomized trial, 262 subjects with the chronic degenerative disease received NAC 1,200 mg for 6 months or a placebo. While seroconversion to influenza virus was similar in the two groups, among virus-infected subjects, symptoms were developed in only 25% of subjects under NAC treatment, versus 79% in the placebo group. Along with these clinical effects, cell-mediated immunity was improved, with a progressive and significant shift from anergy to normoergy following NAC treatment.

NAC has been currently used in many conditions to restore or protect against GSH depletion and has a wide safety margin[7]. Such protective activities can be explained by the protective effect of NAC against oxidative stress and glutathione depletion.

NAC acts as a precursor of GSH inside cells, as it is deacetylated to produce L-cysteine (L-cys), the rate-limiting amino acid in GSH synthesis[4,7]. It may so activate mechanisms mediated by GSH replenishment within cells. A relevant one is nucleophilicity: sulfhydryl groups (-SH) react with electrophilic metabolites, so DNA reactive metabolites and intermediates are bound and blocked[27]. In addition, NAC scavenges ROS (especially of hypochlorous acid and hydroxyl radicals [•OH])[27], and several reactive nitrogen species that play a role in the oxidation of lipids, proteins and DNA[28].

In parallel, NAC also inhibits the activation of NFκB, which mediates oxidative stress and biochemical pathways upregulating pro-inflammatory genes[4]. Inhibition of ROS-dependent activation of NFκB (induced by endosomal Toll-like receptor 3/hemagglutinin [TLR3/HA]) during influenza infection is the basis for prevention of pro-inflammatory cytokine increased production[29].

In addition, NAC has anti-inflammatory activity independently of its antioxidant activity. For example, NAC counteracts the release of Na, K-ATPase (NKA), a marker for cell necrosis, inhibiting neurogenic inflammation induced by a lipopolysaccharide[30].

It is known that ROS is involved in the pathogenesis of the acute lung injury of acute respiratory distress syndrome (ARDS). A deficient content in GSH in the alveolar epithelial lining fluid of patients with ARDS may represent a predisposing factor, which may explain NAC's protective effect[4]. A meta-analysis on the effects of NAC in patients with ARDS published in 2019 found that it shortened intensive care unit stay (-4.47 days; 95% CI: -8.79 to -0.14; p=0.04; I2 = 46%) compared to placebo, although overall mortality was not reduced. The heterogeneity and the limited number of studies did not allow to pool data of glutathione levels[31]. Ortolani et al[32] reported that NAC 50 mg/kg for 6 days protected the lungs of ARDS

patients, as evaluated by measuring the expired ethane and malondialdehyde, oxidized GSH dimer, and GSH in the epithelial lining fluid.

In a randomized, double-blind, placebo-controlled, prospective clinical trial in five ICUs, performed on ARDS patients, red blood cell GSH was repleted by intravenous NAC (70 mg/kg body weight), every 8 hours for 10 days; this was associated with a decreased number of days of acute lung injury and increased cardiac index[33]. In another study, ARDS patients hospitalized in ICU who received NAC (150 mg/kg on the first day, followed by 50 mg/kg for 3 days) had improved clinical outcomes, while extracellular total antioxidant power and total thiol molecules were increased[34].

Finally, 1,200 or 1,800 mg/day NAC counteracted systemic oxidative stress induced by low flow oxygen administration (oxidized erythrocyte glutathione, decreased thiol proteins and increased carbonyl proteins) in stable COPD patients[35].

Prothrombotic State

Experimental data suggest that NAC may act on the mechanisms leading to the prothrombotic state observed in severe COVID-19. NAC potentiates the vasodilator and antiaggregatory effects of endothelial nitric oxide. The mechanism seems to be linked to the ability of NAC to counteract the deficiency of the extracellular antioxidant enzyme glutathione peroxidase 3, which is associated with a deficiency of bioactive NO[36]. Intravenous NAC was demonstrated *in vitro* and *in vivo* to promote lysis of arterial thrombi that are resistant to conventional methods; the main molecular target of the antithrombotic activity of NAC is the Von Willebrand Factor (VWF) that cross-links platelets in arterial thrombi[37]. In addition, NAC reduced soluble plasma-type VWF multimers *in vitro*, degraded ultra-large VWF multimer strings extruded from activated endothelial cells, and inhibited VWF-dependent platelet aggregation and collagen binding. In mice, injected NAC resolved thrombi produced by ionophore treatment and reduced plasma VWF multimers[38].

Clinical Experience with NAC in COVID-19

Although a rationale for using NAC in the prevention or adjunctive treatment of COVID-19 has been thoroughly described, few clinical experiences were published. In a descriptive cross-sectional study on 164 patients with confirmed COVID-19, it was observed that moderate–severe patients who received NAC 1 gm IV TDS with standard therapy had an average hospital stay duration of 12 days, higher rate of discharge, the average duration of oxygen therapy of 8 days, lower number of deaths and reduced transfer to critical care facilities[39]. A randomized study evaluated NAC efficacy in the treatment of moderate COVID-19-associated pneumonia. Forty-six adult patients received standard therapy (hydroxychloroquine, azithromycin, enoxaparin, dexamethasone, tocilizumab) or standard therapy with iv. NAC 1200–1500 mg/day. 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. The rate of C-reactive protein reduction and the decrease of hospitalization duration in the group of patients who received NAC was statistically significantly higher than in the standard treatment group[40]. In a pilot study, 92 patients with mild–moderate COVID-19-associated ARDS were treated with standard-of-care treatment and either 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[41]. A retrospective study included consecutive patients hospitalized with moderate or severe COVID-19 pneumonia. Patients who received standard of care were compared with patients who additionally received NAC 600 mg b.i.d. orally 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 treatment significantly reduced 14-day and 28-day mortality in patients with severe disease ($p<0.001$, respectively). NAC improved the PO_2/FiO_2 ratio over time and decreased the white blood cell, C-reactive protein, D-dimers and LDH levels[42].

Contrary to these promising findings, a double-blind, randomized, placebo-controlled, single-center trial on 135 patients with confirmed or suspected severe COVID-19, with oxyhemo-

globin saturation <94% or respiratory rate >24 breaths/minute, failed to demonstrate the efficacy of NAC to reduce the need for mechanical ventilation. Among the reasons for this treatment failure, the Authors list the administration regimen and/or baseline severity of the disease[43]. These results need to be confirmed by properly designed prospective clinical trials.

Even if no clinical data are available, it may be speculated that the use of NAC could be taken into consideration for milder or earlier viral infections, where the anti-inflammatory activity could have major clinical effects. However, NAC could be beneficial to prevent oxidative stress due to high inspiratory O_2 fraction (FiO_2), as demonstrated in ARDS or COPD patients needing low-flow oxygen.

Conclusions

Several experimental and clinical data suggest a possible role of NAC as adjuvant therapy in the prevention or treatment of COVID-19. This well-known molecule, with a good safety profile, with a well-established role in treating liver failure due to GSH depletion induced by acetaminophen intoxication, is a good candidate to take into consideration also in the treatment of viral infections. Improvement of cell-mediated immunity, replenishment of GSH activity in cells, antioxidant and anti-inflammatory effects were the basis for better outcomes in patients with a viral infection, namely, COVID-19. The final demonstration of its efficacy requires clinical studies enrolling high numbers of patients, as COVID-19 is a little-known, multifactorial disease. The statistical significance level of results is not obtained by small samples.

It would be important to optimize NAC's use in different settings by identifying eligible conditions and defining a long-term protocol, which could completely elicit the antioxidant efficacy of NAC.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Authors' Contribution

All authors contributed to ideation, manuscript drafting and review.

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