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N-Acetylcysteine for coronavirus disease-19: A potential adjuvant therapy

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ABSTRACT

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV2) infection or known as coronavirus disease 2019 (COVID-19) is a highly infectious disease that has been declared as a world pandemic by WHO. Although the majority of patients only experience mild symptoms, older patients and those with comorbidities are in the risk of falling into critically ill and even death. This is thought to correlate with systemic inflammatory response and oxidative stress imbalance. N-acetylcysteine (NAC) is recognized as a potent mucolytic, yet its lesser-known function as an antioxidant is a precursor of glutathione. Basic aspects and either *in vivo* or *in vitro* studies showed various mechanisms of NAC acting as a counterbalance in viral infections and its role in decreasing inflammation and oxidative stress. High-dose NAC is reported to be effective as an antioxidant in pneumonia, influenza, sepsis, and acute respiratory distress syndrome. Early evidence in COVID-19 patients showed that NAC could be beneficial. This review gives the scientific background in considering NAC as an adjuvant treatment for COVID-19.

Keywords: Antioxidant; coronavirus disease-19; glutathione; N-acetylcysteine; oxidative stress

INTRODUCTION

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV2) is a novel coronavirus that causes an ongoing world pandemic of coronavirus disease 2019 (COVID-19) (1). Common symptoms are respiratory symptoms such as fever, cough, dyspnea, fatigue, and muscle sore. Majority of COVID-19 patients experience mild to moderate symptoms; meanwhile, 9-14% experience severe symptoms and 5% fall into critical conditions such as respiratory failure, acute respiratory distress syndrome (ARDS), septic shock, and multiple organ dysfunctions (2,3). ARDS and sepsis that happen in COVID-19 patients are induced by severe inflammatory reaction that involves cytokines and chemokines storm (4-5). Higher risks of ARDS and sepsis that ultimately will lead to deaths are found in older patients and those with comorbidities such as diabetes mellitus, cardiovascular diseases, chronic lung diseases, and cancer (6).

One of the biologic processes that are often found in older people or those with comorbidities is the decreasing level of endogen glutathione (GSH) due to chronic inflammation from oxidative stress and inflammatory cytokines (7). Previous studies reported a declining of GSH level in COVID-19 patients caused by oxidative stress and antioxidant imbalance (8). GSH is a commonly found antioxidant in human body cells, especially in the epithelial lining fluid (ELF) of airway that helps to reduce the inflammatory process in the lungs (9). We review how oxidative stress happens in COVID-19 and the role of N-acetyl cysteine (NAC) as a precursor of GSH and antioxidant in adjuvant treatment of COVID-19.

OXIDATIVE STRESS IN COVID-19

Oxidative stress is a phenomenon caused by an imbalance between production and accumulation of reactive oxygen species (ROS) in cells and tissues that will lead to cell damage (10). The pathophysiology of inflammation caused by viral infection is a complex chain reaction, but it has been proposed that viral infection is an insult that initiating inflammation that involves the activation of cellular immunity and release of inflammatory mediators and intra and extracellular toxic oxygen free radicals (11). Stress oxidative is also thought to be related to the pathogenesis, progressivity, and clinical severity of SARS-CoV2 infection (12,13), Animal study showed an increasing level of ROS and antioxidant imbalance in SARS-CoV-induced ARDS (14). Oxidized environment ROS and antioxidant depletion, including GSH, are needed by the virus to replicate and to evade the immune system (12).

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One of the mechanisms that trigger ROS production is the activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) by angiotensin II (Ang II) (15,16). It has been known before that SARS-CoV2 strongly binds to ACE2 receptor compared to SARS-CoV (17). ACE2 bioavailability decreases due to the binding, thus causing Ang II binds to type 1 angiotensin (AT1R) instead, stimulating the activation of NOX, ROS production, and inflammatory responses (18,19). NADPH oxidase activation is confirmed by the overexpression of NOX2 in COVID-19 patients (20). On the contrary, NOX inhibition in animal macrophages showed a reduction of oxidative stress and improvement of the disease (21).

SARS-CoV2 infection also causing an imbalance of redox by inducing down-regulation of nuclear factor erythroid 2 related factor 2 (Nrf2). The transcription factor Nrf2 plays a role in adaptation of cells under oxidative stresses environment. In the oxidative environment, Nrf2 stimulates transcription of the target genes with antioxidant response and redox homeostasis (22,23). Activation of these genes prevents expression of the inflammatory cytokines and activation of the macrophage inflammasomes (24,25). A study in lung biopsies from COVID-19 patients shown suppressing Nrf2 pathway; conversely, induction of Nrf2 increases antioxidant elements and reduces the inflammatory response (26).

ROS production is also stimulated by pro-inflammatory mediators (27). SARS-CoV2 phagocytosis by phagocytic cells such as neutrophils and macrophages activate transcription factors to produce a large amount of ROS and reactive nitrogen and chlorine species including superoxide, hydrogen peroxide, hydroxyl free radical, nitric oxide, peroxynitrite, and hypochlorous acid to kill the virus (28,29). Besides, the nonphagocytic cells can also produce ROS in response to pro-inflammatory cytokines (30). Some studies showed elevated cytokine pro-inflammatory production in severe COVID-19 patients, including interferon (IFN) γ, monocyte chemoattractant protein-1, granulocyte-macrophage colony-stimulating factor, macrophage inflammatory protein 1α, tumor necrosis factor-alpha (TNF-α), and pro-inflammatory chemokines (31-34). These pro-inflammatory mediators disorderly reactivate mononuclear phagocytes such as macrophages that lead to hyperinflammation (35). Shao et al. observed an increase in oxidative stress-sensitive gene expression in mononuclear peripheral blood cells of SARS-CoV-infected patients. This result shows that oxidative stress and SARS-COV infection are interdependent (36).

Just as oxidative stress can be induced by inflammation, it can also stimulate inflammation through the activation of complex pathways. ROS activation is triggered by the activation of transcription factor NF-κB. Oxidative stress could also induce the activation of NOD-like receptor protein 3 (NLRP3) inflammasome, a protein that triggers innate immune activation through the maturation of pro-inflammatory cytokines (37-39). NLRP3 was observed as a predisposing factor for cytokine storms in COVID-19 patients (34). Activation of the inflammasome also triggers pyroptosis and cell damage (40).

Other mechanisms associated with oxidative stress in COVID-19 are hemoglobinopathy and iron dysmetabolism (41). This mechanism occurs due to the SARS-CoV2 virus attacking the hemoglobin of erythrocyte cells, thereby releasing free Fe ions in the blood and increasing blood ferritin levels (42,43).

THE MECHANISM OF NAC AS AN ANTIOXIDANT

GSH, a tripeptide compound γ-L-glutamyl-L-cysteinyl-glycine or GSH, is the most important antioxidant produced by living cells. A study showed that severity of COVID-19 clinical manifestations might be associated with decreased GSH levels and increased ROS. Severe COVID-19 cases are associated to lower GSH levels, higher ROS levels, and higher redox status (ROS/GSH ratio) than mild-moderate cases (7). Cysteine in GSH has a sulfhydryl/thiol group (-SH), which has the ability to reduce and conjugate in the removal of other peroxides and xenobiotics (44). Cysteine is also a substrate that determines the rate of GSH synthesis. That is, when there is oxidative stress in COVID-19, GSH synthesis will increase through the Nrf2 activator and, of course, requires the availability of adequate cysteine (45). NAC works as an oxygen-free radical scavenger and also reload depleted GSH stores, enhancing the endogenous antioxidant defense. In experimental animals infected with influenza, NAC can promote GSH production (46). N-acetyl cysteine works as an antioxidant directly or indirectly by releasing its cysteine or thiol groups or by breaking sulfide bonds. NAC easily penetrates cells where it is deacetylated to L-cys so that it can be a GSH precursor in the cell (47).

Although studies related to oxidative stress on SARS-CoV2 are still limited, similar studies for the SARS virus can be used as a comparison. The cytokine profile in the inflammatory response that occurs in SARS is almost similar as that in a patient with COVID-19, which generates an immune response involving diverse pro-inflammatory cytokines (interleukins, TNF, and IFNs). Type-I IFNs are suppressed during SARS-CoV infection which at last antagonizes IFN, causing delayed IFN. NAC can strengthen the role of toll-like receptors 7 and antiviral signaling protein in restoring type-I IFN production in COVID-19, thus reducing the process of oxidative stress (48). The antioxidant effect of NAC can also be in the form of inhibition of the activation of the transcription factor NF-κB as demonstrated in *in vitro* influenza (A and B) models (49). Activated NF-κB will produce various inflammatory cytokines which then further activate cellular immunity, infiltrate macrophages and neutrophils, and trigger cytokine storm.

The action mechanism of NAC can also originate from inhibition of SARS-CoV2 binding to its receptor. The envelope protein of SARS-CoV has sequence similarity with that of SARS-CoV2. It consists of a triple cysteine structure connected through disulfide bonds and NAC may cleave these bonds. This may decrease viral infectivity (50). *In vivo* study has shown how NAC can inhibit ACE. Administration of isosorbide dinitrate which has vasodilator activity was administered for 48 h, then at 24 h NAC was added. There was a significant depletion of Ang II plasma concentrations after 2 h NAC addition (51). Another *in vitro* study also demonstrated inhibition of Ang II production by reducing Ang II binding with Ang II type 1 receptor in a dose-dependent manner (52). Several action mechanisms of NAC in treating COVID-19 can be seen in Figure 1.

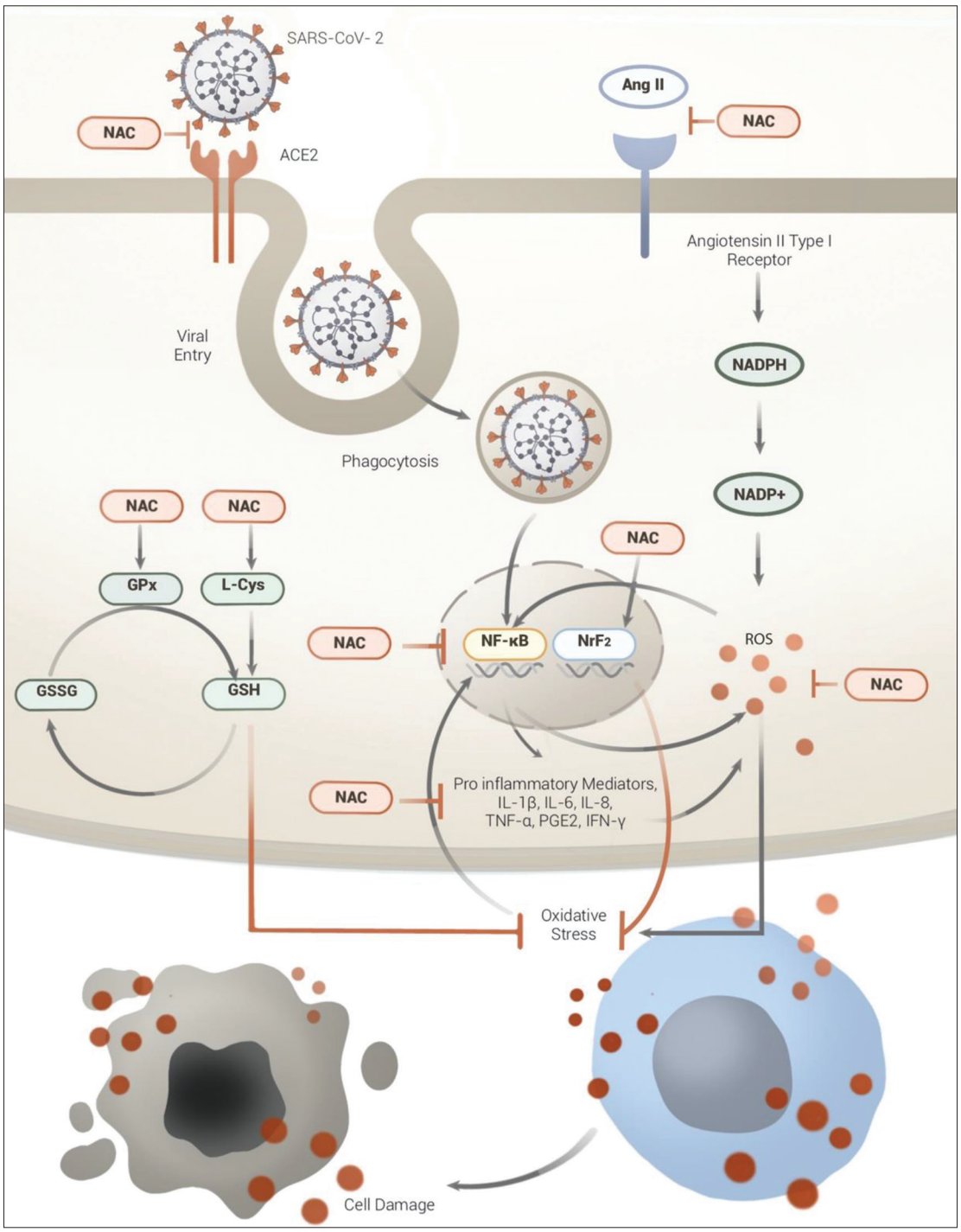

FIGURE 1. Schematic representation of potential mechanisms of NAC as antioxidant and anti-inflammatory in SARS-CoV2 infection. NAC: N-acetylcysteine, SARS-CoV2: Severe acute respiratory syndrome coronavirus 2, ACE2: Angiotensin-converting enzyme 2, Ang II: Angiotensin II, GPx: Glutathione peroxidase, GSSG: Glutathione disulfide, GSH: Glutathione, L-Cys: L-Cysteine, NF-κB: Nuclear factor-κB, NrF2: Nuclear factor erythroid 2-related factor 2, NADPH: Reduced nicotinamide-adenine dinucleotide phosphate, ROS: Reactive oxygen species, TNF-α: Tumors necrosis factor-alpha, IL: Interleukin, PGE2: Prostaglandin E2, IFN-γ: Interferon-gamma.

APPLICATION AND TRIAL NAC IN COVID-19

NAC in COVID-19 is administered empirically based on its efficacy from previous studies on influenza, pneumonia as well as severe cases such as acute lung injury or ARDS. Multicenter, a double-blind trial in Italy, reported that administration of NAC 600 mg twice daily for 6 months during winter shows a significant attenuation of influenza and influenza-like episodes, particularly in high-risk individuals. There were 25% of patients under NAC arm had symptoms while 79% symptomatic patients in the placebo arm. NAC reduced the symptoms even though did not prevent the disease (53). Another small RCT study performed by giving high doses of NAC 1200 mg/day for 10 days in community-acquired pneumonia patients has shown improvement in oxidative stress and inflammatory variables but not radiological changes compared to standard care only. No clinical outcomes were reported (54).

A systematic review of assigning NAC to ARDS included eight trials totaling 289 patients, concluding that NAC shortened intensive care unit length of stay but did not decrease the overall mortality. Duration of mechanical ventilation, GSH levels, and hypoxemia severity could not be

TABLE 1. Clinical trials using systemic NAC as a therapeutic agent for COVID-19

Study	Clinical trial ID	Intervention	Primary outcome
A study of NAC in patients with COVID-19 infection (57)	NCT04374461 Phase 2	NAC IV 6 g/day	Number of patients who are successfully extubated and/or transferred out of critical care due to clinical improvement and discharged from the hospital due to clinical improvement
Efficacy of NAC in preventing COVID-19 from progressing to severe disease (58)	NCT04419025 Phase 4	Inpatients: • NAC 25 mg/kg oral q 4 h until discharge • NAC 1200 mg oral Outpatients: NAC 2400 mg oral then 1200 mg oral twice a day × 2 weeks	• Decrease in dyspnea measured by respiratory rate • Hospital length of stay • Need for mechanical ventilation • Length of time intubated • Need for hospitalization • Outpatients on NAC needing admission to the hospital • Recovery disposition
Inflammatory regulation effect of NAC on COVID-19 treatment (INFECT-19) (59)	NCT04455243 Phase 3	NAC 150 mg/kg every 12 h for 14 days (oral/intravenous)	Time to recovery
A study to evaluate OP-101 (Dendrimer N-acetyl-cysteine) in severe coronavirus disease 2019 (COVID-19) patients (PRANA) (60)	NCT04458298	OP-101 (Dendrimer N-acetyl-cysteine) 2-8 mg/kg	Number of participants with treatment-emergent adverse events

NAC: N-acetylcysteine

further analyzed in meta-analysis (55). Another meta-analysis observing NAC in sepsis and systemic inflammatory response in adult showed that NAC did not show shortening on length of stay and duration of mechanical ventilation, also did not lower the incidence of new organ failure. Early administration of NAC did not prevent the progression of severe oxidative stress and inflammation; otherwise, late administration might be associated with cardiovascular instability (56).

The NAC clinical trial on COVID-19 at the time of this review is written is still in progress. At present, 10 clinical trials are registered in ClinicalTrials.gov, in which NAC is being evaluated as adjuvant therapy for COVID-19. Among these, there are four clinical trials, are solely NAC without being mixed with other drugs (Table 1) (57-60). Apart from oral and intravenous administrations, NAC can also be given by nebulization. Nebulized NAC can reduce mucus density and improve oxygen saturation (61). Phase 3 research regarding nebulized NAC in COVID-19 is currently underway (62). Another study is the Efficacy and Safety of Nebulized Heparin-NAC in COVID-19 Patients by Evaluation of Pulmonary Function Improvement (HOPE) in ventilated COVID-19 patients. The study aims to increase ventilator-free days in hospitalized patients with moderate-severe COVID-19 (63). The results of these studies will greatly influence the clinical application of NAC use in COVID-19.

CONCLUSIONS

SARS-CoV2 infection or COVID-19 is associated with inflammation and oxidative stress imbalance. GSH is an antioxidant that is widely found in the body and plays a role in protecting cells from oxidative stress. NAC has antioxidant properties directly or indirectly through the release of cysteine groups as a precursor compound in the GSH synthesis process. Previous studies, preliminary research data on COVID-19, and reviews of the mechanisms of oxidative stress and inflammation in COVID-19 suggest that NAC can be beneficial as adjuvant therapy in COVID-19. Clinical trials regarding the role of NAC in COVID-19 are still needed.

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