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REVIEW

N-Acetylcysteine to Combat COVID-19: An Evidence Review

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Abstract: The novel coronavirus disease (COVID-19) is caused by a virus (SARS-Cov-2) and is known for inducing multisystem organ dysfunction associated with significant morbidity and mortality. Current therapeutic strategies for COVID-19 have failed to effectively reduce mortality rate, especially for elderly patients. A newly developed vaccine against SARS-Cov-2 has been reported to induce the production of neutralizing antibodies in young volunteers. However, the vaccine has shown limited benefit in the elderly, suggesting an age-dependent immune response. As a result, exploring new applications of existing medications could potentially provide valuable treatments for COVID-19. N-acetylcysteine (NAC) has been used in clinical practice to treat critically ill septic patients, and more recently for COVID-19 patients. NAC has antioxidant, anti-inflammatory and immune-modulating characteristics that may prove beneficial in the treatment and prevention of SARS-Cov-2. This review offers a thorough analysis of NAC and discusses its potential use for treatment of COVID-19.

Keywords: N-acetylcysteine, SARS-Cov-2, COVID-19

Introduction

According to the CDC, most SARS-Cov-2 infected individuals can recover from the disease at home. However, this virus can also cause serious illness in immune-compromised individuals, elderly patients, and in those with certain preexisting health conditions, such as hypertension, diabetes, and cardiovascular disease.[1] It takes approximately 7 days to develop computed tomography (CT)-confirmed pneumonia (COVID-19) from the onset symptoms, such as fever or dry cough, and another 2 days to progress to acute respiratory distress syndrome (ARDS).[2] ARDS is the major cause of death for COVID-19 patients and is associated with dysregulated host immune responses following viral infection.

One of the early immune responses during viral infection is the production of cytokines and chemokines from immune cells. High levels of IL-8, a strong chemoattractant for neutrophils, has been detected early in infected SARS patients.[3] Once activated by infection, neutrophils are rapidly recruited to sites of inflammation in the lungs, where they produce and secrete cytokines, enzymes, including elastase (NE), reactive oxygen species (ROS) by oxidative burst, and finally release DNA to form neutrophil extracellular traps (NETs).[4] In severe COVID-19 patients, an increased number of neutrophils has been associated with disease severity,[5] most likely due to the production of large amounts of proinflammatory cytokines, creating a "cytokine storm".

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In neutrophils, NE can degrade a wide variety of architecturally and functionally important molecules, such as clotting factors and complement proteins.[6] NE activity may, in part, explain the significant increase of D-dimer and pulmonary hemorrhage observed in COVID-19 patients.[2] Additionally, NET-bound NE can degrade local plasminogen without generating plasmin onto fibrin, thus resulting in impaired fibrinolysis. This suggests that NE-bound NETs have the potential to serve as a platform for activation and formation of intravascular coagulation,[7,8] which partly explains why pulmonary embolism usually occurs in critical COVID-19 patients in the intensive care unit (ICU).[9] In some critically ill COVID-19 patients, the coexistence of thrombosis and hemorrhage was observed,[10] indicating that suppression of NE production by stabilizing neutrophils could be beneficial for either condition. For instance, inhibiting neutrophil activation by an IL-8 antibody can effectively combat acute lung injury.[11,12] In other words, any measure that can suppress neutrophil activation might improve the outcomes of COVID-19 patients.

Cellular immunity is also required for a host to fight a viral infection, which is regulated by an oxidant-antioxidant balance. This balance is maintained by antioxidants including glutathione. In the immune cells of senior or immune-compromised individuals, ROS is increased due to decreased glutathione, which causes dysregulation of immune responses, particularly of T cell-mediated functions. This may explain the depressed cell-mediated immunity and increased mortality found in elderly persons as a result of infectious diseases, such as pneumonia.[13,14] In fact, in addition to depressed functions, the number of lymphocytes, including both CD4+ and CD8+ T cells, was found to decrease linearly with age.[15] Furthermore, a reduced number of T cells as a result of apoptosis was also observed in critical COVID-19 patients, which further compromised cellular immunity and was associated with the higher mortality for these populations.[2,16] Therefore, replenishing certain antioxidants may restore the normal responses of immune cells through inhibiting T cell apoptosis, potentially reducing incidence or severity of pneumonia due to virus infection.

Combining count changes of neutrophils and lymphocytes, several groups have recently revealed that a high neutrophil-to-lymphocyte ratio (NLR) predicts a more severe progression of the disease and worse outcomes for COVID-19 patients,[17–19] suggesting that NLR may be used as a prognostic marker and a therapeutic guide during acute COVID-19 infection. Therefore, in addition to administration of anti-viral drugs, inhibiting neutrophil activation and protecting T cells could provide an effective therapeutic option for treating COVID-19 patients.

Theoretically, an effective vaccine would be the best solution to combat SARS-cov-2 infection. A recent study showed that a recombinant adenovirus type-5 (Ad5) vaccine was capable of inducing neutralizing antibodies at day 14 post-vaccination,[20] suggesting that a quick control of the COVID-19 pandemic is a possibility. However, this study also found that the neutralizing antibodies were reduced between the ages of 45 to 60 when compared with younger people. Since younger people are widely considered the major carriers and spreaders of SARS-Cov-2, effective vaccines are still desperately needed to reduce virus transmission. Meanwhile, exogenous neutralizing antibodies may help those not responsive to the SARS-Cov-2 vaccines. Unfortunately, both vaccines and neutralizing antibodies are still under development. Therefore, a multimodal approach may be necessary when treating COVID-19 in elderly patients or in those with preexisting conditions.

Evidence and Discussions

N-Acetylcysteine, a Forgotten Immune-Modulating Agent

N-acetylcysteine (NAC), a precursor of the antioxidant glutathione, has been used to loosen thick mucus in the lungs and treat acetaminophen overdose for decades. However, NAC can also boost the immune system, suppress viral replication, and reduce inflammation. Despite these valuable features, NAC has been mostly overlooked throughout SARS-Cov and MERS-Cov epidemics, as well as the current COVID-19 pandemic.

In 1997, De Flora et al demonstrated that oral administration of NAC (600mg, bid) significantly improved cell-mediated immunity, shifting from anergy to normoergy in seniors (Figure 1A).[21] Anergy represents a lack of reaction from immune cells to foreign substances, such as bacteria and viruses. Unsurprisingly, NAC treatment significantly decreased the frequency of influenza, as well as the severity and duration of most symptoms (Figure 1B). Although the infection rates of influenza virus (H1N1 Singapore 6/86) were similar in the two groups, only 25% of virus-infected subjects in NAC group developed flu symptoms, contrasting with 79% of the subjects in the placebo group. As a result, NAC may improve compromised cellular immunity and prevent development of certain respiratory virus-caused

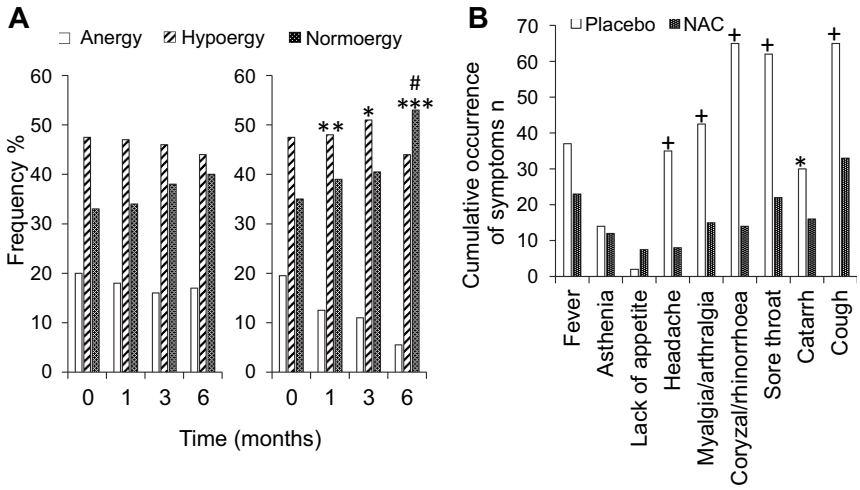

Figure 1 (**A**) Effect of NAC treatment on cell-mediated immunity. Left: Placebo group; Right: NAC-treated group. *p <0.05; **p<0.01; ***p<0.001, significance of difference in the frequency of anergy, within the NAC group, after 1, 3 and 6 months, compared to the start of the study (time 0); #p<0.05, significance of difference in the frequency of anergy between the NAC group and the placebo group. (**B**) Effect of NAC treatment on the cumulative occurrence of individual influenza-like signs and symptoms. *p<0.05; +p<0.0001, significance of difference between the frequency of symptoms in the NAC group and the placebo group. Reproduced with permission from De Flora S, Grassi C, Carati L. Attenuation of influenza-like symptomatology and improvement of cell-mediated immunity with long-term N-acetylcysteine treatment. *European Respiratory Journal*. 1997;10: 1535–1541.[21]

diseases, thus raising the question: Can NAC administration benefit COVID-19 patients?

NAC Offers the Following Key Features to Combat COVID-19

Anti-Virus Functions of NAC

RNA viruses need active NF-κB pathway support within host cells in order to replicate. For human coronaviruses (HCoV-229E), suppression of NF-κB significantly reduced the replication rate.[22] Therefore, drugs that inhibit NF-κB activation could potentially reduce viral replication.

NAC has been demonstrated to inhibit NF-κB, as well as the replication of human influenza viruses (H5N1, Vietnam/VN1203 strain) in human lung epithelial cells in a dose dependent manner (5 to 15 mM) (Figure 2). NAC also reduced the production of pro-inflammatory cytokines (IL-8, CXCL10, CCL5 and IL-6), thus reducing chemotactic migration of monocytes.[23] In addition, NAC has also been showed to inhibit replication of other viruses, such as human immunodeficiency virus (HIV)[24] and respiratory syncytial virus (RSV).[25] This means that, theoretically, NAC has the potential to inhibit SARS-Cov-2 as well because of its ability to negatively regulate NF-κB.

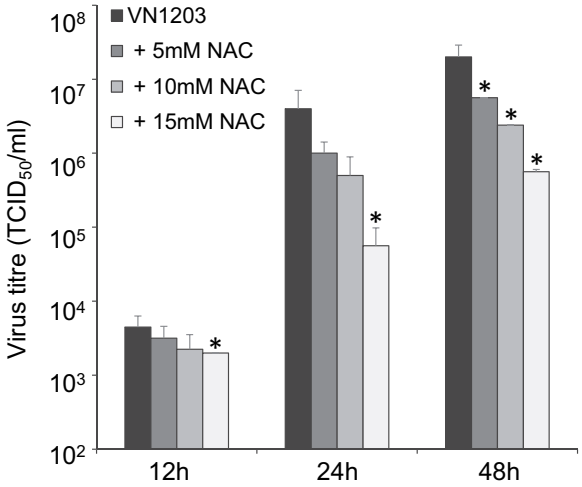

Figure 2 Influence of NAC on H5N1 virus replication in A549 cells. A549 cells were infected with A/Vietnam/1203/04 (VN1203) at a MOI of 0.01. NAC treatment (0 mM NAC: dark grey bars, 5 mM NAC: middle grey bars, 10 mM NAC: light grey bars, 15 mM NAC: white bars) was performed continuously starting 24 h prior to infection. H5N1 titres were determined 12, 24 and 48 h post-infection. *p<0.05 relative to untreated virus control. Reprinted from *Biochemical Pharmacology*, 79, Geiler J, Michaelis M, Naczk P, et al. N-acetyl-L-cysteine (NAC) inhibits virus replication and expression of pro-inflammatory molecules in A549 cells infected with highly pathogenic H5N1 influenza A virus. 413–420, Copyright (2010), with permission from Elsevier.[23]

NAC Could Also Be a Direct Inhibitor of SARS-Cov-2

In SARS-Cov-2, main protease (Mpro) is required for viral replication. As a result, many researchers have sought to develop inhibitors specifically targeting Mpro. Zhang et al provided the crystal structure of Mpro and designed the small compound 13b, which can efficiently inhibit SARS-CoV-2 replication in human lung cells.[26] However, it will likely take

years for this promising Mpro inhibitor to advance to clinical application. Guthappa suggested that NAC may bind to Cys-145, an active site of Mpro, which could potentially inhibit its protease activity and then inhibit viral replication.[27] Thus, NAC could serve as a first-line drug specifically for SARS-Cov-2 due to its structural characteristics.

Immune-Modulating Properties of NAC

It has been demonstrated that NAC can change the redox balance towards reduced status inside neutrophils by replenishing reduced glutathione (GSH), which suppresses NF-κB activation at concentrations of 10mM or more, resulting in modulation of cytokine production and chemotactic signals.[28] Moreover, neutrophils from healthy volunteers taking NAC (600 mg daily) for 14 days showed lower rates of oxidative burst and chemotaxis. NAC also suppressed elastase release from neutrophils induced by formyl-methionyl-leucyl-phenylalanine (fMLP) in a dose dependent manner, as the elastase was completely abolished by 10mM NAC (Figure 3). Monocyte chemotaxis can also be inhibited by clinically accessible concentrations of NAC.[29] When it comes to considering even higher doses of NAC, another study showed that oral administration of 1200 mg of NAC significantly reduced oxidative bursts from neutrophils induced by the stimulants C. albicans, fMLP, and PMA. Interestingly, NAC did not compromise other functions of neutrophils, such as phagocytosis and bacterial killing.[30] Collectively this data supports the notion that 1200 mg of oral NAC can effectively reduce ROS production without compromising phagocytosis of SARS-Cov-2 in neutrophils.

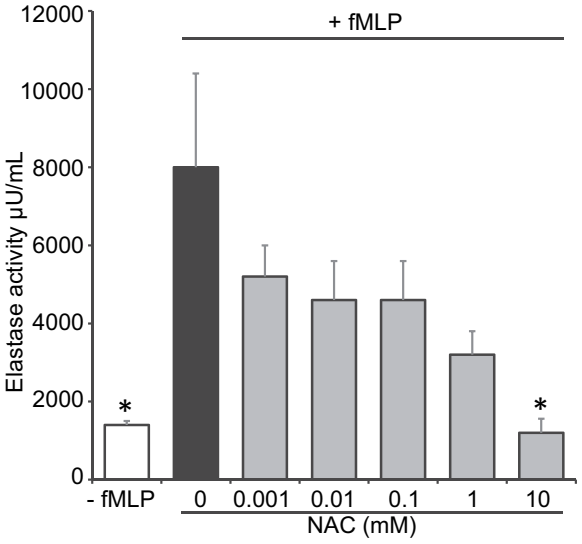

Figure 3 The effect of 30 min incubation of neutrophils with different NAC concentrations on the fMLP (10^{-7} M induced release of elastase by neutrophils in vitro. *p<0.05 when compared to the preincubation without NAC. Reprinted from *Pharmacological Research*, 53, Sadowskaa AM, Manuel-y-Keenoy B, Vertongen T, et al. Effect of N-acetylcysteine on neutrophil activation markers in healthy volunteers: In vivo and in vitro study. 216–225, Copyright (2006), with permission from Elsevier.[28]

In severe COVID-19 patients, a SARS-cov-2 infection frequently causes lymphopenia, especially for T cells.[31] NAC can increase intracellular GSH in human T cells and block apoptosis induced by pro-apoptosis Fas antigen/Fas ligand lethal signal[32] that is upregulated during virus infections, such as HIV, HCV and influenza. Lai et al demonstrated that 2400 mg of oral NAC (1200 mg, bid) quickly increased glutathione levels in lymphocytes during a chronic inflammatory disease, which was not achieved by a low-dose NAC (600 mg, bid).[33] We therefore expect that high-dose oral NAC (1200 mg, bid) can improve adaptive immunity by elevating glutathione levels in lymphocytes, in addition to modulating neutrophil functions during COVID-19 development.

NAC Can Reduce the Incidence of Pneumonia

Given that oral NAC (600mg, bid) significantly decreased the frequency and severity of influenza, oral NAC may reduce the incidence of pneumonia as well. One study has demonstrated that about 37% of mechanically ventilated patients develop pneumonia, namely, ventilator-associated-pneumonia (VAP) in an intensive care unit. NAC (600 mg, bid) treated patients developed significantly less clinically confirmed pneumonia compared with placebo group patients (26.6% VS 46.6%).[34] Another study showed that oral (600mg, bid) NAC significantly reduced the levels of TNF and malondialdehyde (MDA) and significantly improved oxidative stress.[35] Modulation of the inflammatory process with antioxidants may have a mitigating effect in the development of pneumonia, potentially improving outcomes if high doses of NAC (1200mg, bid) are utilized.

Improve Lung Function to Reduce Mortality Rate by Intravenous NAC

Virus induced "Cytokine storm" has been closely linked with the mortality in COVID-19 patients.[36] In addition to secreting cytokines, neutrophils also produce ROS radicals. ROS are chemically reactive oxygen-containing species, such as superoxide, peroxides, hydroxyl radical, singlet oxygen and alpha-oxygen, etc. Superoxide anion radicals ($O2^{-}$) can cause injury directly and can be

converted into more damaging oxidant species such as hydroxyl radical (OH^-) and hypochlorous acid (HOCl).[37] Of the two, OH^- has been demonstrated as the key reactive oxygen species to cause pulmonary edema during acute lung injury.[38] Hypoxemia secondary to Adult Respiratory Distress Syndrome (ARDS) and pulmonary edema may occur during COVID-19 infection.[39] Besides causing tissue injury, ROS can also activate the NF-kB pathway to amplify inflammation through upregulation of expression of multiple genes, such as IL-6, TNF-α, and chemokines. NAC, a powerful scavenger of OH^-,[40] could effectively prevent cytokine storm and ROS-induced pulmonary edema and respiratory failure.

In patients with mild-to-moderate acute lung injury, intravenous (IV) NAC treatment (40mg/kg/day) for 3 days significantly improved systemic oxygenation, reduced the need for ventilatory support and also slightly reduced the mortality rate,[41] suggesting that higher concentrations of IV NAC could be administrated, potentially improving clinical outcomes.

A case report revealed the significance of NAC treatment for a patient with septic shock from an influenza (H1N1) infection. Together with oseltamivir, intravenous infusion of NAC at 100 mg/kg/day for 3 days rapidly improved the patient's sepsis conditions with resolution of lung infiltrates. However, the patient relapsed after cessation of the NAC infusion. Then, reinstating the infusion of NAC at the same dose rapidly improved the patient's conditions again, until the viruses were eventually eradicated, and the patient was discharged.[42] This case suggests that high concentration and enough exposure time of NAC is the key to treat virus-caused critical conditions, including pneumonia-mediated sepsis.

Another promising study revealed that in ARDS and acute ALI patients, IV NAC at a loading dose of 150 mg/kg at the first day, followed by a dose of 50 mg/kg/day for 3 days, not only improved oxygenation, but also decreased mortality rate (35.7% VS 76.9%) compared to control patients ($p < 0.05$).[43] Although this cohort is relatively small, its results are dramatic, further suggesting that IV NAC can be used to treat severe COVID-19 and reduce mortality, given enough dosage and treating time.

Case Reports and Clinical Trials Using NAC to Treat COVID-19 Patients

A recent case report demonstrated that using low-dose hydroxychloroquine (HCQ) and IV NAC had a positive impact on a 54-year-old male COVID-19 patient, with a history of hypertension, hyperlipidemia, and obesity. Multi-system end-organ damage was diagnosed and the patient was given a low-dose oral HCQ (total 600 mg) in combination with IV NAC, at a loading dose of 75 mg/kg for 4 hours, then 35 mg/kg for 16 hours, followed by 17 mg/kg for 24 hours. The patient gradually recovered (NLR from 16.7 to 2.4) despite pulmonary embolism and short-term mechanical ventilation. He was then released from intensive care on day 7 and eventually discharged on post-admission day 12.[44]

In another case report, a 64-year-old male COVID-19 patient developed respiratory failure on day 13 post-admission, despite being treated with antibiotics, antiviral and respiratory support. Together with other treatments, a large dose (10–15 g) of NAC inhalation repeated for 11 days significantly improved his critical conditions. The patient was eventually discharged after 26 days of mechanical ventilation and 46 days of hospitalization.[45] Another two COVID-19 patients with dyspnea were effectively treated with oral and IV glutathione, NAC and alpha lipoic acid,[46] further suggesting that remediation of oxidative stress could be a key in combating COVID-19.

Recently, in a larger cohort study, Ibrahim et al have demonstrated that IV NAC significantly improved disease conditions in 10 severe respirator-dependent COVID-19 patients, aged from 38 to 71 years, including one with Glucose-6-phosphate dehydrogenase (G6PD) deficiency. IV NAC administration significantly reduced inflammatory markers, such as C-reactive protein (CRP) and ferritin, and also improved lung functions. Eight patients were eventually discharged, and two remaining patients showed improved conditions by the date of publication.[47] This clinical practice further proves the effectiveness of NAC in COVID-19 treatment. Since NAC is a stronger antioxidant and less expensive than glutathione, the significance of NAC-mediated treatments for COVID-19 patients should be emphasized, at least as part of a multimodal approach.

To date, there are 6 clinical trials using NAC regarding COVID-19 treatments, 4 of them (NCT04545008; NCT04419025; NCT04455243; NCT04466657) have not recruited patients yet. In the other 2 trials that are recruiting patients, one of them (NCT04370288) was designed with the combination of NAC, Methylene Blue and vitamin C, making the interpretation of NAC effect difficult. And another study (NCT04374461) is scheduled for completion date in May 2021. Without effective treatments, COVID-19 could make severe consequences in terms of

morbidity and mortality. As a dietary supplement, NAC has been used increasingly worldwide. During this COVID-19 pandemic, NAC can potentially prevent development of critical pneumonia for the people sensitive to SARS-CoV-2 infections. It also provides the potential references of how to use NAC for ongoing clinical trials. In addition, NAC features and its successful application for treating COVID-19 may encourage patients to enroll into the clinical trials using NAC.

Routes and Doses of NAC Administration

IV Infusion of NAC

To treat acetaminophen overdose for adults based on a FDA approved 3-bag regime, NAC (Molecular weight: 163) is administrated intravenously, initially 150 mg/kg in 200 mL of 5% dextrose for 60 minutes (first bag), followed by 50 mg/kg in 500 mL of 5% dextrose for 4 hours (second bag), and then 100 mg/kg in 1000 mL of 5% dextrose for 16 hours (third bag).[48] Given that the average American male (20 years or older) weighs about 90 kg with about 7000 mL of blood, 13.5 g of NAC in 200 mL (414 mM) of 5% dextrose can be infused in one hour. Based on the calculations of two studies,[49,50] the approximate NAC concentration in blood should be about 1 mM during infusion of the first bag, which is enough to neutralize the potent oxidant species, suppress oxidative burst, and substantially reduce neutrophil chemotaxis and cytokine storm.

Oral Administration of NAC

The gut is the largest immune organ we have,[51] carrying 70% of all lymphocytes in the body. Absorbed in the small intestine, oral NAC interacts with epithelial cells and immune cells, potentially boosting our immune system to combat virus infection. One capsule of 600 mg NAC can reach a level of 16 μM NAC in the peripheral blood in half an hour after administration. Although it has been labeled as "low bioavailability" for decades, if administrated within 8–10 hours of acetaminophen overdose, oral administration of NAC displays the same capacity of detoxification as given by the IV route.[52]

NAC Inhalation

Under FDA guideline, to loosen mucus, MAYO clinic suggests inhaling 3 to 5 milliliters (mL) of a 20% solution or 6 to 10 mL of a 10% solution using a nebulizer, three or four times a day. For NAC, 10% is equivalent to 613 mM. Highly concentrated NAC can effectively reduce viral replication and significantly alleviate pneumocyte damage, as well as excessive immune responses.

Availability and Cost of NAC

The affordable generic NAC has been used increasingly as a dietary supplement in the US and Europe. For example, a 600 mg NAC capsule costs about 0.07–0.1 dollars on the market. The other acetylcysteine formulations are also commercially available in the US as generic and brand-name drugs, such as Mucomyst for nebulizer administration (prescription needed, 10mL of 10% NAC costs about $6 with insurance, and about $3.8 with an online coupon) and Acetadote for intravenous administration.

The Strategy of NAC Administration

To protect those who have not contracted SARS-Cov-2, oral NAC (600 mg, bid) could be an effective and economical measurement to modulate their immune systems against potential infection. Once onset symptoms appear, such as fever or dry cough, oral NAC (1200 mg, bid) could be taken to alleviate symptoms and accelerate recovery from virus infection.

For relatively severe patients without airway obstructions, an inhalable formula of NAC can be used with a nebulizer. Patients with allergies or asthma should take antihistamine before or during NAC inhalation, to prevent adverse reactions. Self-treatment with oral or inhalable NAC could help many SARS-Cov-2 infected patients safely recover at home.

Once patients develop clinically confirmed pneumonia or dyspnea, in addition to regular therapy, such as Remdesivir,[53] IV NAC should be given intermittently or continuously. This could then prevent development of ARDS, which often entails invasive ventilation and intensive care unit support. For example, NAC can be infused at a dose of 100 mg/kg for at least 3 days, which equals to about 1/3 of the total dose during a 3-bag regime. There is no difference between intermittent and continuous infusion of NAC regarding patient outcomes.[54]

When a patient develops ARDS, along with regular antiviral therapy, 150 mg/kg at the first day, followed by a dose of 100 mg/kg/day for at least 3 days, should be administrated to avoid irreversibly fatal multiple organ failure (MOF). Once MOF or critical sepsis occurs, patients likely will not benefit from any NAC administration.[55] A brief overview of a NAC therapeutic strategy to combat COVID-19 has been summarized in Figure 4.

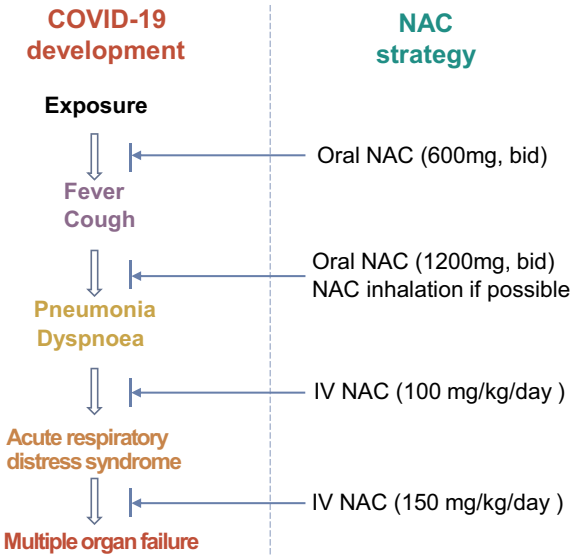

Figure 4 A brief overview of a NAC therapeutic strategy to combat COVID-19.

Conclusions

N-acetylcysteine (NAC) is inexpensive, has very low toxicity, has been FDA approved for many years, and has the potential to improve therapeutic strategies for COVID-19. NAC administered intravenously, orally, or inhaled, may suppress SARS-CoV-2 replication and may improve outcomes if used timely. Potential therapeutic benefits of NAC include, extracellularly scavenging ROS radicals, replenishing intracellular GSH, suppression of cytokine storm, and T cell protection, thus mitigating inflammation and tissue injury. NAC administration in combination with other antiviral agents may dramatically reduce hospital admission rate, mechanical ventilation and mortality.

Disclosure

The authors report no conflicts of interest for this work.

References

1. Jamwal S, Gautam A, Elsworth J, et al. An updated insight into the molecular pathogenesis, secondary complications and potential therapeutics of COVID-19 pandemic. *Life Sci*. 2020;257:118105. doi:10.1016/j.lfs.2020.118105
2. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020;395 (10223):497–506. doi:10.1016/S0140-6736(20)30183-5
3. Huang KJ, Su IJ, Theron M, et al. An interferon-γ-related cytokine storm in SARS patients. *J Med Virol*. 2005;75:185–194. doi:10.1002/jmv.20255
4. Stegelmeier AA, van Vloten JP, Mould RC, et al. Myeloid Cells during Viral Infections and Inflammation. *Viruses*. 2019;11(2):168. doi:10.3390/v11020168
5. Schulte-Schrepping J, Reusch N, Paclik D, et al. Severe COVID-19 Is Marked by a Dysregulated Myeloid Cell Compartment. *Cell*. 2020;182(6):1419–1440. doi:10.1016/j.cell.2020.08.001
6. Janoff A. Elastase in tissue injury. *Ann Rev Med*. 1985;36:207–216. doi:10.1146/annurev.me.36.020185.001231
7. Thierry AR. Anti-protease Treatments Targeting Plasmin(ogen) and Neutrophil Elastase May Be Beneficial in Fighting COVID-19. *Physiol Rev*. 2020;100(4):1597–1598. doi:10.1152/physrev.00019.2020
8. Cruz DB, Helms J, Aquino LR, et al. DNA-bound elastase of neutrophil extracellular traps degrades plasminogen, reduces plasmin formation, and decreases fibrinolysis: proof of concept in septic shock plasma. *FASEB J*. 2020. doi:10.1096/fj.201901363RRR
9. Leonard-Lorant I, Delabranche X, Severac F, et al. Acute Pulmonary Embolism in Patients with COVID-19 at CT Angiography and Relationship to d-Dimer Levels. *Radiology*. 2020;296(3):E189–E191. doi:10.1148/radiol.2020201561
10. Xu J, Wang L, Zhao L, et al. Risk assessment of venous thromboembolism and bleeding in COVID-19 patients. *Res Square*. 2020. doi:10.21203/rs.3.rs-18340/v1
11. Mukaida N, Matsumoto T, Yokoi K, et al. Inhibition of neutrophil-mediated acute inflammatory injury by an antibody against interleukin-8 (IL-8). *Inflammation Res*. 1998;47:151–157. doi:10.1007/s000110050308
12. Bao Z, Ye Q, Gong W, et al. Humanized monoclonal antibody against the chemokine CXCL-8 (IL-8) effectively prevents acute lung injury. *Int Immunopharmacol*. 2010;10(2):259–263. doi:10.1016/j.intimp.2009.11.005
13. Wayne SJ, Rhyne RL, Garry PJ, et al. Cell-Mediated Immunity as a Predictor of Morbidity and Mortality in Subjects Over 60. *J Gerontol*. 1990;45(2):45–48. doi:10.1093/geronj/45.2.M45
14. Ohrui T. Interventions to prevent pneumonia in older adults. *Geriatr Gerontol Int*. 2004;4(s1):92–95. doi:10.1111/j.1447-0594.2004.00162.x
15. Saule P, Trauet J, Dutriez V, et al. Accumulation of memory T cells from childhood to old age, Central and effector memory cells in CD4 + versus effector memory and terminally differentiated memory cells in CD8+ compartment. *Mech Ageing Dev*. 2006;127(3):274–281. doi:10.1016/j.mad.2005.11.001
16. Li H, Wang S, Zhong F, et al. Age-dependent risks of Incidence and Mortality of COVID-19 in Hubei Province and Other Parts of China. *Fron Med*. 2015:214. doi:10.3389/fmed.2020.00190
17. Zhang B, Zhou X, Zhu C, et al. Immune phenotyping based on neutrophil-to-lymphocyte ratio and IgG predicts disease severity and outcome for patients with COVID-19. *medRxiv*. 2020. doi:10.1101/2020.03.12.20035048
18. Ma Y, Shi N, Fan Y, et al. Predictive Value of the Neutrophil-to-Lymphocyte Ratio (NLR) for diagnosis and worse clinical course of the COVID-19: findings from ten provinces in China. *Lancet*. 2020. doi:10.2139/ssrn.3569838
19. Qin C, Zhou L, Hu Z, et al. Dysregulation of immune response in patients with COVID-19 in Wuhan, China. *Clin Infect Dis*. 2015;14:148. doi:10.1093/cid/ciaa248
20. Zhu F, Li Y, Guan X, et al. Safety, tolerability, and immunogenicity of a recombinant adenovirus type-5 vectored COVID-19 vaccine: a dose-escalation, open-label, non-randomised, first-in-human trial. *Lancet*. 2020. doi:10.1016/S0140-6736(20)31208-3
21. Flora SD, Grassi C, Carati L. Attenuation of influenza-like symptomatology and improvement of cell-mediated immunity with long-term N-acetylcysteine treatment. *Eur Respiratory J*. 1997;10 (7):1535–1541. doi:10.1183/09031936.97.10071535
22. Poppe M, Wittig S, Jurida L, et al. The NF-κB-dependent and -independent transcriptome and chromatin landscapes of human coronavirus 229E-infected cells. *PLoS Pathog*. 2017;13(3):e1006286. doi:10.1371/journal.ppat.1006286

23. Geiler J, Michaelis M, Naczk P, et al. N-acetyl-L-cysteine (NAC) inhibits virus replication and expression of pro-inflammatory molecules in A549 cells infected with highly pathogenic H5N1 influenza A virus. *Biochem Pharmacol*. 2010;79(3):413–420. doi:10.1016/j.bcp.2009.08.025
24. Ho W, Douglas SD. Glutathione and N-Acetylcysteine suppression of human immunodeficiency virus replication in human Monocyte/Macrophages in vitro. *AIDS Res Hum Retroviruses*. 1992;8(7):1249–1253. doi:10.1089/aid.1992.8.1249
25. Mata M, Sarrion I, Armengot M, et al. Respiratory syncytial virus inhibits ciliagenesis in differentiated normal human bronchial epithelial cells: effectiveness of N-Acetylcysteine. *PLoS One*. 2012;7(10):e48037. doi:10.1371/journal.pone.0048037
26. Zhang L, Lin D, Sun X, et al. Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α-ketoamide inhibitors. *Science*. 2020;368:409–412.
27. Guthappa R. Molecular Docking Studies of N-Acetyl Cysteine, Zinc Acetyl Cysteine and Niclosamide on SARS Cov 2 Protease and Its Comparison with Hydroxychloroquine. *Chemarxiv*. 2020. doi:10.26434/chemrxiv.12161493.v1
28. Sadowskaa AM, Manuel-y-Keenoy B, Vertongen T, et al. Effect of N-acetylcysteine on neutrophil activation markers in healthy volunteers: in vivo and in vitro study. *Pharmacol Res*. 2006;53(3):216–225. doi:10.1016/j.phrs.2005.11.003
29. Kharazmi A. Nielsen H and Schiotx PO. N-acetylcysteine inhibits human neutrophil and monocyte chemotaxis and oxidative metabolism. *Int J Lmmunopharmac*. 1988;10(1):39–46. doi:10.1016/0192-0561(88)90148-8
30. Allegra L, Sasso MD, Bovio C, et al. Human Neutrophil Oxidative Bursts and their in vitro Modulation by Different N-Acetylcysteine Concentrations. *Drug Res*. 2002;52(9):669–676.
31. Tavakolpour S, Rakhshandehroo T, Wei EX, Rashidian M. Lymphopenia during the COVID-19 infection: what it shows and what can be learned. *Immunol Lett*. 2020;225:31–32. doi:10.1016/j.imlet.2020.06.013
32. Chiba T, Takahashi S, Sato N, et al. Fas-mediated apoptosis is modulated by intracellular glutathione in human T cells. *Eur J Immunol*. 1996;26(5):1164–1169. doi:10.1002/eji.1830260530
33. Lai Z, Hanczko R, Bonilla E, et al. N-acetylcysteine reduces disease activity by blocking mammalian target of rapamycin in T cells from systemic lupus erythematosus patients: A randomized, double-blind, placebo-controlled trial. *Arthritis Rheumatology*. 2012;64(9):2937–2946. doi:10.1002/art.34502
34. Sharafkhah M, Abdolrazaghnejad A, Zarinfar N, et al. Safety and efficacy of N-acetylcysteine for prophylaxis of ventilator-associated pneumonia: a randomized, double blind, placebo-controlled clinical trial. *Mojtaba Med Gas Res*. 2018;8(1):19–23. doi:10.4103/2045-9912.229599
35. Zhang Q, Ju Y, Ma Y, et al. N-acetylcysteine improves oxidative stress and inflammatory response in patients with community acquired pneumonia A randomized controlled trial. *Medicine*. 2018;97:e13087.
36. Ragab D, Eldin H, Taeimah M, et al. The COVID-19 cytokine storm; What we know so far. *Front Immunol*. 2020;11. doi:10.3389/fimmu.2020.01446
37. Hassett P, Curley GF, Contreras M, et al. Overexpression of pulmonary extracellular superoxide dismutase attenuates endotoxin induced acute lung injury. *Intensive Care Med*. 2011;37:1680–1687.
38. Fox RB. Prevention of granulocyte-mediated oxidant lung injury in rats by a hydroxyl radical scavenger, dimethylthiourea. *J Clin Invest*. 1984;74(4):1456–1464. doi:10.1172/JCI111558
39. Li L, Huang Q, Wang DC, et al. Acute lung injury in patients with COVID-19 infection. *Clin Transl Med*. 2020. doi:10.1002/ctm2.16
40. Aruoma OI, Halliwell B, Hoey BM, et al. The antioxidant action of N-acetylcysteine: its reaction with hydrogen peroxide, hydroxyl radical, superoxide, and hypochlorous acid. *Free Radic Biol Med*. 1989;6(6):593–597. doi:10.1016/0891-5849(89)90066-X
41. Suter PM, Domenighetti G, Schaller MD, et al. N-Acetylcysteine enhances recovery from acute lung injury in man: a randomized, double-blind, placebo-controlled clinical study. *Chest*. 1994;105(1):190–194. doi:10.1378/chest.105.1.190
42. Lai K, Ng W, Chan PK, et al. High-Dose N-Acetylcysteine Therapy for Novel H1N1 Influenza Pneumonia. *Ann Intern Med*. 2010;152(10):687. doi:10.7326/0003-4819-152-10-201005180-00017
43. Moradi M, Mojtahedzadeh M, Mandegari A, et al. The role of glutathione-S-transferase polymorphisms on clinical outcome of ALI/ARDS patient treated with N-acetylcysteine. *Respir Med*. 2009;103(3):434–441. doi:10.1016/j.rmed.2008.09.013
44. Puyo C, Kreig D, Saddi V, et al. Case Report: use of hydroxychloroquine and N-acetylcysteine for treatment of a COVID-19 positive patient [version 1; peer review: awaiting peer review]. *F1000Research*. 2020;9:491.
45. Liu Y, Luo G, Qian X, et al. Experience of N-acetylcysteine airway management in the successful treatment of one case of critical condition with COVID-19. *Researchsquare*. 2020. doi:10.21203/rs.3.rs-34193/v1
46. Nasia A, McArdleb S, Gaudernackc G, et al. Reactive oxygen species as an initiator of toxic innate immune responses in retort to SARS-CoV-2 in an ageing population, consider N-acetylcysteine as early therapeutic intervention. *Toxicol Rep*. 2020;7:768–771. doi:10.1016/j.toxrep.2020.06.003
47. Ibrahim H, Perl A, Smith D, et al. Therapeutic blockade of inflammation in severe COVID-19 infection with intravenous n-acetylcysteine. *Clin Immunol*. 2020;219:108544. doi:10.1016/j.clim.2020.108544
48. ACETADOTE® (acetylcysteine) Injection. NDA 21-539/S-004. FDA. 2006
49. Dósa E, Helta K, Radovits T, et al. Dose escalation study of intravenous and intra-arterial N-acetylcysteine for the prevention of oto- and nephrotoxicity of cisplatin with a contrast-induced nephropathy model in patients with renal insufficiency. *Fluids and Barriers of the CNS*. 2017;14(1):26. doi:10.1186/s12987-017-0075-0
50. Hong SY, Gil HW, Yang JO, et al. Effect of High-Dose Intravenous N-acetylcysteine on the Concentration of Plasma Sulfur-Containing Amino Acids. *Korean J Intern Med*. 2005;20:217–223. doi:10.3904/kjim.2005.20.3.217
51. MacDonald TT. The gut is still the biggest lymphoid organ in the body. *Nature*. 2008;1(4):246–247.
52. Green JL, Heard KJ, Reynolds KM, et al. Oral and Intravenous Acetylcysteine for Treatment of Acetaminophen Toxicity: A Systematic Review and Meta-analysis. *West J Emerg Med*. 2013;14(3):218–226. doi:10.5811/westjem.2012.4.6885
53. Grein J, Ohmagari N, Shin D, et al. Compassionate Use of Remdesivir for Patients with Severe Covid-19. *NEJM*. 2020;382(24):2327–2336. doi:10.1056/NEJMoa2007016
54. Yazdi AP, Razavi M, Sheikh S, et al. Clinical Trial Assessment of Intermittent and Continuous Infusion Dose of N-Acetylcysteine on Redox Status of the Body in Patients with Sepsis Admitted to the ICU. *J Intensive Care Med*. 2019. doi:10.1177/0885066618823152
55. Molnar Z, Shearer E, Lowe D, et al. N-Acetylcysteine treatment to prevent the progression of multisystem organ failure: a prospective, randomized, placebo-controlled study. *Crit Care Med*. 1999;27(6):1100–1104. doi:10.1097/00003246-199906000-00028

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The primary aim of *World Journal of Virology* (*WJV*, *World J Virol*) is to provide scholars and readers from various fields of virology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

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REVIEW

Bottom-up analysis of emergent properties of N-acetylcysteine as an adjuvant therapy for COVID-19

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Author contributions: Dominari A and Hathaway III D defined the topic and designed the study, Dominari A coordinated the writing of the manuscript, Alvarez A and Talalaev M provided critical reviews, all authors contributed to the literature search, all authors wrote the original manuscript, all authors assisted in reviewing and editing the manuscript, all authors consented for publication of the finalized manuscript.

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Abstract

N-acetylcysteine (NAC) is an abundantly available antioxidant with a wide range of antidotal properties currently best studied for its use in treating acetaminophen overdose. It has a robustly established safety profile with easily tolerated side effects and presents the Food and Drug Administration's approval for use in treating acetaminophen overdose patients. It has been proven efficacious in off-label uses, such as in respiratory diseases, heart disease, cancer, human immunodeficiency virus infection, and seasonal influenza. Clinical trials have recently shown that NAC's capacity to replenish glutathione stores may significantly improve coronavirus disease 2019 (COVID-19) outcomes, especially in high risk individuals. Interestingly, individuals with glucose 6-phosphate dehydrogenase deficiency have been shown to experience even greater benefit. The same study has concluded that NAC's ability to mitigate the impact of the cytokine storm and prevent elevation of liver enzymes, C-reactive protein, and ferritin is associated with higher success rates weaning from the ventilator and return to normal function in COVID-19 patients. Considering the background knowledge of biochemistry, current uses of NAC in clinical practice, and newly acquired evidence on its potential efficacy against COVID-19, it is worthwhile to investigate further whether this agent can be used as a treatment or adjuvant for COVID-19.

Key Words: N-acetylcysteine; Antioxidant; COVID-19; SARS-CoV-2; Treatment

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Core Tip: N-acetylcysteine is a long known antioxidant that is currently best studied for its use as an antidote for acetaminophen overdose. Its off-label use in various diseases, such as chronic respiratory disease, heart disease, cancer, human immunodeficiency virus infection, and seasonal influenza, has shown promising results, as have recent clinical trials investigating the potential benefits of N-acetylcysteine in patients with coronavirus disease 2019.

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INTRODUCTION

N-acetylcysteine (NAC) is a glutathione precursor derived from L-cysteine, long known for its antioxidant properties. NAC has a variety of clinical benefits, seen in cough, dry eyes, and influenza. It is also commonly used as an antidote for acetaminophen overdose and as a means to reduce nitrate tolerance. This medication has been recommended by the World Health Organization as an antidote in poisoning since the 1960s. NAC is also a common ingredient found in certain cosmetics and vitamin supplements[1].

NAC has been proposed as a potential prophylactic or adjuvant for coronavirus disease-19 (COVID-19) therapy, a cost-effective alternative for mild to severe cases. NAC is routinely used in the prevention and adjuvant treatment in conditions with thick and tenacious mucus production, such as pneumonia, cystic fibrosis, chronic bronchitis, and postoperative pulmonary complications. It has unbound sulfhydryl groups that break disulfide bonds of the glycoprotein matrix within the mucus, which helps dissolve the mucus, making NAC a potent mucolytic. NAC is not only responsible for managing the redox state by replenishing the thiol stores, but it is also a cysteine precursor, making it a durable antioxidant[2].

The number of Americans who have perished from COVID-19 is nearly double that of World War I and almost two to three times that of Nagasaki's atomic bombing. Therefore, it is vital to use the best therapeutic approaches possible to help contain COVID-19. There are currently numerous studies being carried out to test the efficacy of NAC in COVID-19 patients. A clinical trial called 'Efficacy and Safety of Nebulized Heparin-NAC in COVID-19 Patients by Evaluation of Pulmonary Function Improvement' investigates whether this method can decrease ventilator use in COVID-19 patients. Another clinical trial called "A study of NAC in Patients With COVID-19 Infection" is testing the number of patients being taken off the ventilator, the number of patients released from the Intensive Care Unit, and the number of patients discharged from the hospital after treatment with NAC (for a complete list of current clinical trials on the use of NAC in COVID-19, please refer to the "Ongoing Clinical Trials" section). NAC could also be immensely beneficial as prophylaxis in front-line workers, but its benefits are yet to be studied. Further testing is necessary for assessing potential medical gain and validation of this therapeutic approach[2,3].

STRUCTURE

NAC is known by many different names, such as acetylcysteine, NAC, or R-mercaptate. The organic compounds class is known as N-acyl-alpha-amino acids[4]. Cysteine is converted to NAC *via* acetylation. Cysteine, among a few other amino acids, is a small molecule, and its structure is NH2-CH (CH2-SH) COOH[5]. Cysteine contains sulfanyl (-SH) in its side chain, which are helpful in the movement of living cells and ions by forming channels. The formation of disulfide bonds between cysteine

are known to unravel different proteins. Cysteine is made of many occupied and unoccupied orbitals such as O-2p, C-2p, S-4s+3d orbitals, N-no ($n > 3$), O-np ($n \geq 3$) and sulfur-ns+md ($n > 4$, $m > 3$), S-3sp, O-2sp[6-8]. Its structure can explain the function and clinical significance of NAC. According to the dynamic rotational isomeric state formalism, there is a frequent timed transition of a molecule from one isomeric state to another isomeric state. The transition rate can be calculated from the molecular dynamics simulations of Gly-Gly-X-Gly-Gly peptides, where X is one of the amino acids. This has been recorded in the lab experiments by the fluorescence tag, by Ramachandran[9].

Molecular dynamics, explained by the dynamic rotational isomeric state formalism, illustrate the torsional transition from Psi to Pi and vice versa. According to the study, these torsional rotations of amino acids are influenced by temperature, molecular weight, and pressure. They studied different amino acids and found that rate constants for different amino acids are reflective of the flexibility of the side chain. These transitions are determined by the carboxyl and amino end of the amino acids. Unlike other amino acids, Cys, Trp, Tyr, and Met don't have specified constants since they are known as "efficient quenchers"; they accept the free electrons into their outermost orbit and become stabilized. This process also gives NAC its antioxidant effects. NAC is a protein, and like other proteins, it is a dynamic molecule. The cysteine component of NAC contributes to this[6,9].

The chemical structure is C5H9NO3S. The IUPAC name for NAC is (2R)-2-acetamido-3-sulfanylpropanoic acid. Its molecular weight is 163.2 g/mol. It is an N-acetyl-L-Amino acid from the N-acetylated derivative of the natural amino acid L-cysteine[6]. NAC is composed of cysteine and an acetyl group attached to the amino group of cysteine[10,11]. It is a white crystalline powder with a slightly acidic odor and a sour taste. It has a specific optical rotation of +5 degrees at 20 °C, and it is stable in ordinary light and temperatures up to 120 °C. NAC is non-hygroscopic, meaning it oxidizes in moist air[12]. NAC exerts its antioxidant effects in multiple ways. It is a precursor of reduced glutathione (GSH) and cysteine *via* a deacetylation reaction. GSH, in turn, has both direct and indirect antioxidant effects. NAC acts as a direct antioxidant on NO_2 and Homeobox. NAC also acts as an antioxidant by breaking the thiolated proteins, a form of organosulfur compound (R-SH). By this action, it releases free thiols as well as reduced proteins like mercapto-albumin[2].

SOURCES

The human body can naturally produce cysteine in small amounts. This production requires adequate amounts of folate, iron, and vitamins B6 and B12. These nutrients can be found in beans, lentils, spinach, bananas, salmon, and tuna. Protein-rich foods are also a good source of cysteine. The top high-cysteine-containing foods include pork, beef, chicken, fish, lentils, oatmeal, low-fat yogurt, sunflower seeds, and cheese[13]. High dietary nitrogen sources are found in both animal sources, fruits, and vegetables. Meat sources include poultry, fish, shellfish, beef cuts such as tenderloin and top sirloin, and pork. The principal dietary sources of acetyl-coenzyme A are egg yolk, liver, kidney, broccoli, and milk. Substantial concentrations of pantothenic acid are also found in chicken, beef, potatoes, and whole-grain[14].

Plant foods rich in nitrogen sources are tofu and soy-based proteins, beans (lentils, black beans, kidney beans), and sesame seeds. According to the Centers for Disease Control and Prevention, leafy green vegetables, such as spinach, lettuce, and beetroot, are the richest nitrate source that can be included in the diet[15].

ANALYSIS AND EXTRACTION

Total NAC from human plasma can be obtained through liquid chromatography-tandem mass spectrometry[16]. Recognition by mass spectrometry can be done through positive electrospray ionization and various reaction surveillance modes. NAC transition pairs and isotope-labeled internal standards are obtained. Trichloroacetic acid has been shown to improve extraction recovery yields. The blank matrix can be used to reduce the effect of endogenous NAC[17].

Lewis *et al*[18] discussed the use of the high-performance liquid chromatography method for NAC in human plasma and urine using a dinitrophenyl derivative of NAC with a Carbon 18-bonded reverse-phase column A mobile methanol phase citrate solution, used to reach a retention time of congruent to 13 min at a flow rate of 1

mL/min. For the NAC assay in urine, there is a slight modification. The assays' sensitivity limits were determined as 60 ng/mL for the plasma and 200 μg/mL for the urine.

NAC's oxidation process yields disulfides and artifacts, making it difficult to perform an assay in biological systems. Also, biological systems have thiols like cysteine and glutathione that have physical and chemical properties like that of NAC. Hence, it is always important to receive NAC in its reduced form quickly. This is possible *via* chemical derivatization of NAC using several electrophilic agents, leading to the formation of secure adducts. These adducts are more easily separated by chromatography than the main compound and display properties like fluorescence, which helps recognize and quantify them. Reagents which are required for derivatization and assay of NAC include: N-(1-Pyrene) maleimide; N-(7-Dimethylamino-4-methylcoumarinyl) maleimide; 4-(Aminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole; Ammonium 7-fluoro-2,1,3-benzoxadiazole; 2,4-Dinitro-l-fluorobenzene; Monobromobimane; and o-Phthalaldehyde. The derivatization is done in basic pH since most of the reagents interact with the thiolate anion of NAC. However, oxidation of NAC increases quickly with basic pH such that the derivatizing agents must interact quickly with the remaining NAC in the sample before thiol is extracted. As thiols are present in the biological samples, it is important to add sufficient reagents to permit quantifiable recovery of the NAC adduct from such biological specimens. The assay protocol for NAC should include the capacity to ascertain the redox condition of the thiol. Acid precipitation and reduction allow for oxidized NAC formation in disulfide forms, and NAC intermingled with disulfides and proteins. This can be done by dividing the soluble and protein components of the specimen by acid precipitation, followed by reducing these constituents with reducing agents like dithiothreitol. Finally, extra NAC derivatives are obtained from the oxidized specimens[19-22].

STORAGE

A study conducted by Siddiqui *et al*[23], 2016, NAC was reported to be the most fragile cell reinforcement agent among endogenous thiol mixes. It was found to be more stable in an aqueous arrangement. It was exposed to dependability reads for 24 h with a 4 h span, and the outcomes were as far as rate debasement. The outcomes recommend that there was a corruption of 0.89% and 0.48% in the solution put away at room temperature and in refrigerated conditions, individually[23]. Unopened vials of acetylcysteine sodium solutions ought to be stored at 15-30 °C. Following the exposure to air, the orally taken solutions should be stored at 2-8 °C to hinder oxidation and should be utilized within 96 h[6]. Acetylcysteine arrangement doesn't contain any antimicrobial operator; therefore, care must be taken to limit the sterile arrangement's pollution. Once opened, the vial should be put away in the fridge, and the opened vial ought to be disposed of after 96 h.

In the long haul (2 mo) steadiness study conducted by He *et al*[24] in mice using analytical methods, N acetylcysteine amide and N acetylcysteine spiked in plasma at -20 °C, with a recovery extending from 103.5% to 111.5% for N- acetylcysteine amide and from 99.7% to 105.4% for NAC, demonstrating that keeping the agent at -20 °C is an option when plasma can't be examined right away. In fluid arrangements (10 mmol/L NH4HCO3, pH: 7.4), recuperation paces of 91.8% to 102.1% were acquired for NAC amide and 4 °C or -20 °C for NAC at room temperature, demonstrating that watery/stock arrangements are steady for long-term studies. This proves that NAC amide was likewise stable in physiological saline at RT and 4 °C (91.0%-116.1%), while less stability was seen in 5% glucose at high fixation at RT (86.6%), recommending that NAC amide ought to be ideally put away at 4 °C when 5% glucose is utilized in future clinical settings[24].

BIOLOGICAL MECHANISMS AND HEALTH BENEFITS

NAC plays several roles in medicine, and different mechanisms of action have been postulated for the various roles. When used for acetaminophen poisoning, it acts by restoring hepatic concentrations of GSH, an antioxidant that metabolizes acetaminophen into nontoxic soluble intermediates. When there is acetaminophen overdose, reduced glutathione stores in the liver are depleted, resulting in the accumulation of the toxic intermediate N-acetyl-p-benzoquinone imine. NAC helps

replenish glutathione stores by being metabolized into L-cysteine, which is a glutathione precursor. It is suggested that the thiol group contained in NAC can also directly inactivate the toxic metabolite[25].

NAC is also used as a mucolytic through the lytic effect of its free sulfhydryl group on the disulfide bonds in mucus, which helps lower the viscosity of mucus. It is found to have positive neuropsychotropic effects through its metabolite L-cysteine, which also serves as a precursor of cysteine, a substrate for the cystine-glutamate antiporter on astrocytes. Increased cystine levels increase glutamate release into the extracellular space. Thus, NAC has been suggested as an adjuvant in the treatment of Parkinson's disease, Alzheimer's disease, neuropathic pain, and stroke[26].

The role of NAC in viral infections has been investigated since the early 1990s. In 1993, Roederer *et al*[27] investigated the role of thiol replenishment therapy in the treatment of human immunodeficiency virus (HIV)/acquired immune deficiency syndrome (AIDS). They showed that NAC can inhibit inflammatory stimulation *in vivo*, including that caused by HIV replication[27]. On the other hand, Geiler *et al*[28] explained that NAC can inhibit H5N1 replication and H5N1-induced production of pro-inflammatory molecules. The mechanism behind these findings is mostly explained by NAC's effect on reactive oxygen species (ROS). ROS is produced *via* multiple pathways during viral infections, including mitochondrial reactions, degradation of lipids and proteins, and, importantly, from respiratory burst reactions in phagocytes. Several viruses such as HIV-1, Respiratory Syncytial Viral, H5N1 have been shown to increase oxidative stress in the host by dysregulating the oxidative stress pathways and causing an escalation of ROS synthesis. While high levels of ROS help in the phagocytosis and apoptosis of infectious organisms, low levels promote viral replication and mutations resulting in the development of resistant strains. ROS also causes significant host cell damage and lysis[29]. NAC scavenges ROS directly through direct interaction with target proteins containing a cysteine residue or thiol group such as Raf-1, MEK, and ERK *via* a thiol-disulfide exchange reaction, and indirectly by increasing synthesis of GSH. This potent antioxidant catalyzes the reduction of hydrogen peroxide to water and oxygen and the reduction of peroxide radicals to alcohols and oxygen. NAC also protects cells from apoptosis by chemically forming inactive adducts or complexes with several 18b-glycyrrhetinic acid derivatives, which induce apoptosis by activation of caspase-8 and caspase-9 and downregulation of anti-apoptotic proteins like c-FLIP, XIAP, and Mcl-1[30].

NAC has various anti-inflammatory actions, including the inhibitory effect on inflammatory cytokines such as CXCL8, CXCL10, CCL5, that are responsible for neutrophil recruitment, Th1 response, and NK and CD8 cell trafficking, as well as on interleukin-6 (IL-6), which is responsible for stimulation of acute-phase responses, hematopoiesis, and immune reactions. It also regulates proinflammatory kinases, such as nuclear factor kappa B (NF-kB) and p38 through activation of GSH and direct antioxidant effect of its free thiol group. NF-kB is a redox-sensitive transcription factor that regulates the expression of pro-inflammatory cytokines, including IL-1, IL-6, and tumor necrosis factor-alpha, as well as genes associated with apoptosis, such as p53, and is activated by increased ROS levels. NAC, a glutathione precursor, inhibits NF-kB by S-glutathionylation of the p65 subunit of NF-kB, resulting in blockage of TNF-alpha activation and nuclear translocation of NF-kB-p65. The latter results in reduced synthesis of inflammatory cytokines[31].

NAC has also been reported to promote lymphocyte proliferation, which is inversely affected by oxidative stress and low GSH levels. T cell exhaustion, which refers to low levels of CD4+ and CD8+ levels, commonly occurs in chronic viral infections and is considered to be caused by inflammatory cytokines, TNF-alpha, IL-6, IL-10. NAC's antioxidant effect helps to improve the redox balance, which helps protect and promote lymphocyte proliferation[32].

Another mechanism of its anti-inflammatory effect is the inhibition of the NLR family pyrin domain containing 3 (NLRP3) inflammasome pathway. NLRP3 inflammasome is a well-known trigger of the cleavage and activation of caspase-1, leading to maturation and secretion of interleukin-1β and interleukin-18. Overactivation of this inflammasome is critical in the pathogenesis of several disorders, such as Crohn disease, atherosclerosis, gout, type 2 diabetes mellitus, and chronic infections. Data from both severe acute respiratory syndrome coronavirus (SARS-CoV)-1 and SARS-CoV-2 patients show evidence of increased NLRP3 inflammasome activity. NAC blocks NLRP3 inflammasome activation by interfering with the priming step required to induce NLRP3 expression. It is also shown to work in a dose-dependent manner to reduce mRNA expression of NLRP3 inflammasome and caspase-1, a large pro-inflammatory enzyme that causes the production of interleukin-1β and interleukin-18, as well as the recruitment of neutrophils[33].

As it has come to be known, NAC has been used in practice for several decades now. It has served as a mucolytic agent, contributing to the breakdown of mucus in the respiratory tract and keeping the tract moist to decrease irritation. By reacting with hydroxyl radicals, superoxide, hydrogen peroxide, and peroxynitrite radicals, NAC helps reduce the disulfide bonds in proteins[34]. Since it is a cysteine pro-drug and a GSH precursor, it can also help scavenge free radicals such as those mentioned above. NAC has anti-inflammatory activity already mentioned in the previous section, and it accomplishes this *via* the inhibition of nuclear factor-kappa light chain enhancer of activated B cell (NF-kB). An example of a disease with oxidative stress implicated in its pathogenesis and progression is chronic obstructive pulmonary disease (COPD). The oxidative species are from the inhalation of cigarette smoke and those formed within the body by inflammatory cells. This leads to an increase in oxidant stress in the lung. NAC's antioxidant property plays a crucial role in COPD patients to reduce their symptoms, acute exacerbations, and the decline in lung function[35].

The known health benefits of NAC are mainly exerted at the cellular level. A study conducted by Kinscherf *et al*[35] in 1994, using healthy human subjects, showed that people with intracellular glutathione levels of 20-30 nmol/mg had higher numbers of CD4+ T cell numbers than people who had higher or lower glutathione levels. Once the patients in the 4-wk observation period moved from the optimal to the suboptimal range, which meant from 20-30 nmol/mg to 10-20 nmol/mg, they ended up with a 30% decrease in CD4+ T cells. This 30% decrease was prevented by using NAC as a treatment. They found that NAC causes a relative increase of CD4+ T cell numbers even though the glutathione levels decrease but not by increasing the glutathione levels either. They discovered that NAC, which determines glutathione levels, has a strong influence not only on cysteine and glutathione levels but also on T cells in the human body[36].

SAFETY PROFILE AND ADVERSE EFFECTS

NAC is administered in the intravenous, oral, and nebulized forms. It is used as adjuvant therapy in respiratory conditions and can be administered in a nebulized form or be directly instilled. The inhaled form can be given by nebulization through a face mask, mouthpiece, or tracheostomy. Alternatively, inhalation through a tent or croupette is also available[37]. Acetylcysteine solutions of 10% and 20% are used in adult, geriatric and pediatric patients receiving the inhaled dosage employing face mask, mouthpiece, or tracheostomy. The 20% solution is diluted with sodium chloride or sterile water for inhalation. The 10% solution can be used undiluted[37].

When administered orally at a dose of 1200 mg/d for six months, De Flora *et al*[37] found that NAC reduced symptoms of influenza in patients over the age of 65 years with chronic degenerative diseases. The NAC recipients suffered from influenza less and only had fewer influenza-like episodes with fewer days confined to bed. Though NAC played no role in viral seroconversion, symptomatic infection episodes were considerably less[37].

The effectiveness and tolerability profile of high-dose NAC was studied in a trial, where NAC at a dose of 1200 mg/d, 600 mg/d, or placebo was given once daily for 10 d to patients with COPD exacerbations Evidence showed that a significant proportion of patients had normalization of C-reactive protein (CRP) levels which was obtained with both NAC 600 and 1200 mg/d compared to placebo. The same study demonstrated NAC's therapeutic superiority in decreasing the IL-8 Levels with a dose of 1200 mg/d rather than 600 mg/d. Both treatment regimens' effects were equally effective in terms of lung function and other clinical outcomes, including the intensity and frequency of cough and Korsakoff sounds. Adverse events were reported only in one patient amongst the 1200 mg/d NAC groups, whereas; two events were seen in the placebo group[38].

Therefore, oral NAC (600 mg/d) could function as a preventive measure in those who are repeatedly exposed to possible SARS-CoV-2 carriers like health workers and those who cannot work at home. Healthcare workers worldwide have become infected while caring for hospitalized patients; therefore, 600 to 1200 mg daily NAC could potentially help to flatten the exponential curve in several countries[39].

In severe cases of COVID-19, ventilator use is common, with roughly 3.2% of all cases requiring mechanical ventilation at some point during the illness. The use of NAC as a prophylactic intervention for mechanical ventilation complications, such as ventilator-associated pneumonia (VAP), has been studied in a randomized controlled trial involving nasogastric administration of 1200 mg NAC daily. It was found that

patients treated with NAC had fewer incidences of VAP and a shorter hospital stay. Also, the complete recovery from VAP was more frequently observed in the NAC group[40].

NAC can also be of benefit in the treatment of patients with acute respiratory distress syndrome. A clinical trial conducted in the United States and Canada found that intravenous NAC (70 mg/kg body weight), when given every 8 h for ten days, effectively reduced glutathione in RBCs, thereby decreasing lung injury. Additionally, it helped increase the cardiac index[41]. Administration of NAC (50 mg/kg body weight in 250 mL of 5% dextrose for 6 d) was found to protect the lung tissue in acute respiratory distress syndrome patients. The effectiveness of NAC was quantified by measuring the expired ethane and malondialdehyde along with glutathione disulfide and GSH in the epithelial lining fluid[42]. In another study, intensive care unit (ICU) patients who received NAC at a dose of 150 mg/kg body weight on the first day, followed by 50 mg/kg for a total of 3 d, appeared to have a better clinical outcome when compared to the placebo group[43].

The use of NAC has been established in a clinical study in which isosorbide dinitrate, given its vasodilator properties, was given to six male participants for a period of 48 h. NAC was administered at 24 h in a dose of 2 g intravenously, followed by 5 mg/kg/h. The plasma concentration of angiotensin II increased for the duration of the first 24 h of isosorbide dinitrate administration, but the levels decreased by 28 ng/L to 14 ng/L ($P < 0.05$) just 2 h after NAC was started[44]. This effect could postulate that NAC's protective effects counteract the harmful effects of angiotensin II in SARS-CoV-2. NAC has an exceptional safety history in clinical trials. The side effects of oral NAC include stomatitis, nausea, vomiting, gastroesophageal reflux. If an anaphylactoid reaction occurs with intravenous NAC, then oral NAC may be used instead[45,46]. Bronchoconstriction and extended coughing, and worsening of asthma were the side effects of nebulized NAC[47,48].

The harmful effects of NAC are mainly dependent on its route of administration. A clinical study investigated the pharmacological profile of a six-month administration of oral NAC in 26 volunteers. The main adverse effects seen were mostly gastrointestinal symptoms; intestinal gas, diarrhea, nausea, and fatigue, with the maximum nontoxic dose being 800 mg/m^2/d[49]. Another trial studied the effects of oral administration of NAC at high doses of up to 8000 mg/d in HIV patients, and no adverse effects were reported[50]. Severe anaphylactoid reactions like hypotension, bronchospasm, and angioedema were noted to occur with initial loading infusions of NAC, which resulted in temporary increased plasma concentrations of NAC. These symptoms were promptly resolved after discontinuation of the drug[51]. Nevertheless, severe systemic reactions are rare. NAC does not require dosage adjustments in renal or hepatic impairment[52]. The risk of sound-alike error can be observed with acetylcysteine, which may be confused with acetylcholine, and mucomyst, which may be confused with Mucinex.

All patients (adult and pediatric) should receive an aerosolized bronchodilator 10-15 min before NAC administration. In adults, 3 to 5 mL of the 20% solution or 6 to 10 mL of the 10% solution is given through nebulization up to 3 or 4 times/d. The standard dosing range for the 20% solution is 1 to 10 mL and 2 to 20 mL for the 10% solution every 2 to 6 h. For inhalation of the 10% or 20% solution in the form of a heavy mist *via* a tent or croupette, the dose must be individualized and may require up to 300 mL solution/treatment. Children and adolescents are usually given the adult dosage, but in infants, 1 to 2 mL of 20% solution or 2 to 4 mL of 10% solution is used. NAC can also be given through direct administration into the tracheostomy in adults. 1 to 2 mL of the 10% or 20% solution is introduced every 1 to 4 h. When administered through a percutaneous intratracheal catheter, 1 to 2 mL of the 20% or 2 to 4 mL of the 10% solution should be instilled every 1 to 4 h *via* a syringe attached to the catheter. In children and adolescents, 1 to 2 mL of 10% to 20% solution can be instilled every 1 to 4 h as needed *via* the endotracheal tube. The dosage remains the same for percutaneous endotracheal instillation[53].

Different adverse events have been reported with NAC, and they range from nausea to death. Although NAC's severe reactions look like anaphylaxis, they are non-immunological and hence classified as anaphylactoid reactions. Other adverse events that have been reported infrequently in studies of NAC include dizziness, fever, vertigo, localized skin rash, dyspnea, tachycardia, hypertension, cardiac arrest[54]. Oral NAC has been rarely associated with serious adverse events. However, repeated high doses may cause nausea, vomiting, diarrhea, and rarely headache, rash, hypotension, and respiratory distress[55].

Urticaria and hepatotoxicity have also been reported. High-dose Intravenous NAC has been associated with anaphylactoid reactions like flushing, rash/pruritus,

angioedema, bronchospasm, nausea/vomiting, hypotension, tachycardia, and respiratory distress[56]. There are also case reports that describe ECG abnormalities, status epilepticus, and a serum sickness-like illness[57-59].

NAC is contraindicated in persons with previous severe anaphylactoid reactions or hypersensitivity reactions associated with its use. Should be cautiously used in pregnant women as it crosses the placental barrier, those with a family history of drug allergy, and patients with asthma or bronchospasm. It should not be used in acute paraquat poisoning. Nebulised NAC should be used cautiously in patients with respiratory insufficiency, an inadequate cough mechanism, or gag reflex depression. At the same time, oral NAC can exacerbate vomiting for which precautions should be taken to use in patients with esophageal varices and peptic ulcers. Acetylcysteine effervescent tablets should also be cautiously used in patients with sodium-restricted diets like hypertension, heart failure, and renal disease[60].

CLINICAL APPLICATIONS

NAC has been used for more than 30 years and is best known for its use in acetaminophen overdose. It can be used in several other diseases like chronic bronchitis, HIV, influenza, heart disease, and several other poisonings. It can be used in acetaminophen overdose and respiratory diseases like pneumonia, tracheobronchitis, cystic fibrosis, tracheostomy patients, postoperative pulmonary complications, and posttraumatic chest conditions. Its off-label uses are acute hepatic failure and prevention of contrast-induced nephropathy[45].

Acetaminophen overdose

The treatment for acetaminophen overdose is NAC. It is proved that NAC's early administration within 8 to 24 h prevents mortality[45]. Interestingly, it has recently been suggested that a shorter 12-h regimen of NAC be used in these patients, instead of the conventional regimen of 20-21 h in duration. The rationale behind this recommendation is the ability to preserve resources in the current shortage conditions while ensuring effective treatment of the most common cause of excessive medicine ingestion[61].

Respiratory diseases

A study by Cotgreave *et al*[61] observed the levels of NAC in the bronchoalveolar lavage of six healthy volunteers following administration of 600 mg of NAC orally for four weeks. Although the levels of NAC, cysteine, and glutathione in the bronchoalveolar lavage fluid did not increase, the levels of protein-bound NAC and both free and total plasma glutathione were shown to rise significantly[62]. On the other hand, a study by Rodenstein *et al*[62] demonstrated that NAC given orally to people with respiratory disorders led to a similar NAC level in the plasma and lung tissue. NAC has been used as a mucolytic agent in chronic bronchitis. Although initial studies like the one by Millar *et al*[63] showed no significant effect in patients with chronic bronchitis, a study by Parr *et al*[64] showed that there is a substantial decrease in the number of incapacitated days in the individuals suffering from chronic bronchitis.

Additionally, Rasmussen *et al*[65] conducted a double-blind, placebo-controlled, six-month comparison study, which showed that the NAC treatment group had a lower number of sick-leave days and exacerbation days. Jackson *et al*[66] conducted a multicenter, double-blind, placebo-controlled study that found that the difficulty in expectoration and cough severity improved and was more evident in patients using NAC. Behr *et al*[67] studied the effect of NAC administration for 12 wk on 18 patients suffering from fibrosing alveolitis, a disease known for the uncontrolled activation of the oxidative stress response, as well as for the reduced levels of GSH in the lower respiratory tract. This treatment led to improved pulmonary function tests and an increase in total and reduced glutathione[68]. NAC has shown some preventive effect of microembolism in a rat model having acute respiratory distress syndrome by decreasing alveolar edema, fibrin deposition, and plasma viscosity.

Cancer

NAC has been proven to have some beneficial effects on cancer and its management. Though evidence is still preliminary, a few studies have shown its efficacy when combined with chemotherapeutic agents. De Flora *et al*[69] have studied NAC's effect on GSH metabolism and the biotransformation of carcinogenic compounds. *In vitro* and *in vivo* studies have shown that NAC counteracted the mutagenicity of direct-acting

compounds and, at high concentrations, inhibited procarcinogens' mutagenicity[70]. This study has also combined NAC with doxorubicin and found that, under certain experimental conditions, it can be highly effective by working synergistically with doxorubicin to reduce tumor formation and prevent metastases. Pre-treatment with NAC increased the non-protein content of P388 Leukemia cells nearly threefold, without negatively affecting the chemotherapeutic activity of doxorubicin against this tumor.

Heart disease

NAC is also useful in heart disease. It affects the levels of homocysteine and possibly even the levels of lipoprotein A. Moreover, it protects against ischemic and reperfusion damage and increases the efficacy of nitroglycerine. Gavish and Breslow *et al*[71] proved that NAC administration to patients with increased lipoprotein levels had reduced plasma lipoprotein levels by 70%. Wiklund *et al*[72] postulated that NAC administration reduces plasma homocysteine levels by 45% but did not show any effect on lipoprotein levels. Bostom *et al*[73] reported that even in dialysis patients who have high homocysteine levels and are refractory to vitamin B supplementation, oral NAC supplementation resulted in a 16% decrease in non-fasting pre-hemodialysis total plasma homocysteine[74]. In combination with nitroglycerin and streptokinase, NAC decreased the oxidative stress and preserved left ventricular function in patients with evolving acute myocardial infarction[75]. In combination with nitroglycerin, NAC should be used with caution because of the adverse effects[76].

Cigarette smoking

Oral supplementation with NAC is necessary for smokers and people exposed to second-hand smoke, as NAC has been proven to decrease smoking-induced mucus cell hyperplasia, epithelial hypertrophy, and the time required for the secretory cells to return to normal[77].

HIV

HIV-positive individuals have low cysteine and GSH levels. Supplementation of NAC in these individuals has been studied, and the results are still unclear. Wu *et al*[76] observed that NAC administration had increased the ability of cells to form T-cell colonies in people with AIDS[78]. Herzenberg *et al*[77] noted that the oral administration of NAC in HIV-infected individuals improves GSH levels and aids in the improvement of survival rates in this population[79]. Sandilands *et al*[80] suggested that NAC administration to HIV-infected individuals prevented the progression to AIDS. Though further evidence is needed to determine NAC's efficacy in HIV-positive individuals, based on the available evidence, NAC supplementation can be considered an essential component of anti-HIV treatment in individuals with low GSH levels[81].

Other uses

NAC usage in individuals with influenza and influenza-like episodes decreased the symptoms but did not prevent the disease. NAC is also used in myoclonic epilepsy, where it has been shown to reduce the myoclonus. Finally, NAC is of benefit in Sjogren syndrome, where it is considered to help improve ocular soreness, irritability, halitosis, and daytime thirst[82].

PREVIOUS HUMAN EXPERIENCE

NAC is a powerful drug used for a variety of treatments, including pulmonary and liver diseases. Different *in vitro* and *in vivo* studies were performed to demonstrate NAC's efficacy as an antioxidant in COPD. Data has shown that oxidative stress acts as an essential pathogenetic factor in altering the lungs of patients with COPD. Open-label and double-blinded clinical studies with patients with and without COPD were used to conclude that the ability of NAC to protect the lungs against toxic agents is through its antioxidant properties. Results show that in patients with COPD, a dose of 600 mg daily accounted for the reduced risk of exacerbations and viscosity of expectorations. After two months of treating patients with NAC, the viscosity improved by 80%, the severity of the cough improved by 71%, and the difficulty of expectoration by 74%. However, a different double-blind, double-dummy, controlled study with 120 patients suggested that 1200 mg was the correct dosage to see improvements in COPD patients[83].

Another study with acute coronary syndrome patients was designed to determine

the effectiveness of rapid intravenous hydration with sodium bicarbonate plus NAC to prevent contrast-induced nephropathy in patients undergoing percutaneous coronary intervention. The study focused on 120 patients that were consequently divided among group A and group B. The first group received an initial intravenous bolus of 5 mL/kg/h of alkaline saline solution with 154 mEq/L of sodium bicarbonate in 5% glucose and H_2O plus 2400 mg of NAC in the same solution. The next day, patients received two doses of 600 mg NAC. In contrast, Group B was treated with perfusion of isotonic saline (0.9%) at a rate of 1 mL/kg/h for 12 h after percutaneous coronary intervention plus two doses of 600 mg NAC orally the next day. After collecting samples and stating that the development of acute contrast-induced nephropathy refers to an increase in serum creatinine concentration of 0.5 mg/dL or more, data analysis was performed. Data indicated that rapid hydration with saline bicarbonate and high doses of NAC before contrast injection helps prevent renal dysfunction, and the rate of contrast-induced nephropathy decreases drastically[84].

The alleviation of polychlorinated biphenyls (PCBs) 52-induced hepatotoxicity with NAC was tested by performing an *in vitro* study in human and rat cells. Human L-02 cells supplemented with 15% fetal bovine serum and 100 U/mL penicillin-streptomycin, in addition to rat Brl-3A cells cultured with 3% fetal bovine serum and 100 U/mL penicillin-streptomycin, were utilized for the investigation. It is known that PCBs may induce human hepatotoxicity since they are a type of persistent chlorinated pollutant. In this study, cells were treated with 40 μmol/L of PCB52 for 48 h after NAC/saline pre-treatment. Exposure to PCB52 Leads to excessive production of ROS-releasing inflammatory mediators, which play an essential role in hepatotoxicity. Consequently, data was analyzed with different laboratory techniques to gather ROS levels. Results show that NAC pretreatment drastically reduced ROS levels in both rat and human cells. NAC ameliorated PCB52 reduction of cell viability, implying that the alleviation of PCB52-induced hepatotoxicity could result from the elimination of ROS[85].

CURRENT CORONAVIRUS DISEASE 2019 MANAGEMENT AND POTENTIAL IMPLICATIONS OF N-ACETYLCYSTEINE AS A SUPPLEMENTARY AGENT

The therapeutic options for COVID-19 have constantly been evolving. Many studies have shown that certain dietary elements and vitamin supplements could be promising[86] and, according to the World Health Organization's International Clinical Trials Registry Platform, there are about 3369 studies on management of COVID-19. Currently, COVID-19 management is based on the severity of the disease, patient age, and history of comorbidities[87] (Table 1). The following drugs are used as a possible therapy though still lacking evidence of efficacy. Chloroquine acts by blocking the cell fusion of the virus and also increases endosomal pH[88]. It is an autoimmune and antimalarial drug used alone or together with remdesivir and has the highest efficacy in controlling coronavirus infection[89]. The use of chloroquine or hydroxychloroquine in combination with azithromycin has been evaluated in several retrospective observational, and uncontrolled studies[90,91]. In patients on first treatment with antiviral drugs like lopinavir or ritonavir, the viral road decreased and helped with the recovery[92]. Rosuvastatin is capable of binding and inhibiting the main protease enzyme of COVID-19. Statins act by reducing chemokine release, levels of adhesion molecules, and by modulating T-cell activity. The use of statins has been postulated to affect mortality in COVID-19[93]. Monoclonal antibodies like tocilizumab act against IL-6 receptors and prevent the development of cytokine storm and severe inflammation[94]. Anakinra is another antibody utilized in the treatment of critically ill patients. By blocking the IL-1 receptor, Anakinra reduces cytokine release triggered by the virus[95]. Treatment with vitamin C enhances the internal production of vasopressors and reduces the need for norepinephrine treatment[96].

The worldwide spread of COVID-19 continues with no effective treatment in the medical armamentarium and with the first Food and Drug Administration's approved vaccines only rolling out since December 2020. It would thus be of benefit to once again look into our current understanding of the pathogenic mechanisms of SARS-CoV-2 infection. More specifically, the significant variability among the responses of different patients to COVID-19 and the importance of excessive inflammatory reaction and redox decompensation observed in critical cases of COVID-19 are both worth highlighting[97].

Table 1 Principles of coronavirus disease 2019 management according to disease severity and presence of comorbidities

Severity	No comorbidities present	Comorbidities present
Mild	Conservative at home	Steroids,or/and plasma therapy
Moderate	Conservative at home	Steroids, or/and plasma therapy
Severe	Hospitalized: Treatment focused on the complication	Intravenous fluid, oxygen, corticosteroids

Angiotensin-converting enzyme (ACE) and ACE2 proteases are present on the surface of many cell types and have the same substrates angiotensin I and angiotensin II, but the opposite activities. ACE increases levels of angiotensin II, thereby mediating vasoconstriction, apoptosis, as well as the induction of oxidative stress and inflammatory reaction. ACE2 is responsible for a decrease in angiotensin II levels and for induction of ang (1-7) peptide. As a result, ACE2 counteracts the pro-inflammatory effects of ACE[97]. By binding ACE2 at its entry into human cells, SARS-CoV-2 decreases ACE2 availability and promotes ACE activity. The latter sets the background for induction of oxidative stress, as angiotensin II stimulates the NADPH oxidase pathway for production of ROS and peroxynitrite anions[98]. The imbalance between ACE and ACE2 can become even more evident in patients with an endogenous tendency towards higher levels of ACE. It is known that ACE/ACE2 ratios can differ among people and ACE-predominant individuals can be susceptible to excessive inflammation[97].

The main defense mechanism against free radical damage is through natural scavenging systems, such as the system of reduced GSH. GSH donates an electron to an unstable molecule, such as ROS, and then becomes reactive and can rapidly bind to another reactive glutathione molecule, forming a glutathione disulfide. This is feasible under normal circumstances because of the abundant concentration of GSH in cells. GSH insufficiency arising either in the context of COVID-19 or as baseline low levels due to other conditions have been postulated to have an association with the overwhelming oxidative stress leading to COVID-19 complications. On one hand, SARS-CoV-2 infection in itself induces the synthesis of free radicals, thereby consuming GSH supplies. Given that intracellular levels of GSH tend to remain relatively stable and are regulated by various environmental stimuli, such as NF-κB, hypoxia, ROS, and reactive nitrogen species, it is no surprise that in a COVID-19 patient, less GSH may be available for other cellular functions. On the other hand, low GSH levels have additionally been identified in a series of pathologic conditions that are currently considered as risk factors for severe COVID-19: older age, male sex, diabetes mellitus, hypertension, obesity, and even certain medications[97].

The extensive study of the above biochemical mechanisms and the failure of antiviral and anti-inflammatory agents to show positive results have led several researchers to explore the effects of NAC as an adjuvant treatment in patients with COVID-19.

In July 2020, a study by Ibrahim *et al*[36] found that having glucose 6-phosphate dehydrogenase (G6PD) deficiency facilitates SARS-CoV-2 infection due to glutathione depletion. NAC can be administered to help replenish glutathione stores. They found that patients with severe COVID-19 benefited from the intravenous (IV) administration of NAC. NAC blocks the hemolysis that G6PD deficiency patients are predisposed to. It also blocks the elevation of liver enzymes, CRP, and ferritin. Blocking these enzymes allowed the G6PD deficient patients to be taken off the ventilator and the veno-venous extracorporeal membrane oxygenator and led to a full recovery. Additionally, NAC was administered to another 9 ventilator-dependent COVID-19 patients who did not have G6PD deficiency. They found that NAC promoted the clinical improvement and reduced CRP levels in all patients and ferritin in 9/10 patients. In COVID-19 patients, there are high serum levels of pro-inflammatory cytokines being reported. IL-6 has also been shown to play an essential role in the cytokine storm that is associated with COVID-19. IL-6 and CRP are one of them, and NAC has been found to reduce the IL-6 dependent CRP elevation during the H1N1 influenza pneumonia. Morbidity and mortality of the human coronavirus, causing lower respiratory tract infections, originates from the host's immune response, which includes the cytokine storm perpetuated by IL-6.

De Alencar *et al*[99] conducted a double-blind, randomized, placebo-controlled trial of NAC for the treatment of severe COVID-19 respiratory disease. The rationale behind this study was the potential for improvement in COVID-19 outcomes through mitigation of oxidative stress. In this trial, 135 patients with severe COVID-19,

saturation < 94%, tachypnea of > 24 breaths/min were included, and received 300 mg/kg NAC or placebo. 23.9% of patients on placebo and 20.6% of patients of NAC received mechanical ventilation (P = 0.675), while the need for ICU admission was 42.3% in the placebo group and 47.1% in the NAC group. The mortality rate and hospital stay were the same for both groups. The study concluded that NAC can be safely tolerated but does not seem to be of benefit to severely ill patients with COVID-19.

Alamdari *et al*[97] studied the effects of methylene blue-vitamin C-NAC (MCN, 1 mg/kg methylene blue, 1500 mg/kg vitamin C, 1500 mg/kg NAC) administration as last resort therapy in five critically ill COVID-19 patients with elevated levels of nitrite, nitrate, and methemoglobin among others. Four out of five patients recovered and were discharged from the ICU, but one patient died from sepsis shortly after initiation[100]. The results of this study demonstrate that treatment with MCN is both safe and feasible. Oxidative stress is shown to play a major role in COVID-19 and the need for earlier initiation of NAC therapy, before critical disease develops, is expressed.

A different application of NAC in COVID-19 has been presented by Melisa *et al*[101]. A patient with critical COVID-19 developed a superinfection with Pseudomonas aeruginosa and Staphylococcus aureus and progressed to respiratory failure with persistent hypercapnia. In addition to standard of care, consisting of antiviral and antibiotic agents, respiratory, and nutritional support, the patient underwent bronchoalveolar lavage with a 10-15 g NAC nebulized inhalation solution. The patient gradually recovered showing that NAC can have a dual role in COVID-19: Mucus dissolving expectorant and antioxidant effects. However, what is lacking right now is the presence of large-scale studies in order to confirm the individual outcomes.

ONGOING CLINICAL TRIALS

The clinical use of NAC in COVID-19 is still under investigation. There are few ongoing trials, but no results have been posted as of the time of this writing. The trials are as follows.

A pilot double-blinded randomized placebo-controlled multicenter clinical trial was posted in July 2020 with an estimated 1180 participants at King Saud University: The study attempts to evaluate NAC therapy's efficacy in the management of adult hospitalized patients with COVID-19, focusing on the regulation of inflammatory response. The current estimated completion date for this trial is on August 30, 2021[99].

Study of NAC in patients with COVID-19: This study has started recruiting patients. The expected time frame is from May 1, 2020 - May 2021. This study has two arms A and B. Arm A has mechanically-ventilated patients and patients managed in the critical care unit. In contrast, arm B has non-mechanically-ventilated, noncritical care patients. Patients in both arms in the experimental group and the intervention group will be treated with NAC administered intravenously at a dose of 6 g/d, along with supportive care and medications specific for COVID-19. The latter will be determined by the physician on an individual basis[100].

Patients in the experimental group will receive treatment for a maximum of three weeks or until the fulfillment of one of the criteria mentioned in the corresponding table. The treatment group will utilize NAC and peripheral blood for both mechanically-ventilated and non-mechanically-ventilated patients. In the NAC treatment group, treatment may be held for ≤ 48 h, if clinically indicated. Patients can resume treatment if the drug was discontinued for no more than 48 h. The peripheral blood used in the treatment group uses a total of 16mL of whole blood collected in CPT tubes at baseline, the first day of Cycle 2 (or as close as feasible, when still coordinating sample collection across patients in a critical-care unit), and at the end of the study[100].

Efficacy of NAC in preventing COVID 19 from progressing to severe disease: This study is a randomized clinical trial and was first started on September 23, 2020, and will run through May 31, 2021, with a sample size of 200 participants[101].

A randomized double-blinded placebo-controlled study to evaluate the safety, efficacy, tolerability, and pharmacokinetics of OP-101 (Dendrimer N-acetyl-cysteine) in severe COVID-19: The anticipated primary completion date is within a week as of this writing, on October 10, 2020, and will be one of the earliest phase 2 trials with anticipated results. The primary outcome in this trial is "treatment-

emergent adverse events", and secondary outcomes include time to improvement based on the World Health Organization 7-point ordinal scale, time to improvement in oxygenation, time to resolution of fever, number of days of resting respiratory rate, and the time to discharge from the clinic or to the point of the National Early Warning Score, which consists of physiological parameters: respiration rate (per minute), SpO2 Scale 1 (%), SpO2 Scale 2 (%), use of air or oxygen, systolic blood pressure (mm Hg), pulse (per minute), consciousness, temperature (°C). Furthermore, this study is unique in assessing the baseline percent change in cytokines, including IL-6, CRP, and ferritin[102] (Tables 2 and 3).

CONCLUSION

NAC is a long-known antioxidant whose main clinical application is in the treatment of acetaminophen overdose. Its mucolytic and anti-inflammatory properties make it useful in chronic bronchitis, and its ability to reduce homocysteine levels is of benefit to people with heart disease. Moreover, it helps mitigate the impact of environmental toxins and malignancy by preventing reactive oxygen species overproduction. NAC use has also shown promising results in the treatment of various viral infections. By increasing glutathione levels, it impedes viral replication and decreases viral load. Several studies have illustrated the antiviral activity of NAC against influenza A strains H3N2 and H5N1. Recently, several studies have attempted to explore the effects of NAC in severe COVID-19 patients and the results vary. Although it seems that the ability of NAC to reduce the formation of pro-inflammatory cytokines and mitigate the impact of cytokine storms could lead to better outcomes in COVID-19 patients, there is currently not enough evidence to support this. Our hopes are that ongoing clinical trials and future studies will be able to confirm both the positive outcomes and safety profile of in COVID-19.

Table 2 Details of clinical trial

	Arm	Intervention/Treatment
NCT04455243	Experimental: Intervention group	Drug N-acetylcysteine is given as 150 mg/kg q 12 h PO or IV every 12 h for 14 d diluted in 200 mL diluent (D5 % NS)
	Placebo comparator: Control group	Matching drug placebo is administered in the same schedule and volume as N-acetylcysteine
NCT04374461	Experimental: Arm A. (1) Transfer out of the critical care unit; (2) Extubation; (3) Toxicity; and (4) Death	Drug NAC. Others: Peripheral blood dosages are given in both groups as mentioned above
	Experimental: Arm B. (1) Discharge from the hospital; (2) Admission to a critical care unit; (3) Intubation; (4) Toxicity; and (5) Death	Drug NAC. Others: Peripheral blood dosage details as mentioned above
NCT04419025	Active Comparator: NAC Patients receiving N-acetylcysteine	Drug: N-acetylcysteine. In-patient: (1) Oral formulation 600 mg capsules of NAC q4 h until discharge; and (2) 1200 mg PO BID × 1-wk post-discharge Outpatient :2400 mg PO × 1 then 1200 mg PO BID × 2 wk
	No Intervention: Control patients not receiving N-acetylcysteine	
NCT04458298	Experimental: Cohort A: OP-101 2 mg/kg. Participants will receive a single intravenous (IV) infusion of OP-101 2 milligram per kilogram (mg/kg) on Day 1	Drug: OP-101 will be administered as an IV infusion
	Experimental: Cohort B: OP-101 4 mg/kg. Participants will receive a single IV infusion of OP-101 4 mg/kg on Day 1	Drug: OP-101 will be administered as an IV infusion
	Experimental: Cohort C: OP-101 8 mg/kg Participants will receive a single IV infusion of OP-101 8 mg/kg on Day 1	Drug: OP-101 will be administered as an IV infusion
	Placebo Comparator: Cohort D: Placebo Participants will receive a single IV infusion of matching placebo on Day 1	Drug: Placebo. Matching placebo infusion will be administered intravenously

PO: Peros; NAC: N-acetylcysteine; NAC: N-acetylcysteine; BID: Bisindie; PO: Peros.

Table 3 Summary of ongoing clinical trials of N-acetyl cysteine and corona virus disease 2019

Nct	Drug or other interventions	Diseases	Location (State, Country)	Status (Recruiting or completed)	Results (Yes or not available)	Phase
NCT04455243	N-acetyl cysteine *vs* placebo	COVID 19	Riyadh, Saudi Arabia	Not yet recruiting	Pending	3
NCT04374461	N-acetyl cysteine *vs* peripheral blood	COVID 19	New York, United States	Trial began May 2020	Pending, expected May 2022	2
NCT04419025	N-acetyl cysteine	COVID 19 SARS COV 2, SARS associated Coronavirus disease, Oxidative stress	Massachusetts, United States	Trial began September 2020	Pending, expected May 2021	4
NCT04458298	OP-101 (Dendrimer N-Acetylcysteine) Placebo	COVID 19	California, United States	Trial began July 2020	Pending, expected February 2021	2

COVID 19: Corona virus disease 2019; SARS COV 2: Severe acute respiratory syndrome coronavirus 2.

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REFERENCES

1 **Šalamon Š**, Kramar B, Marolt TP, Poljšak B, Milisav I. Medical and Dietary Uses of N-Acetylcysteine. *Antioxidants (Basel)* 2019; **8** [PMID: 31035402 DOI: 10.3390/antiox8050111]
2 **Aldini G**, Altomare A, Baron G, Vistoli G, Carini M, Borsani L, Sergio F. N-Acetylcysteine as an antioxidant and disulphide breaking agent: the reasons why. *Free Radic Res* 2018; **52**: 751-762

[PMID: 29742938 DOI: 10.1080/10715762.2018.1468564]
3 **Jorge-Aarón RM**, Rosa-Ester MP. N-acetylcysteine as a potential treatment for COVID-19. *Future Microbiol* 2020; **15**: 959-962 [PMID: 32662664 DOI: 10.2217/fmb-2020-0074]
4 **Zafarullah M**, Li WQ, Sylvester J, Ahmad M. Molecular mechanisms of N-acetylcysteine actions. *Cell Mol Life Sci* 2003; **60**: 6-20 [PMID: 12613655 DOI: 10.1007/s000180300001]
5 **Fliege R**, Metzler M. Electrophilic properties of patulin. N-acetylcysteine and glutathione adducts. *Chem Res Toxicol* 2000; **13**: 373-381 [PMID: 10813654 DOI: 10.1021/tx9901480]
6 **National Center for Biotechnology Information**. PubChem Compound Summary for CID 12035, Acetylcysteine [cited March 10, 2021]. Available from: https:/pubchem.ncbi.nlm.nih.gov/compound/Acetylcysteine
7 **Maul R**, Preuss M, Ortmann F, Hannewald K, Bechstedt F. Electronic excitations of glycine, alanine, and cysteine conformers from first-principles calculations. *J Phys Chem A* 2007; **111**: 4370-4377 [PMID: 17461555 DOI: 10.1021/jp068294j]
8 **Beerbom MM**, Gargagliano R, Schlaf R. Determination of the electronic structure of self-assembled L-cysteine/Au interfaces using photoemission spectroscopy. *Langmuir* 2005; **21**: 3551-3558 [PMID: 15807601 DOI: 10.1021/La040083n]
9 **Bayrak CS**, Erman B. Conformational transitions in the Ramachandran space of amino acids using the dynamic rotational isomeric state (DRIS) model. *Mol Biosyst* 2014; **10**: 663-671 [PMID: 24442235 DOI: 10.1039/c3mb70433e]
10 **Poole LB**. The basics of thiols and cysteines in redox biology and chemistry. *Free Radic Biol Med* 2015; **80**: 148-157 [PMID: 25433365 DOI: 10.1016/j.freeradbiomed.2014.11.013]
11 **Medina-Navarro R**, Durán-Reyes G, Díaz-Flores M, Vilar-Rojas C. Protein antioxidant response to the stress and the relationship between molecular structure and antioxidant function. *PLoS One* 2010; **5**: e8971 [PMID: 20126468 DOI: 10.1371/journal.pone.0008971]
12 **Sisombath NS**, Jalilehvand F. Similarities between N-Acetylcysteine and Glutathione in Binding to Lead(II) Ions. *Chem Res Toxicol* 2015; **28**: 2313-2324 [PMID: 26624959 DOI: 10.1021/acs.chemrestox.5b00323]
13 **Górska-Warsewicz H**, Laskowski W, Kulykovets O, Kudlińska-Chylak A, Czeczotko M, Rejman K. Food Products as Sources of Protein and Amino Acids-The Case of Poland. *Nutrients* 2018; **10** [PMID: 30551657 DOI: 10.3390/nu10121977]
14 **Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate**, Other B Vitamins, and Choline. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. Washington (DC): National Academies Press (US) [cited March 10, 2021]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK114310/
15 **Agency for Toxic Substances and Disease Registry**. Public Health Statement for Nitrate and Nitrite, January 21, 2015 [cited March 10, 2021]. Available from: https://www.atsdr.cdc.gov/phs/phs.asp?id=1448&tid=258
16 **Lu C**, Liu G, Jia J, Gui Y, Liu Y, Zhang M, Li S, Yu C. Liquid chromatography tandem mass spectrometry method for determination of N-acetylcysteine in human plasma using an isotope-labeled internal standard. *Biomed Chromatogr* 2011; **25**: 427-431 [PMID: 21374646 DOI: 10.1002/bmc.1465]
17 **Kågedal B**, Källberg M. Reversed-phase ion-pair high-performance liquid chromatography of mercaptoacetate and N-acetylcysteine after derivatization with N-(1-pyrene)maleimide and N-(7-dimethylamino-4-methyl-3-coumarinyl)maleimide. *J Chromatogr* 1982; **229**: 409-415 [PMID: 7096475 DOI: 10.1016/s0378-4347(00)84283-8]
18 **Lewis PA**, Woodward AJ, Maddock J. High-performance liquid chromatographic assay for N-acetylcysteine in plasma and urine. *J Pharm Sci* 1984; **73**: 996-998 [PMID: 6470970 DOI: 10.1002/jps.2600730736]
19 **Toyo'oka T**, Imai K. High-performance liquid chromatography and fluorometric detection of biologically important thiols, derivatized with ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulphonate (SBD-F). *J Chromatogr* 1983; **282**: 495-500 [PMID: 6671013 DOI: 10.1016/s0021-9673(00)91626-1]
20 **Lewis PA**, Woodward AJ, Maddock J. Improved method for the determination of N-acetylcysteine in human plasma by high-performance liquid chromatography. *J Chromatogr* 1985; **327**: 261-267 [PMID: 4030959 DOI: 10.1016/s0021-9673(01)81655-1]
21 **Cotgreave IA**, Moldéus P. Methodologies for the analysis of reduced and oxidized N-acetylcysteine in biological systems. *Biopharm Drug Dispos* 1987; **8**: 365-375 [PMID: 3620595 DOI: 10.1002/bdd.2510080407]
22 **Gabard B**, Mascher H. Endogenous plasma N-acetylcysteine and single dose oral bioavailability from two different formulations as determined by a new analytical method. *Biopharm Drug Dispos* 1991; **12**: 343-353 [PMID: 1878531 DOI: 10.1002/bdd.2510120504]
23 **Siddiqui MR**, Wabaidur SM, Ola MS, AlOthman ZA, Rafiquee MZ, Khan MA. High-Throughput UPLC-MS Method for the Determination of N-Acetyl-l-Cysteine: Application in Tissue Distribution Study in Wistar Rats. *J Chromatogr Sci* 2016; **54**: 1244-1252 [PMID: 27102930 DOI: 10.1093/chromsci/bmw060]
24 **He R**, Zheng W, Ginman T, Ottosson H, Norgren S, Zhao Y, Hassan M. Pharmacokinetic profile of N-acetylcysteine amide and its main metabolite in mice using new analytical method. *Eur J Pharm Sci* 2020; **143**: 105158 [PMID: 31740394 DOI: 10.1016/j.ejps.2019.105158]

25 **Hodgman MJ**, Garrard AR. A review of acetaminophen poisoning. *Crit Care Clin* 2012; **28**: 499-516 [PMID: 22998987 DOI: 10.1016/j.ccc.2012.07.006]

26 **Tardiolo G**, Bramanti P, Mazzon E. Overview on the Effects of *N*-Acetylcysteine in Neurodegenerative Diseases. *Molecules* 2018; **23** [PMID: 30551603 DOI: 10.3390/molecules23123305]

27 **Roederer M**, Ela SW, Staal FJ, Herzenberg LA. N-acetylcysteine: a new approach to anti-HIV therapy. *AIDS Res Hum Retroviruses* 1992; **8**: 209-217 [PMID: 1540408 DOI: 10.1089/aid.1992.8.209]

28 **Geiler J**, Michaelis M, Naczk P, Leutz A, Langer K, Doerr HW, Cinatl J Jr. N-acetyl-L-cysteine (NAC) inhibits virus replication and expression of pro-inflammatory molecules in A549 cells infected with highly pathogenic H5N1 influenza A virus. *Biochem Pharmacol* 2010; **79**: 413-420 [PMID: 19732754 DOI: 10.1016/j.bcp.2009.08.025]

29 **Molteni CG**, Principi N, Esposito S. Reactive oxygen and nitrogen species during viral infections. *Free Radic Res* 2014; **48**: 1163-1169 [PMID: 25039433 DOI: 10.3109/10715762.2014.945443]

30 **Sun SY**. N-acetylcysteine, reactive oxygen species and beyond. *Cancer Biol Ther* 2010; **9**: 109-110 [PMID: 19949311 DOI: 10.4161/cbt.9.2.10583]

31 **Gordon JW**, Shaw JA, Kirshenbaum LA. Multiple facets of NF-κB in the heart: to be or not to NF-κB. *Circ Res* 2011; **108**: 1122-1132 [PMID: 21527742 DOI: 10.1161/CIRCRESAHA.110.226928]

32 **Liu Y**, Yao W, Xu J, Qiu Y, Cao F, Li S, Yang S, Yang H, Wu Z, Hou Y. The anti-inflammatory effects of acetaminophen and N-acetylcysteine through suppression of the NLRP3 inflammasome pathway in LPS-challenged piglet mononuclear phagocytes. *Innate Immun* 2015; **21**: 587-597 [PMID: 25575547 DOI: 10.1177/1753425914566205]

33 **Samuni Y**, Goldstein S, Dean OM, Berk M. The chemistry and biological activities of N-acetylcysteine. *Biochim Biophys Acta* 2013; **1830**: 4117-4129 [PMID: 23618697 DOI: 10.1016/j.bbagen.2013.04.016]

34 **Dekhuijzen PN**. Antioxidant properties of N-acetylcysteine: their relevance in relation to chronic obstructive pulmonary disease. *Eur Respir J* 2004; **23**: 629-636 [PMID: 15083766 DOI: 10.1183/09031936.04.00016804]

35 **Kinscherf R**, Fischbach T, Mihm S, Roth S, Hohenhaus-Sievert E, Weiss C, Edler L, Bärtsch P, Dröge W. Effect of glutathione depletion and oral N-acetyl-cysteine treatment on CD4+ and CD8+ cells. *FASEB J* 1994; **8**: 448-451 [PMID: 7909525]

36 **Ibrahim H**, Perl A, Smith D, Lewis T, Kon Z, Goldenberg R, Yarta K, Staniloae C, Williams M. Therapeutic blockade of inflammation in severe COVID-19 infection with intravenous N-acetylcysteine. *Clin Immunol* 2020; **219**: 108544 [PMID: 32707089 DOI: 10.1016/j.clim.2020.108544]

37 **De Flora S**, Grassi C, Carati L. Attenuation of influenza-like symptomatology and improvement of cell-mediated immunity with long-term N-acetylcysteine treatment. *Eur Respir J* 1997; **10**: 1535-1541 [PMID: 9230243 DOI: 10.1183/09031936.97.10071535]

38 **Zuin R**, Palamidese A, Negrin R, Catozzo L, Scarda A, Balbinot M. High-dose N-acetylcysteine in patients with exacerbations of chronic obstructive pulmonary disease. *Clin Drug Investig* 2005; **25**: 401-408 [PMID: 17532680 DOI: 10.2165/00044011-200525060-00005]

39 **Poe FL**, Corn J. N-Acetylcysteine: A potential therapeutic agent for SARS-CoV-2. *Med Hypotheses* 2020; **143**: 109862 [PMID: 32504923 DOI: 10.1016/j.mehy.2020.109862]

40 **Meng L**, Qiu H, Wan L, Ai Y, Xue Z, Guo Q, Deshpande R, Zhang L, Meng J, Tong C, Liu H, Xiong L. Intubation and Ventilation amid the COVID-19 Outbreak: Wuhan's Experience. *Anesthesiology* 2020; **132**: 1317-1332 [PMID: 32195705 DOI: 10.1097/ALN.0000000000003296]

41 **Bernard GR**, Wheeler AP, Arons MM, Morris PE, Paz HL, Russell JA, Wright PE. A trial of antioxidants N-acetylcysteine and procysteine in ARDS. The Antioxidant in ARDS Study Group. *Chest* 1997; **112**: 164-172 [PMID: 9228372 DOI: 10.1378/chest.112.1.164]

42 **Ortolani O**, Conti A, De Gaudio AR, Masoni M, Novelli G. Protective effects of N-acetylcysteine and rutin on the lipid peroxidation of the lung epithelium during the adult respiratory distress syndrome. *Shock* 2000; **13**: 14-18 [PMID: 10638663 DOI: 10.1097/00024382-200013010-00003]

43 **Soltan-Sharifi MS**, Mojtahedzadeh M, Najafi A, Reza Khajavi M, Reza Rouini M, Moradi M, Mohammadirad A, Abdollahi M. Improvement by N-acetylcysteine of acute respiratory distress syndrome through increasing intracellular glutathione, and extracellular thiol molecules and anti-oxidant power: evidence for underlying toxicological mechanisms. *Hum Exp Toxicol* 2007; **26**: 697-703 [PMID: 17984140 DOI: 10.1177/0960327107083452]

44 **Boesgaard S**, Aldershvile J, Poulsen HE, Christensen S, Dige-Petersen H, Giese J. N-acetylcysteine inhibits angiotensin converting enzyme in vivo. *J Pharmacol Exp Ther* 1993; **265**: 1239-1244 [PMID: 8389858]

45 **Ershad M**, Naji A, Vearrier D. N Acetylcysteine. [Updated 2020 June 28]. In: StatPearls [Internet] [cited 10 March 2021]. Treasure Island (FL): StatPearls Publishing. Available from: https://www.ncbi.nlm.nih.gov/books/NBK537183/

46 **Yoon E**, Babar A, Choudhary M, Kutner M, Pyrsopoulos N. Acetaminophen-Induced Hepatotoxicity: a Comprehensive Update. *J Clin Transl Hepatol* 2016; **4**: 131-142 [PMID: 27350943 DOI: 10.14218/JCTH.2015.00052]

47 **Masoompour SM**, Anushiravani A, Tafaroj Norouz A. Evaluation of the Effect of Nebulized N-Acetylcysteine on Respiratory Secretions in Mechanically Ventilated Patients: Randomized Clinical Trial. *Iran J Med Sci* 2015; **40**: 309-315 [PMID: 26170516]

48 **Reinero CR**, Lee-Fowler TM, Dodam JR, Cohn LA, DeClue AE, Guntur VP. Endotracheal nebulization of N-acetylcysteine increases airway resistance in cats with experimental asthma. *J Feline Med Surg* 2011; **13**: 69-73 [PMID: 21145769 DOI: 10.1016/j.jfms.2010.09.010]

49 **Pendyala L**, Creaven PJ. Pharmacokinetic and pharmacodynamic studies of N-acetylcysteine, a potential chemopreventive agent during a phase I trial. *Cancer Epidemiol Biomarkers Prev* 1995; **4**: 245-251 [PMID: 7606199]

50 **De Rosa SC**, Zaretsky MD, Dubs JG, Roederer M, Anderson M, Green A, Mitra D, Watanabe N, Nakamura H, Tjioe I, Deresinski SC, Moore WA, Ela SW, Parks D, Herzenberg LA. N-acetylcysteine replenishes glutathione in HIV infection. *Eur J Clin Invest* 2000; **30**: 915-929 [PMID: 11029607 DOI: 10.1046/j.1365-2362.2000.00736.x]

51 **Atkuri KR**, Mantovani JJ, Herzenberg LA. N-Acetylcysteine--a safe antidote for cysteine/glutathione deficiency. *Curr Opin Pharmacol* 2007; **7**: 355-359 [PMID: 17602868 DOI: 10.1016/j.coph.2007.04.005]

52 **Heard KJ**. Acetylcysteine for acetaminophen poisoning. *N Engl J Med* 2008; **359**: 285-292 [PMID: 18635433 DOI: 10.1056/NEJMct0708278]

53 **Dailymed**. Acetylcysteine Solution, 2020 [cited 10 March 2021]. Available from: https://dailymed.nlm.nih.gov/dailymed/

54 **Miller LF**, Rumack BH. Clinical safety of high oral doses of acetylcysteine. *Semin Oncol* 1983; **10**: 76-85 [PMID: 6340205]

55 **Kearns SR**, O'Briain DE, Sheehan KM, Kelly C, Bouchier-Hayes D. N-acetylcysteine protects striated muscle in a model of compartment syndrome. *Clin Orthop Relat Res* 2010; **468**: 2251-2259 [PMID: 20309660 DOI: 10.1007/s11999-010-1287-7]

56 **Bonfiglio MF**, Traeger SM, Hulisz DT, Martin BR. Anaphylactoid reaction to intravenous acetylcysteine associated with electrocardiographic abnormalities. *Ann Pharmacother* 1992; **26**: 22-25 [PMID: 1606339 DOI: 10.1177/106002809202600105]

57 **Bailey B**, Blais R, Letarte A. Status epilepticus after a massive intravenous N-acetylcysteine overdose leading to intracranial hypertension and death. *Ann Emerg Med* 2004; **44**: 401-406 [PMID: 15459624 DOI: 10.1016/j.annemergmed.2004.05.014]

58 **Mohammed S**, Jamal AZ, Robison LR. Serum sickness-like illness associated with N-acetylcysteine therapy. *Ann Pharmacother* 1994; **28**: 285 [PMID: 8173157 DOI: 10.1177/106002809402800230]

59 **Prescribers Digital Reference**. Acetylcysteine-Drug Summary, 2020 [cited 10 March 2021]. Available from: https://www.pdr.net/drug-summary/Acetylcysteine-acetylcysteine-668

60 **Goodnough R**, Canseco K. Truncated IV acetylcysteine treatment duration has potential to safely preserve resources during the COVID-19 pandemic. *Clin Toxicol (Phila)* 2021; **59**: 69 [PMID: 32345063 DOI: 10.1080/15563650.2020.1758327]

61 **Cotgreave IA**, Eklund A, Larsson K, Moldéus PW. No penetration of orally administered N-acetylcysteine into bronchoalveolar lavage fluid. *Eur J Respir Dis* 1987; **70**: 73-77 [PMID: 3817074]

62 **Rodenstein D**, DeCoster A, Gazzaniga A. Pharmacokinetics of oral acetylcysteine: absorption, binding and metabolism in patients with respiratory disorders. *Clin Pharmacokinet* 1978; **3**: 247-254 [PMID: 657688 DOI: 10.2165/00003088-197803030-00005]

63 **Millar AB**, Pavia D, Agnew JE, Lopez-Vidriero MT, Lauque D, Clarke SW. Effect of oral N-acetylcysteine on mucus clearance. *Br J Dis Chest* 1985; **79**: 262-266 [PMID: 3893512]

64 **Parr GD**, Huitson A. Oral Fabrol (oral N-acetyl-cysteine) in chronic bronchitis. *Br J Dis Chest* 1987; **81**: 341-348 [PMID: 3329530 DOI: 10.1016/0007-0971(87)90182-3]

65 **Rasmussen JB**, Glennow C. Reduction in days of illness after long-term treatment with N-acetylcysteine controlled-release tablets in patients with chronic bronchitis. *Eur Respir J* 1988; **1**: 351-355 [PMID: 3294038]

66 **Jackson IM**, Barnes J, Cooksey P. Efficacy and tolerability of oral acetylcysteine (Fabrol) in chronic bronchitis: a double-blind placebo controlled study. *J Int Med Res* 1984; **12**: 198-206 [PMID: 6376210 DOI: 10.1177/030006058401200312]

67 **Behr J**, Maier K, Degenkolb B, Krombach F, Vogelmeier C. Antioxidative and clinical effects of high-dose N-acetylcysteine in fibrosing alveolitis. Adjunctive therapy to maintenance immunosuppression. *Am J Respir Crit Care Med* 1997; **156**: 1897-1901 [PMID: 9412572 DOI: 10.1164/ajrccm.156.6.9706065]

68 **Wegener T**, Sandhagen B, Saldeen T. Effect of N-acetylcysteine on pulmonary damage due to microembolism in the rat. *Eur J Respir Dis* 1987; **70**: 205-212 [PMID: 3582517]

69 **De Flora S**, Bennicelli C, Camoirano A, Serra D, Romano M, Rossi GA, Morelli A, De Flora A. In vivo effects of N-acetylcysteine on glutathione metabolism and on the biotransformation of carcinogenic and/or mutagenic compounds. *Carcinogenesis* 1985; **6**: 1735-1745 [PMID: 3905042 DOI: 10.1093/carcin/6.12.1735]

70 **Doroshow JH**, Locker GY, Ifrim I, Myers CE. Prevention of doxorubicin cardiac toxicity in the mouse by N-acetylcysteine. *J Clin Invest* 1981; **68**: 1053-1064 [PMID: 7287901 DOI: 10.1172/jci110328]

71 **Gavish D**, Breslow JL. Lipoprotein(a) reduction by N-acetylcysteine. *Lancet* 1991; **337**: 203-204 [PMID: 1670844 DOI: 10.1016/0140-6736(91)92161-t]

72 **Wiklund O**, Fager G, Andersson A, Lundstam U, Masson P, Hultberg B. N-acetylcysteine treatment lowers plasma homocysteine but not serum lipoprotein(a) levels. *Atherosclerosis* 1996; **119**: 99-106 [PMID: 8929261 DOI: 10.1016/0021-9150(95)05635-1]

73 **Bostom AG**, Shemin D, Yoburn D, Fisher DH, Nadeau MR, Selhub J. Lack of effect of oral N-acetylcysteine on the acute dialysis-related lowering of total plasma homocysteine in hemodialysis patients. *Atherosclerosis* 1996; **120**: 241-244 [PMID: 8645365 DOI: 10.1016/0021-9150(95)05705-6]

74 **Arstall MA**, Yang J, Stafford I, Betts WH, Horowitz JD. N-acetylcysteine in combination with nitroglycerin and streptokinase for the treatment of evolving acute myocardial infarction. Safety and biochemical effects. *Circulation* 1995; **92**: 2855-2862 [PMID: 7586252 DOI: 10.1161/01.cir.92.10.2855]

75 **Rogers DF**, Godfrey RW, Majumdar S, Jeffery PK. Oral N-acetylcysteine speeds reversal of cigarette smoke-induced mucous cell hyperplasia in the rat. *Exp Lung Res* 1988; **14**: 19-35 [PMID: 3342780 DOI: 10.3109/01902148809062848]

76 **Wu J**, Levy EM, Black PH. 2-Mercaptoethanol and n-acetylcysteine enhance T cell colony formation in AIDS and ARC. *Clin Exp Immunol* 1989; **77**: 7-10 [PMID: 2527652]

77 **Herzenberg LA**, De Rosa SC, Dubs JG, Roederer M, Anderson MT, Ela SW, Deresinski SC, Herzenberg LA. Glutathione deficiency is associated with impaired survival in HIV disease. *Proc Natl Acad Sci U S A* 1997; **94**: 1967-1972 [PMID: 9050888 DOI: 10.1073/pnas.94.5.1967]

78 **Dröge W**, Eck HP, Mihm S. HIV-induced cysteine deficiency and T-cell dysfunction--a rationale for treatment with N-acetylcysteine. *Immunol Today* 1992; **13**: 211-214 [PMID: 1378279 DOI: 10.1016/0167-5699(92)90156-2]

79 **Kelly GS**. Clinical applications of N-acetylcysteine. *Altern Med Rev* 1998; **3**: 114-127 [PMID: 9577247]

80 **Sandilands EA**, Bateman DN. Adverse reactions associated with acetylcysteine. *Clin Toxicol (Phila)* 2009; **47**: 81-88 [PMID: 19280424 DOI: 10.1080/15563650802665587]

81 **Recio-Mayoral A**, Chaparro M, Prado B, Cózar R, Méndez I, Banerjee D, Kaski JC, Cubero J, Cruz JM. The reno-protective effect of hydration with sodium bicarbonate plus N-acetylcysteine in patients undergoing emergency percutaneous coronary intervention: the RENO Study. *J Am Coll Cardiol* 2007; **49**: 1283-1288 [PMID: 17394959 DOI: 10.1016/j.jacc.2006.11.034]

82 **Zhou WT**, Wang LB, Yu H, Zhang KK, Chen LJ, Wang Q, Xie XL. N-acetylcysteine alleviates PCB52-induced hepatotoxicity by repressing oxidative stress and inflammatory responses. *PeerJ* 2020; **8**: e9720 [PMID: 32864221 DOI: 10.7717/peerj.9720]

83 **Galmés S**, Serra F, Palou A. Current State of Evidence: Influence of Nutritional and Nutrigenetic Factors on Immunity in the COVID-19 Pandemic Framework. *Nutrients* 2020; **12** [PMID: 32911778 DOI: 10.3390/nu12092738]

84 **Nicola M**, O'Neill N, Sohrabi C, Khan M, Agha M, Agha R. Evidence based management guideline for the COVID-19 pandemic - Review article. *Int J Surg* 2020; **77**: 206-216 [PMID: 32289472 DOI: 10.1016/j.ijsu.2020.04.001]

85 **Di Franco S**, Alfieri A, Petrou S, Damiani G, Passavanti MB, Pace MC, Leone S, Fiore M. Current status of COVID-19 treatment: An opinion review. *World J Virol* 2020; **9**: 27-37 [PMID: 33024717 DOI: 10.5501/wjv.v9.i3.27]

86 **Wang M**, Cao R, Zhang L, Yang X, Liu J, Xu M, Shi Z, Hu Z, Zhong W, Xiao G. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Res* 2020; **30**: 269-271 [PMID: 32020029 DOI: 10.1038/s41422-020-0282-0]

87 **Gautret P**, Lagier JC, Parola P, Hoang VT, Meddeb L, Mailhe M, Doudier B, Courjon J, Giordanengo V, Vieira VE, Tissot Dupont H, Honoré S, Colson P, Chabrière E, La Scola B, Rolain JM, Brouqui P, Raoult D. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. *Int J Antimicrob Agents* 2020; **56**: 105949 [PMID: 32205204 DOI: 10.1016/j.ijantimicag.2020.105949]

88 **Gautret P**, Lagier JC, Parola P, Hoang VT, Meddeb L, Sevestre J, Mailhe M, Doudier B, Aubry C, Amrane S, Seng P, Hocquart M, Eldin C, Finance J, Vieira VE, Tissot-Dupont HT, Honoré S, Stein A, Million M, Colson P, La Scola B, Veit V, Jacquier A, Deharo JC, Drancourt M, Fournier PE, Rolain JM, Brouqui P, Raoult D. Clinical and microbiological effect of a combination of hydroxychloroquine and azithromycin in 80 COVID-19 patients with at least a six-day follow up: A pilot observational study. *Travel Med Infect Dis* 2020; **34**: 101663 [PMID: 32289548 DOI: 10.1016/j.tmaid.2020.101663]

89 **Chu CM**, Cheng VC, Hung IF, Wong MM, Chan KH, Chan KS, Kao RY, Poon LL, Wong CL, Guan Y, Peiris JS, Yuen KY; HKU/UCH SARS Study Group. Role of lopinavir/ritonavir in the treatment of SARS: initial virological and clinical findings. *Thorax* 2004; **59**: 252-256 [PMID: 14985565 DOI: 10.1136/thorax.2003.012658]

90 **Zhang XJ**, Qin JJ, Cheng X, Shen L, Zhao YC, Yuan Y, Lei F, Chen MM, Yang H, Bai L, Song X, Lin L, Xia M, Zhou F, Zhou J, She ZG, Zhu L, Ma X, Xu Q, Ye P, Chen G, Liu L, Mao W, Yan Y, Xiao B, Lu Z, Peng G, Liu M, Yang J, Yang L, Zhang C, Lu H, Xia X, Wang D, Liao X, Wei X, Zhang BH, Zhang X, Zhao GN, Zhang P, Liu PP, Loomba R, Ji YX, Xia J, Wang Y, Cai J, Guo J, Li H. In-Hospital Use of Statins Is Associated with a Reduced Risk of Mortality among Individuals with COVID-19. *Cell Metab* 2020; **32**: 176-187. e4 [PMID: 32592657 DOI: 10.1016/j.cmet.2020.06.015]

91 **Zhang C**, Wu Z, Li JW, Zhao H, Wang GQ. Cytokine release syndrome in severe COVID-19: interleukin-6 receptor antagonist tocilizumab may be the key to reduce mortality. *Int J Antimicrob Agents* 2020; **55**: 105954 [PMID: 32234467 DOI: 10.1016/j.ijantimicag.2020.105954]

92 **Mehta P**, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ; HLH Across Speciality

Collaboration, UK. COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet* 2020; **395**: 1033-1034 [PMID: 32192578 DOI: 10.1016/S0140-6736(20)30628-0]

93 **Carr AC**, Shaw GM, Fowler AA, Natarajan R. Ascorbate-dependent vasopressor synthesis: a rationale for vitamin C administration in severe sepsis and septic shock? *Crit Care* 2015; **19**: 418 [PMID: 26612352 DOI: 10.1186/s13054-015-1131-2]

94 **Silvagno F**, Vernone A, Pescarmona GP. The Role of Glutathione in Protecting against the Severe Inflammatory Response Triggered by COVID-19. *Antioxidants (Basel)* 2020; **9** [PMID: 32708578 DOI: 10.3390/antiox9070624]

95 **Kuba K**, Imai Y, Rao S, Gao H, Guo F, Guan B, Huan Y, Yang P, Zhang Y, Deng W, Bao L, Zhang B, Liu G, Wang Z, Chappell M, Liu Y, Zheng D, Leibbrandt A, Wada T, Slutsky AS, Liu D, Qin C, Jiang C, Penninger JM. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. *Nat Med* 2005; **11**: 875-879 [PMID: 16007097 DOI: 10.1038/nm1267]

96 **de Alencar JCG**, Moreira CL, Müller AD, Chaves CE, Fukuhara MA, da Silva EA, Miyamoto MFS, Pinto VB, Bueno CG, Lazar F, Gomez LM, Menezes MCS, Marchini JFM, Marino LO, Brandão RA, Souza HP; Covid Register Group. Double-blind, randomized, placebo-controlled trial with N-acetylcysteine for treatment of severe acute respiratory syndrome caused by COVID-19. *Clin Infect Dis* 2020 [PMID: 32964918 DOI: 10.1093/cid/ciaa1443]

97 **Alamdari DH**, Moghaddam AB, Amini S, Keramati MR, Zarmehri AM, Alamdari AH, Damsaz M, Banpour H, Yarahmadi A, Koliakos G. Application of methylene blue -vitamin C -N-acetyl cysteine for treatment of critically ill COVID-19 patients, report of a phase-I clinical trial. *Eur J Pharmacol* 2020; **885**: 173494 [PMID: 32828741 DOI: 10.1016/j.ejphar.2020.173494]

98 **Liu Y**, Wang M, Luo G, Qian X, Wu C, Zhang Y, Chen B, Leung EL, Tang Y. Experience of N-acetylcysteine airway management in the successful treatment of one case of critical condition with COVID-19: A case report. *Medicine (Baltimore)* 2020; **99**: e22577 [PMID: 33080692 DOI: 10.1097/MD.0000000000022577]

99 **Tariq Alhawassi**. Inflammatory Regulation Effect of NAC on COVID-19 Treatment (INFECT-19), 2020 [cited March 10, 2021]. In: United States National Library of Medicine. Available from: https://clinicaltrials.gov/ct2/show/NCT04455243

100 **Vardhana**, Santosha. A Study of N-acetylcysteine in Patients With COVID-19 Infection. May 1 2020 - May 2021 [cited March 10, 2021]. In: United States National Library of Medicine. Available from: https://clinicaltrials.gov/ct2/show/NCT04374461

101 **Melisa Lai-Becker**, Melisa, Kuhn, Duncan. Efficacy of N-Acetylcysteine (NAC) in Preventing COVID-19 From Progressing to Severe Disease. September 23 2020 - May 31, 2021 [cited March 10, 2021]. In: United States National Library of Medicine. Available from: https://clinicaltrials.gov/ct2/show/NCT04419025

102 A Study to Evaluate OP-101 (Dendrimer N-acetyl-cysteine) in Severe Coronavirus Disease 2019 (COVID-19) Patients (PRANA). July 1, 2020 - November 10, 2020 [cited March 10, 2021]. In: United States National Library of Medicine. Available from: https://clinicaltrials.gov/ct2/show/NCT04458298

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