



Therapeutic blockade of inflammation in severe COVID-19 infection with intravenous N-acetylcysteine

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ABSTRACT

Glucose 6-phosphate dehydrogenase (G6PD) deficiency facilitates human coronavirus infection due to glutathione depletion. G6PD deficiency may especially predispose to hemolysis upon coronavirus disease-2019 (COVID-19) infection when employing pro-oxidant therapy. However, glutathione depletion is reversible by N-acetylcysteine (NAC) administration. We describe a severe case of COVID-19 infection in a G6PD-deficient patient treated with hydroxychloroquine who benefited from intravenous (IV) NAC beyond reversal of hemolysis. NAC blocked hemolysis and elevation of liver enzymes, C-reactive protein (CRP), and ferritin and allowed removal from respirator and veno-venous extracorporeal membrane oxygenator and full recovery of the G6PD-deficient patient. NAC was also administered to 9 additional respirator-dependent COVID-19-infected patients without G6PD deficiency. NAC elicited clinical improvement and markedly reduced CRP in all patients and ferritin in 9/10 patients. NAC mechanism of action may involve the blockade of viral infection and the ensuing cytokine storm that warrant follow-up confirmatory studies in the setting of controlled clinical trials.

1. Introduction

New York is currently the epicenter of the current COVID-19 pandemic caused by SARS-CoV-2. Up until this report, cases of hydroxychloroquine-induced hemolysis in G6PD-deficiency patients affected by COVID-19 have been lacking despite the widespread use of hydroxychloroquine as a treatment option for COVID-19. Hydroxychloroquine oxidative properties decreases glutathione (GSH) levels. Normally, cellular GSH can be regenerated from its oxidized form at the expense of reduced nicotinamide adenine dinucleotide phosphate (NADPH), which is an essential product of the pentose phosphate pathway (PPP). G6PD is a rate-limiting enzyme of NADPH synthesis by the PPP [1]. Thus, diminished production of NADPH leads to a profound depletion of GSH and the subsequent risk of hemolysis in G6PD-deficient patients. NAC can reverse the depletion of GSH imposed by diminished production of NADPH through the PPP [2]. Here, we report a case of hydroxychloroquine-induced severe hemolysis in a G6PD-deficient patient with COVID-19 infection and the successful treatment with IV NAC.

2. NAC treatment of G6PD-deficient patient and nine additional respirator-dependent subjects

A 44-year-old man presented to NYU Langone emergency department on March 20th 2020 with a 5-day history of fever, cough, and shortness of breath. He was earlier diagnosed with G6PD deficiency after hemolytic reaction to sulfa drugs. On admission, physical exam was notable for a body mass index of 37, blood pressure of 138/81 mmHg, pulse of 102 beats/min, respiratory rate of 25 per minute, and temperature of 39.5C. His oxygen (O₂) saturation was 85% on room air and 94% on 4 l of O₂ via nasal cannula. Patient tested positive for SARS-CoV-2 by PCR. Upon admission, his inflammatory markers, such as C-reactive protein (CRP), ferritin, and D-dimer, neutrophil to lymphocyte ratio (NLR) were elevated. His liver function tests, hemoglobin (Hb), and white blood cell count were normal (Table 1). Patient was started on hydroxychloroquine on March 21st and received only one dose of 400 mg. His respiratory status continued to worsen and required intubation on March 24th. Despite intubation and maximum ventilation

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Table 1

Laboratory test values of G6PD-deficient patient upon admission for COVID-19 infection before administration of hydroxychloroquine.

Variable	Admission value	Reference value
G6PD U/g Hemoglobin	0.5	> 9
White blood cells $\times 10^3 / \mu\text{L}$	5.3	4.2–9.1
Hemoglobin mg/dL	12.6	13.7–17.5
Platelets $\times 10^3 / \mu\text{L}$	205	150–400
Neutrophil %	73	34–68
Lymphocyte %	18	22–53
C-reactive protein mg/L	45	0–5
Ferritin ng/ml	491	22–248
D-dimer ng/ml	520	< 230
Bilirubin, total mg/dL	1.0	0.2–1.2
Bilirubin, direct mg/dL	0.5	0–0.5
Interleukin-6 pg/ml	20	< 5

settings the patient respiratory status continued to deteriorate requiring veno-venous extracorporeal membrane oxygenator (VV ECMO) which was started on March 26th.

On March 29th, his Hb level dropped (7.9 g/dL) and his direct (6.0 mg/dL) and total bilirubin have become dramatically elevated (9.0 mg/dL). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) peaked at 263 U/L and 338 U/L, respectively. Further testing revealed low G6PD level (0.5 U/g Hb) and haptoglobin (2 mg/dL). Blood smear showed bell cells suggesting G6PD-deficiency hemolysis. The patient was started on IV NAC on March 30th (30,000 mg divided into three doses over 24 h). This was followed by immediate improvement in hemolysis indices (Fig. 1A). ALT and AST improved to 100 U/L and 62 U/L, respectively. On April 7th, one week after IV NAC discontinuation, total and direct bilirubin started rising again and IV NAC was re-started at 600 mg every 12 h for one week. Again, IV NAC administration was associated with resolution of hemolysis as evident by sustained reduction in bilirubin (total and direct) (Fig. 1A) and an increase in haptoglobin. Patient oxygenation continued to improve and his VV ECMO was discontinued on April 9th. Ten days after discontinuation of the second round of IV NAC, a slight increase in both total and direct bilirubin was noted, IV NAC was started again on April 25th at 600 mg every 12 h, this again was associated with reduction in total and direct bilirubin (Fig. 1A). Patient continued to improve clinically and was discharged to rehab on April 27th and was then discharged home on April 30th. Of note, the patient was treated with steroids starting March 30th. Interestingly, we observed a reduction in inflammatory markers (CRP and ferritin) that coincided with IV NAC administration (Fig. 1B). Additionally, IV NAC was associated with a decrease in NLR that was sustained after the first dose (Fig. 1C).

Due to this successful outcome, IV NAC was given to 9 consecutive COVID-19 patients without G6PD deficiency (Table 2). Eight of the nine patients required VV ECMO. We have observed a significant overall reduction in inflammatory markers (CRP and ferritin) during IV NAC administration. A rebound of inflammation was noted in six patients following discontinuation of NAC (Supplementary Figs. S1–S6). In the other three patients IV NAC was associated with decrease in CRP and ferritin without rebound increase after discontinuation. The median CRP level during IV NAC administration 55 mg/dL [interquartile range (29–109)] was significantly lower than during the periods without IV

NAC (either before administration 143 mg/dL (46–235), or after IV NAC discontinuation 69 mg/dL (27–114) (Fig. S7). Table 2 displays peak CRP levels before NAC as well as CRP level at the completion of NAC administration for each individual patient.

3. Discussion

We describe a remarkable benefit of IV NAC in severe COVID-19 infection. As expected, IV NAC initially mitigated the hemolysis in a G6PD-deficient, COVID-19-infected patient treated with hydroxychloroquine for a single day. We also observed an obvious drop in inflammatory markers following initial NAC administration. This was followed by rebound rises of CRP and ferritin upon discontinuation of NAC. Re-started IV NAC for two additional intervals resulted in repeated drops in CRP and ferritin levels (Fig. 1B). NAC administration allowed the discontinuation of ECMO and eventual discharge of the patient to his home.

G6PD deficiency was first described in the setting of hemolysis induced by the anti-malarial medication primaquine [3]. In red blood cells, G6PD is essential for the PPP to produce NADPH, which is used by glutathione reductase to regenerate GSH from oxidized glutathione (GSSG) [1]. The depletion of GSH in G6PD deficient cells can be reversed by NAC that helps replenish cellular GSH [4]. An *in-vitro* study showed that G6PD deficient cells were more susceptible to infection by the human coronavirus (HCoV 229E) [5]. The association between G6PD deficiency and COVID 19 has not yet been reported despite the widespread use of hydroxychloroquine. This could be due to the minimal oxidative stress that hydroxychloroquine exerts compared to chloroquine and primaquine [6]. Additionally, G6PD deficiency can vary in severity based on specific genetic polymorphisms. Nonetheless, our case shows that caution should be exerted when considering hydroxychloroquine as a treatment option for these patients. Reports from other epicenters where G6PD deficiency is far more prevalent -such as Italy- may shed more light on a potentially overlooked association.

The CRP and ferritin responses to IV NAC were favorable in our patient, and we observed similar benefit in nine additional patients without G6PD deficiency. Anti-viral [7,8] and anti-inflammatory properties of NAC have been well documented [9–11]. The morbidity and mortality of human coronaviruses causing lower respiratory tract infections appears to stem from the exuberant immune response of the host. High serum levels of pro-inflammatory cytokines have been reported. Interleukin-6 (IL-6) has been proposed to play an essential role in COVID-19 associated cytokine storm [12]. NAC has been found to reduce IL-6-dependent CRP elevation during H1N1 influenza pneumonia [13]. NAC is a cell-permeable precursor of reduced GSH. Pre-clinical studies have shown that GSH-capped nanoclusters inhibit coronavirus replication through blockage of viral RNA synthesis and budding [7]. Furthermore, an *in vitro* study showed that NAC was able to reduce H5N1 viral replication [8]. Apparently, host cell infection by COVID-19 depends on the interaction between the receptor binding domain (RBD) of the viral spike glycoprotein S2 subdomain and the peptidase domain of the angiotensin converting enzyme 2 (ACE2) receptor [14]. The S2 subdomain of SARS-CoV-1, which lies 6 amino acids away from the fusion peptide, is flanked by two cysteine residues that are essential for membrane fusion [15], is conserved across all coronaviruses (Fig. 2). The post- translational disulfide bond between

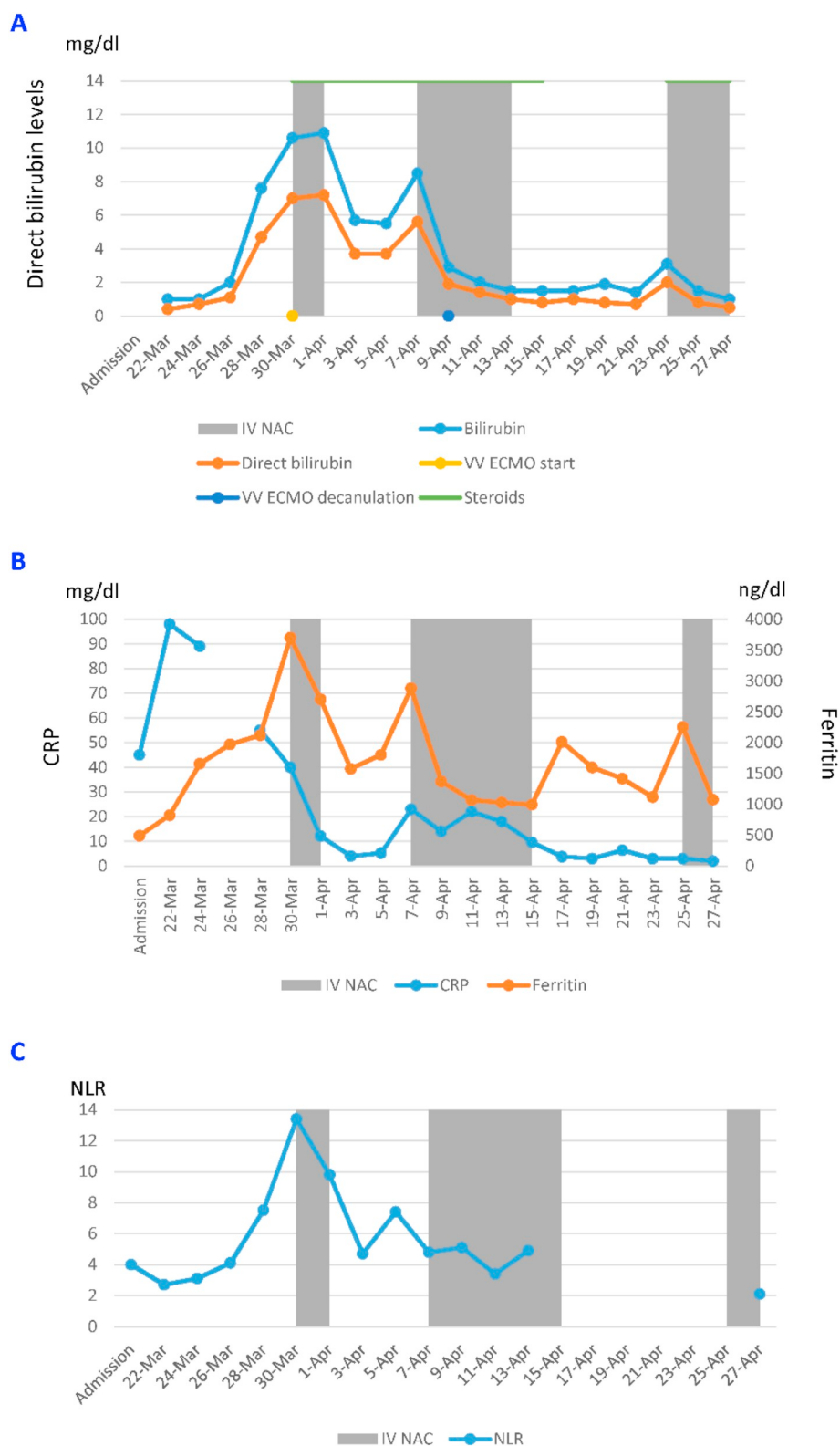


Fig. 1. Effect of IV NAC on clinical and laboratory outcomes in a G6PD-deficient patient infected by COVID-19. Gray shaded areas represent intervals of IV NAC administration. Initiation and termination of CC-ECMO are indicated along the horizontal axis with yellow and blue dots, respectively. A) Display of total and direct bilirubin levels. B) Tracking of CRP and ferritin levels. C) Monitoring of neutrophil/lymphocyte ratio (NLR). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2

Effect of IV NAC on inflammation assessed by serum levels of CRP (mg/ml) and ferritin (ng/ml) and clinical outcome of COVID-19 infection in 9 patients without G6PD deficiency. *, $p = .0022$; **, $p = .0301$, using two-tailed paired t -test.

Patient (Age/Gender)	CRP before NAC	CRP after NAC	Ferritin before NAC	Ferritin after NAC	NAC duration	NAC dose (mg)	ECMO	Outcome
1 (44/M)	89	14	3700	1500	2 days	30,000	Yes	Discharged home
2 (44/M)	90	13	9000	2000	2 days	20,000	Yes	Discharged Home
3 (48/M)	243	72	5900	2700	7 days	600 every 12 h	Yes	Discharged Home
4 (38/M)	280	26	4900	900	9 days	600 every 12 h	Yes	Hospitalized
5 (38/M)	46	5	1100	800	4 days	600 every 12 h	Yes	Discharged home
6 (42/M)	235	31	4000	2500	5 days	600 every 12 h	Yes	Hospitalized
7 (48/F)	99	45	300	330	4 days	600 every 12 h	Yes	Discharged Home
8 (48/M)	307	23	2700	1100	6 days	600 every 12 h	Yes	Discharged Home
9 (71/M)	145	71	2200	1800	5 days	600 every 12 h	No	Discharged home
10 (65/M)	63	11	2800	1800	4 days	600 every 12 h	Yes	Discharged home
Mean $\pm$ SD	160 $\pm$ 97	31 $\pm$ 24*	3630 $\pm$ 2526	1543 $\pm$ 762**				

SARS-Cov-2 156 CLGDIARDLIC 167
 SARS-CoV-1 CLGDINARDLIC
 Bat CoV ZXC21 CLGDISARDLIC
 Bat CoV ZC45 CLGGSARDLIC

Fig. 2. Highly Conserved motif in the S2 subdomain of some coronaviruses (including SARS-CoV1 and SARS-CoV2). This motif, that lies six residues away from the fusion peptide, is flanked by two highly conserved cysteine residues between which a disulfide bond is essential for membrane fusion¹. More CoVs sequences available in [15].

the two cysteine residues (C156 and C167) is apparently essential for fusion complex exposure and the subsequent membrane fusion [15], which may be disrupted by NAC. Moreover, NAC blocks mTOR [9] which is a central regulator of inflammation within the immune system (Fig. 3) [16–18] and required for binding of its substrates LARP1 and FKBP7 to viral N and ORF8 proteins [19].

We propose that NAC restrains the pro-inflammatory metabolic pathways that control oxidative stress and mTOR-dependent generation of cytokine storm emanating from the immune system [20]. mTOR blockade also abrogates the production of oxidized apolipoprotein H, also known as β 2-glycoprotein I (β 2GPI) by hepatocytes [21]. Oxidized β 2GPI is the primary antigen that elicits the formation of antiphospholipid antibodies (aPL) in patients with antiphospholipid syndrome [22]. Direct blockade of mTOR with sirolimus also attenuates aPL production in patients with lupus [23]. Thus, oxidation of β 2GPI induces not only aPL but also promotes cardiovascular disease [24] in the setting of COVID-19 infection [25–27]. IL-6, the primary cytokine that drives inflammation in COVID-19 infected patients, elicits mitochondrial oxidative stress at complex I of the mitochondrial electron transport chain (ETC). In turn, this leads to redox-dependent activation of mTORC1. Further downstream, uncontrolled activation of mTORC1

promotes inflammation [28]. NAC inhibits oxidative stress by serving as a cell-permeable amino acid precursor of the main intracellular antioxidant, GSH. Acting outside the cell, NAC may break disulfide bonds within ACE2 that serves as the cellular receptor for COVID-19 [15]. NAC may also block COVID-19 binding by disrupting disulfide bond within its receptor-binding domain [29]. In addition to epithelial, endothelial, and myocardial cells [30,31], ACE2 is expressed on T lymphocytes [32], macrophages [33], and hepatocytes [34–37]. ACE2 controls the expression of pro-inflammatory transcription factor Stat3 [38–43], which also modulates the production of reactive oxygen intermediates by complex I of the mitochondrial electron transport chain (ETC) [44]. ACE2 also attenuates signaling through mTORC1 [45–48] (Fig. 3). In turn, mTOR-dependent promoter hypomethylation causes increased expression of ACE2 which may underlie severe infection and poor outcomes in patients with preexisting comorbidities, such as lupus and cancer [49–51]. Our findings support the notion that mTOR blockade with sirolimus is expected to improve the clinical outcome of COVID-19 infection [52]. Several anti-inflammatory medications have been shown to mitigate the cytokine storm in COVID-19 infection, such as corticosteroids [53], colchicine [54], imatinib [55], and complement C3 inhibitor AMY-101 [56]. However, the safety of mTOR blockade stands out based on its propensity to extend overall lifespan [57].

IV NAC has long been used to safely treat patients with acetaminophen overdose [58,59], or ARDS [60,61]. NAC was also found to reduce CRP levels in several controlled clinical trials [10,11]. CRP elevation is a prominent risk factor for disease progression in patients infected with COVID-19 [62,63]. Whether these anti-inflammatory changes were specific to the use of NAC is difficult to discern from our study due to sporadic use of steroids and other anti-inflammatory drugs. However, it is conceivable that NAC has beneficial effects in reducing inflammation in patients infected with COVID 19. Our observations warrant prospective studies to look at the clinical and laboratory effects of NAC and to evaluate the role of IV NAC –if any– in the treatment of severe COVID 19 patients.

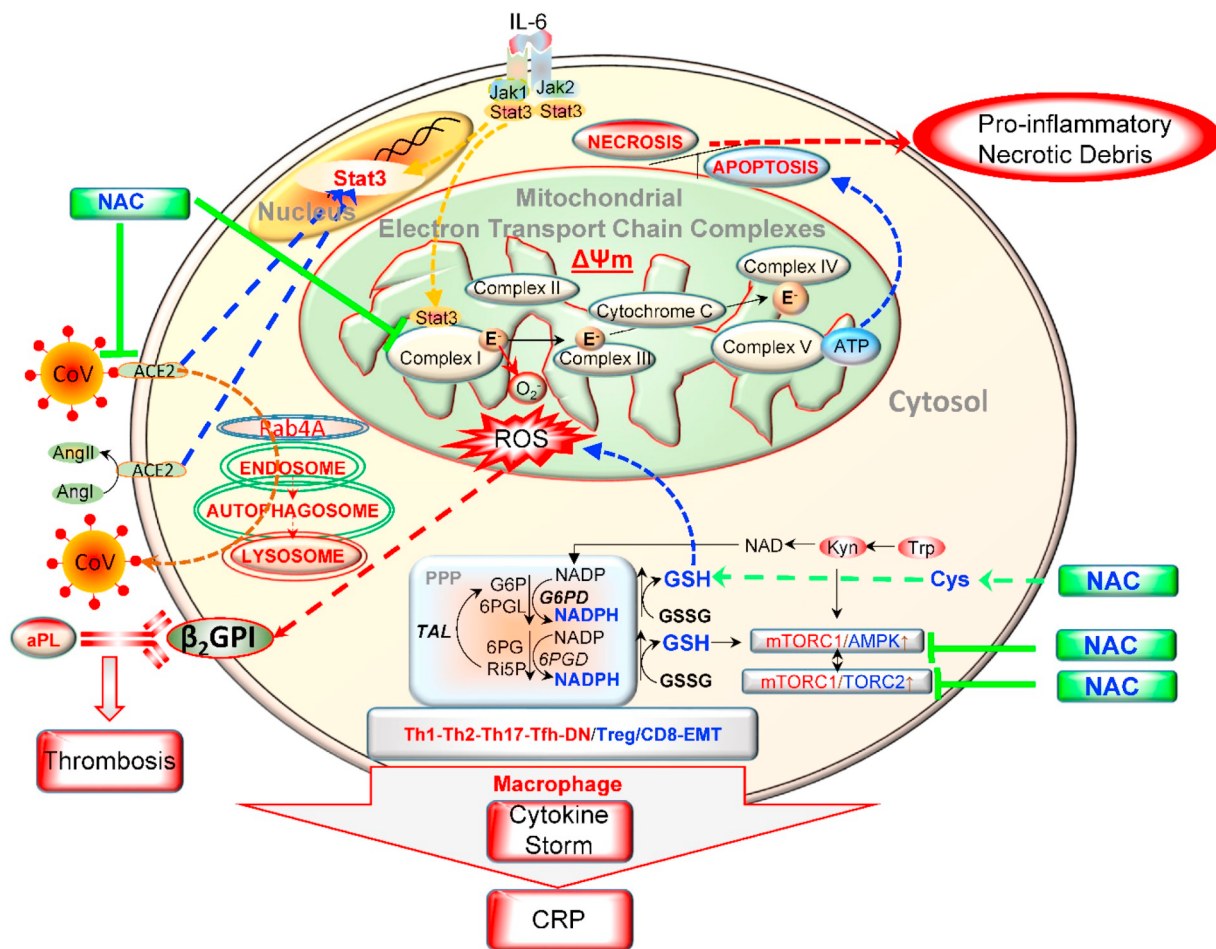


Fig. 3. Schematic diagram of metabolic pathways that control oxidative stress and mTOR-dependent generation of cytokine storm. The depicted surface receptors and transducers exemplify those that operate in T cells and underlie pro-inflammatory lineage development as well as hepatocytes that secrete apolipoprotein H, also known as β 2-glycoprotein I (β 2GPI). Oxidized β 2GPI is the primary antigen that elicits the formation of antiphospholipid antibodies (aPL) in patients with antiphospholipid syndrome [22]. Thus, oxidation of β 2GPI induces not only aPL but also promotes cardiovascular disease [24] in the setting of COVID-19 infection [25–27]. IL-6, the primary cytokine that drives inflammation in COVID-19 infected patients, elicits mitochondrial oxidative stress at complex I of the mitochondrial electron transport chain (ETC). In turn, this leads to redox-dependent activation of mTORC1. Further downstream, uncontrolled activation of mTORC1 promotes inflammation [28]. NAC inhibits oxidative stress by serving as a cell-permeable amino acid precursor of the main intracellular antioxidant, GSH. Acting outside the cell, NAC may break disulfide bonds within ACE2 that serves as the cellular receptor for COVID-19 [15]. NAC may also block COVID-19 binding by disrupting disulfide bond within its receptor-binding domain [29]. In addition to epithelial, endothelial, and myocardial cells [30,31], ACE2 is expressed on T lymphocytes [32], macrophages [33], and hepatocytes [34–37]. ACE2 controls the expression of pro-inflammatory transcription factor Stat3 [38–43], which also modulates the production of reactive oxygen intermediates by complex I of the mitochondrial electron transport chain (ETC) [44]. ACE2 also attenuates signaling through mTORC1 [45–48].

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.clim.2020.108544>.

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