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N-Acetylcysteine: A potential therapeutic agent for SARS-CoV-2

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ABSTRACT

COVID-19, a respiratory disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), continues to spread across the globe. Predisposing factors such as age, diabetes, cardiovascular disease, and lowered immune function increase the risk of disease severity. T cell exhaustion, high viral load, and high levels of TNF-α, IL1β, IL6, IL10 have been associated with severe SARS-CoV-2. Cytokine and antigen overstimulation are potentially responsible for poor humoral response to the virus. Lower cellular redox status, which leads to pro-inflammatory states mediated by TNF-α is also potentially implicated. *In vivo, in vitro*, and human clinical trials have demonstrated N-acetylcysteine (NAC) as an effective method of improving redox status, especially when under oxidative stress. In human clinical trials, NAC has been used to replenish glutathione stores and increase the proliferative response of T cells. NAC has also been shown to inhibit the NLRP3 inflammasome pathway (IL1β and IL18) *in vitro*, and decrease plasma TNF-α in human clinical trials. Mediation of the viral load could occur through NAC's ability to increase cellular redox status via maximizing the rate limiting step of glutathione synthesis, and thereby potentially decreasing the effects of virally induced oxidative stress and cell death. We hypothesize that NAC could act as a potential therapeutic agent in the treatment of COVID-19 through a variety of potential mechanisms, including increasing glutathione, improving T cell response, and modulating inflammation. In this article, we present evidence to support the use of NAC as a potential therapeutic agent in the treatment of COVID-19.

Introduction

COVID-19, a respiratory disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), continues to spread across the globe. SARS-CoV-2 is an enveloped positive-sense, single stranded-RNA virus in the betacoronavirus genus [1,2]. SARS-CoV-2 is genomically similar to severe acute respiratory coronavirus 1 (SARS-CoV-1), which led to an outbreak of severe acute respiratory syndrome (SARS) in the early 2000s. No cases of SARS have been reported globally since 2004 [1].

Similar to SARS-CoV-1, SARS-CoV-2 gains entry into cells through spike protein affinity for angiotensin converting enzyme 2 receptors (ACE2) and uses host cell serine protease TMPRSS2 to mediate entry [3–5]. ACE2 receptors are expressed in lung, heart, kidney, and intestinal tissue and primarily function physiologically in the maturation of angiotensin [6].

The pathogenicity of the SARS-CoV-1 stemmed from nod like receptor, pyrin domain containing 3 (NLRP3) inflammasome activation in monocytes and macrophages [7,8]. This led to high levels of cytokines: interleukin-1β (ILβ), interleukin-18 (IL18), tumor necrosis factor alpha (TNF-α); and an immunopathological response associated with acute respiratory distress syndrome (ARDS), cytokine storms, organ damage, and death [7,9–11].

Although SARS-CoV-2 is 78% genomically similar to SARS-CoV-1, there are variations in the open reading frames (ORFs) involved in the inflammatory monocyte and macrophage response [3,12]. Research is underway to elucidate these mechanisms further. In severe COVID-19, there are increased levels of cytokines interleukin-6 (IL6), interleukin-10 (IL10), and TNF-α [13]. In some cases, these increased cytokine levels create a "cytokine storm" and cause significant damage to lung tissue [14].

Clinically, COVID-19 is mild in most cases, with severe cases characterized by pneumonia and critical cases characterized by ARDS, sepsis, and multiple organ failure [14]. Older adults, aged 65 years and older, appear to be most susceptible to COVID-19 [14,15]. The most susceptible persons to SARS-CoV-2 are the elderly: the typical profile of the critically ill patient is 65 years and older, presents with comorbidities, and ARDS [9]. These patients had a mortality rate of 67% from the time of admittance to 28 days later [9].

N-acetylcysteine (NAC) is a precursor of glutathione and acts as a powerful antioxidant and free radical scavenger in the body [16]. NAC has been used for paracetamol toxicity and conditions with viscous

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mucous secretions [17]. NAC may be administered orally, intravenously, or nebulized.

Hypothesis

We hypothesize that NAC could act as a potential therapeutic agent in the treatment of COVID-19 through a variety of potential mechanisms, including increasing glutathione [18], improving T cell response [19–21], and modulating inflammation [22,23]. In this article, we present evidence to support the use of NAC as a treatment for COVID-19.

Evaluation of hypothesis

SARS-CoV-2 and the elderly

SARS-CoV-2 is especially virulent in elderly populations. The virus gains entry primarily via ACE2 receptors on alveolar type 2 epithelium [24]. Hypertension, a known risk factor for developing more severe COVID-19 disease, is more common in older adults [25,26]. In adults with a history of taking angiotensin converting enzyme inhibitors and angiotensin receptor blockers to treat hypertension, there is a possible increase in ACE2 receptors [27]. Although the relationship has yet to be clearly defined, viral infectivity may be increased due to increased expression of ACE2 secondary to treatment with ACEI and ARBs in the elderly [28].

In SARS-CoV-1, the immune response was dominated by a Th1 response, but in SARS-CoV-2 there are both Th1 and Th2 responses [29]. In SARS-CoV-1, an inflammatory monocyte and macrophage cascade activated via the NLRP3 inflammasome pathway [7,8]. This resulted in pro-inflammatory cytokines IL1β and IL18, and an immunopathological response causing injury to lung tissues [7]. In SARS-CoV-2, there is a remarkable increase of cytokines IL6, IL10, and TNF-α [15], however, similar to SARS-CoV-1, there appears to be elevated levels of IL1β, which suggests possible pro-inflammatory macrophage and monocyte activity activated by the NLRP3 inflammasome pathway [15,30].

T cell exhaustion has been noted in severe SARS-CoV-2, illustrated by high levels of programmed cell death protein 1 (PD1) and low CD4+ and CD8+ counts [15]. T cell exhaustion leads to poor CD4+ and CD8+ responses, which limits viral clearance via humoral immunity [31]. The likely cause of T cell exhaustion in SARS-CoV-2 is hypothesized to be from excessive stimulation of T cells by viral antigen and cytokines (IL6, IL10, TNF-α) [15]. Notwithstanding SARS-CoV-2 infection, the elderly have an increased risk of mortality due to age related pro-inflammatory changes [32]. One mechanism suggested is increased TNF-α induced apoptosis that occurs in the elderly [33]. In severe SARS-CoV-2, Diao et al. suggest that age related pro-inflammatory changes to TNF-α expression may be a causative factor in T cell exhaustion [15]. Additionally, elderly adults may also have decreased redox potential via lower glutathione levels [34–36]. Lowered redox status of a cell increases susceptibility to oxidative stress that may lead to cell death and viral release [37].

NAC and glutathione

Glutathione conjugates oxidative species through phase 2 detoxification. It is a central oxidative species mediator that impacts cell cycle and apoptosis [38,39]. L-cysteine is the rate-limiting substrate in the intracellular synthesis of glutathione [39]. NAC directly affects the amino acid pool of extracellular cystine and intracellular cysteine through a series of redox reactions in the plasma [40]. NAC increases extracellular cysteine, and through transport channels increases intracellular cysteine levels [18,38,41]. During oxidative stress, NAC will increase glutathione synthesis [18,42]. Without oxidative stress, the pool of cysteine and cystine appears to primarily mediate cellular stress through thiols other than glutathione [18,38].

Individuals 60 years and older demonstrate lower plasma glutathione levels and increased oxidative stress [43]. Those with diabetes have lower glutathione levels compared to control subjects [35,43]. Dietary supplementation of cysteine and glycine can increase glutathione levels and reduce oxidative stress in the elderly [36] and persons with diabetes [35].

NAC and CD4 and CD8

Glutathione concentrations affect the proliferative capacity of lymphocytes: low concentrations lead to less lymphocyte proliferation and high concentrations lead to more lymphocyte proliferation [44]. T cell exhaustion occurs when lymphocytes (CD4+ and CD8+) counts are low, and can be measured through program cell death protein 1 (PD1) [31]. It is commonly seen in chronic viral infections [31].

PD1 was elevated in severe COVID-19 cases [15]. Diao et al. referred to this state, in conjunction with significantly lowered CD4+ and CD8+ counts, as functional T cell exhaustion [15]. The investigators posited that functional T cell exhaustion is an etiological factor in severe SARS-CoV-2 infection. They determined the observed T cell exhaustion is associated with and likely caused by overstimulation of cytokines (IL6, IL10, TNF-α).

Lymphocyte proliferation has been noted with the administration of NAC. In patients with HIV, oral NAC increased both whole blood glutathione levels and lymphocyte count (CD4+ and CD8+) [20,21]. NAC has also shown to decrease PD-1 levels and increase the longevity of CD8+ cells *in vitro* [19].

NAC and NLRP3

Data from SARS-CoV-1 indicates that this coronavirus mediates the NLRP3 inflammasome pathway in infected cells by ORFs 3a and 8b [7,45,46]. This pathway has been indicated in inflammatory cell death (necroptosis) and is likely responsible for the pathological findings found in lung biopsy of SARS-1 patients [45,47].

Likewise in SARS-CoV-2, there is an elevated level of IL1β suggesting NLRP3 activation in macrophages and monocytes [29,30,48]. TNF-α is usually increased early in the inflammatory response [49,50], and can be secreted by monocytes, macrophages, and alveolar epithelium [50]. TNF-α attaches to receptor sites and depending on the redox potential of the cell, may induce an apoptotic or a proliferative pathway [51].

In vitro, NAC interrupts the NLRP3 inflammasome pathway in a dose dependent manner through effects on mRNA expression of NLRP3 and caspase-1 activation [22]. NAC lowers IL1β, IL18 [22]. NAC has been shown to lower mucin production, and IL6 and TNF-α *in vitro* [52]. NAC inhibits the downstream activities post TNF-α receptor activation [52], and while under oxidative stress NAC inhibits gene expression of TNF-α and IL-6 [23].

Clinical trials

There have been several clinical trials investigating the use of NAC in respiratory illness in humans. Intravenous NAC has been used clinically for the treatment of ARDS. In a *meta*-analysis of randomized clinical trials investigating the use of NAC as a treatment for ARDS, administration of NAC resulted in a decreased length of stay in intensive care units, however, there was no change in overall short term mortality. The *meta*-analysis found no adverse reactions with doses similar to those used in drug-induced liver injury [53]. In both *in vivo* and human trials, nebulized NAC may improve arterial oxygen tension [54,55]; and attenuate pulmonary fibrosis [56], and ARDS [57].

In a randomized clinical trial, oral NAC demonstrated decreases in TNF-α and no adverse reactions at 1200 mg daily, however there were no changes in computed tomography scores between those treated with NAC and the control group [58]. In another trial involving multiple

elder care facilities, oral NAC was investigated as a prophylactic and therapeutic agent for influenza. Participants who were given 1200 mg daily for six months experienced fewer influenza and influenza-like episodes, decreased severity of illness, and fewer days confined to bed [59]. In this trial, NAC did not alter viral seroconversion but participants taking NAC were less likely to develop a symptomatic infection [59]. These findings are especially impactful given the higher-risk study population.

Ventilator use is common in severe cases of COVID-19, with approximately 3% of all cases requiring intubation and invasive ventilation at some point during the course of illness [60]. NAC has been studied as a prophylactic intervention for ventilator associated pneumonia (VAP), a complication of the use of mechanical ventilation [61]. In a randomized, double-blind, placebo-controlled trial of 60 participants receiving nasogastric administration of 1200 mg NAC daily, participants in the treatment group were less likely to develop VAP and had a shorter duration of hospital and ICU stay. Additionally, the incidence of complete recovery from VAP was higher in the NAC group.

NAC has an excellent safety record in clinical trials. Oral NAC can cause stomatitis, nausea, vomiting, gastroesophageal reflux [17,62]. Oral NAC may be used if there has been an anaphylactoid reaction to IV NAC [17]. Nebulized NAC may cause bronchoconstriction and prolonged coughing and may worsen asthma [55,63].

Serious adverse reactions with IV NAC are rare, loading dose dependent, and primarily occur in drug-induced liver injury [17,64]. In drug-induced liver injury from paracetamol, 10 g of NAC is typically loaded in 15 min. This can result in upwards of 31% risk of anaphylactoid reactions ranging from pruritus, rash, angioedema, bronchospasm, and hypotension [65]. With an alternate loading schedule the overall risk of anaphylactic reaction can be lowered to 5% [65]. Anaphylactoid reactions occur with IV NAC and can be managed with attentive care [17]. But as noted in the ARDS *meta*-analysis, no adverse reactions were recorded with a similar loading dosage used in drug-induced liver injury [53].

Discussion

NAC has shown activity in a variety of potential therapeutic target pathways involved in the pathophysiology of SARS-CoV-2 infection. The pathogenic factors of SARS-CoV-2 that could possibly be mediated by NAC are (1) T cell exhaustion, which manifests as lower counts and decreased functional capacity of CD4+ and CD8+ cells; (2) pro-inflammatory state via increase in TNF-α, IL1β, IL18; and (3) modulation of viral activity through increased glutathione.

The virus is especially virulent in the elderly population. Certain physiological conditions, including diabetes, cardiovascular disease, and lowered immune function that may affect the severity of SARS-CoV-2 are more common in older adults. Lowered redox status, common in high-risk groups including older adults and those with uncontrolled diabetes, causes alterations in the TNF-α receptor activity towards a pro-inflammatory state [33,51]. NAC has been shown to replenish glutathione stores and increase the proliferative response of T cells. NAC has also been shown to inhibit the NLRP3 inflammasome pathway (IL1β and IL18) *in vitro*, and decrease plasma TNF-α. Mediation of the viral load could occur through the ability of NAC to increase cellular redox status by maximizing the rate limiting step of glutathione synthesis, and thereby decreasing the effects of virally induced oxidative stress and cell death.

To test these hypotheses further, we recommend both *in vitro* and *in vivo* studies investigating NAC. *In vitro* studies may include investigations into the action of NAC on certain cell types such alveolar type 2 epithelial cells, monocytes, or macrophages, as well changes in receptor and cytokine expression in these cell types. Other studies could investigate the action of NAC in human cell lines with low redox potential.

Human clinical trials would build on current evidence for the use of NAC in other infectious respiratory illnesses. Hospital-based clinical trials could administer nebulized, oral, or IV NAC during various stages of illness, perhaps utilizing grouping identified by Diao et al. Similar to clinical trials in influenza, ARDS, and VAP, outcome measures could include change in SARS-CoV-2 associated cytokines, change in lymphocyte count and activation, change in ICU status, and overall mortality.

While no clinical trials investigating the use of NAC in COVID-19, trials completed in using NAC for influenza, ARDS, and VAP have shown promising results in reducing disease severity and duration of hospital stay. Currently, a protocol for using both NAC and heparin has been developed by a Seattle-based biotherapeutics researcher. The protocol is available for use by clinicians but at the date of this publication, data from studies using this protocol have not been published [66].

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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