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EDITORIAL

- 30 New Year's greeting and overview of *World Journal of Virology* in 2021

Wang DM, Chen EQ, El-Bendary M

REVIEW

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

Dominari A, Hathaway III D, Kapasi A, Paul T, Makkar SS, Castaneda V, Gara S, Singh BM, Agadi K, Butt M, Retnakumar V, Chittajallu S, Taugir R, Sana MK, KC M, Razzack S, Moallem N, Alvarez A, Talalaev M

MINIREVIEWS

- 53 Are nucleotide inhibitors, already used for treating hepatitis C virus infection, a potential option for the treatment of COVID-19 compared with standard of care? A literature review

Spera AM

- 62 Trends in the management of infectious disease under SARS-CoV-2 era: From pathophysiological comparison of COVID-19 and influenza

Seki M

META-ANALYSIS

- 69 Epidemiological characterization and geographic distribution of human immunodeficiency virus/acquired immunodeficiency syndrome infection in North African countries

Daw MA, Ahmed MO

ABOUT COVER

Editorial Board Member, Sharof M Tugizov, DSc, PhD, Professor, Department of Medicine, University of California, Division of Infectious Diseases, San Francisco, CA 94143, United States. sharof.tugizov@ucsf.edu

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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.

WJV mainly publishes articles reporting research results obtained in the field of virology and covering a wide range of topics including arbovirus infections, viral bronchiolitis, central nervous system viral diseases, coinfection, DNA virus infections, viral encephalitis, viral eye infections, chronic fatigue syndrome, animal viral hepatitis, human viral hepatitis, viral meningitis, opportunistic infections, viral pneumonia, RNA virus infections, sexually transmitted diseases, viral skin diseases, slow virus diseases, tumor virus infections, viremia, and zoonoses.

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

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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-mercaptopate. 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-sulfanypropanoic 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 NO₂ 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-1-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-glycyrrhetic 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- κ B) and p38 through activation of GSH and direct antioxidant effect of its free thiol group. NF- κ B is a redox-sensitive transcription factor that regulates the expression of pro-inflammatory cytokines, including IL-1, IL-6, and tumor necrosis factor- α , as well as genes associated with apoptosis, such as p53, and is activated by increased ROS levels. NAC, a glutathione precursor, inhibits NF- κ B by S-glutathionylation of the p65 subunit of NF- κ B, resulting in blockage of TNF- α activation and nuclear translocation of NF- κ B-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- α , 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-1 β 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-1 β 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- κ B). 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 Rales 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/m²/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 H₂O 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 load 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- κ B, 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-acetylcysteine) 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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