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BRIEF REVIEW OF N-ACETYLCYSTEINE AS ANTIVIRAL AGENT: POTENTIAL APPLICATION IN COVID-19

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Abstract
Novel Coronavirus disease 2019 – COVID-19, was first identified amongst an outbreak of respiratory illness cases in Wuhan City, Hubei Province, China. Research for effective therapies including antiviral agents, immunotherapies, and vaccines are being investigated and developed as potential therapies.
Acetylcysteine a precursor in the formation of the antioxidant Glutathione (GSH) in the body is an important determinant of cellular redox status in endothelial cells, in maintaining intracellular GSH/Glutathione oxidized (GSSG) homeostasis, hence represents one of the most important antioxidant defense systems in lung cells, also, used in the prophylaxis or therapy of Virus Diseases. There are multiple reasons for maintaining adequate GSH levels in lungs. Researchers have demonstrated that, immunomodulatory agents that have increased survival in combination with influenza antivirals in murine models include N-acetylcysteine. These anti-oxidant capacities of NAC are mostly indirect, via a pro-glutathione effect where NAC provides L-cysteine residues required for glutathione synthesis. Therefore, antioxidants like NAC represent a potential additional treatment option that could be considered either as adjuvant therapy.
According to the latest study by Jason Kim *et al*, March 2020, it is indicated glutathione, as top hits and highly ranked for potential benefit against SARS-CoV-2; and have also warranted further investigation for potential benefit against SARS-CoV-2.
Still, its clinical effectiveness needs further investigations, since most of the results in this area of research are derived from in vitro and in vivo studies. Further research as antiviral agent may provide a new therapeutic strategy for the treatment of viral infections such as COVID 19.
Keywords: COVID-19, World Health Organization (WHO), N-Acetylcysteine, Antiviral, reactive oxygen intermediates, glutathione, antioxidant

Introduction

"Novel Coronavirus Disease 2019 (COVID-19), an infectious disease caused by a novel beta-coronavirus, known as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2; formerly called 2019-nCoV), which was first identified amid an outbreak of respiratory illness cases in Wuhan City, Hubei Province, China, pneumonia of unknown cause detected in Wuhan, China was first reported to the WHO Country Office in China on 31st December 2019".[1, 2, 3]

The disease causes respiratory illness (like the flu) with symptoms such as a cough, fever, and in more severe cases, difficulty breathing. COVID-19 spreads chiefly through contact with an infected person when they cough or sneeze. It also spreads when a person touches a surface or objects that has the virus on it, and then touches their eyes, nose, or mouth. Coronaviruses consists of a vast family of viruses, 7 of which are known to cause disease in humans. Some coronaviruses that typically infect animals, such as bats, have been known to evolve to infect humans. SARS-CoV-2 is probably one such virus, asserted to have originated in a large animal and seafood market. The latest figures as on 17th March 2020, suggest that nearly 180,00 people have been infected worldwide, with about 7200 deaths. The World Health Organization (WHO) designated COVID 19 a "public health emergency of international concern" on 30th January 2020 and declared it a pandemic on 11th March.[2, 3] Either for the prevention nor for the treatment, there have been no drugs or vaccines proven to be effective, however, research for effective therapies for COVID-19 infection is a complex process, although numerous antiviral agents, immunotherapies,

and vaccines are being investigated and developed as potential therapies or as adjuvant therapies.[2, 3, 4]

WHO has released a scientific brief on the off-label use of medicines for COVID 19. A number of medicines have been suggested as potential investigational therapies, many of which are now being or will soon be studied in clinical trials, including the SOLIDARITY trial co-sponsored by WHO and participating countries.[2, 3, 4] The current COVID 19 emergency warrants the urgent development of potential systemic plan of action to protect people at high risk of infection-particularly close contacts and health-care workers, among others, even if more enriched data on antiviral therapies is yet to come. WHO encourages countries to prepare hospitals and health facilities, protect their health personnel, and decide what social distancing measures need to be implemented and for how long, among other actions. As per WHO data, "WHO has described four levels of COVID 19 transmission with varying public health and social measures depending on the local evolution of the COVID-19 pandemic outbreak. Public health and social measures are measures or actions by individuals, institutions, communities, local and national governments and international bodies to slow or stop the spread of COVID 19. These measures to reduce transmission of COVID 19 include individual and environmental measures, detecting and isolating cases, contact-tracing and quarantine, social and physical distancing measures including for mass gatherings, international travel measures, and vaccines and treatments. While vaccines and specific medications are not yet available for COVID 19, other public health and social measures play an essential role in reducing the number of infections and saving lives. Social and physical distancing measures aim to slow the spread of disease by stopping chains of transmission of COVID-19 and preventing new ones from appearing. These measures secure physical distance between people (of at least one metre), and reduce contact with contaminated surfaces, while encouraging and sustaining virtual social connection within families and communities. Measures for the general public include introducing flexible work arrangements such as teleworking, distance learning, reducing and avoiding crowding, closure of non-essential facilities and services, shielding and protection for vulnerable groups, local or national movement restrictions and staying-at home measures, and coordinated reorganization of health care and social services networks to protect hospitals. The measures are used in conjunction with individual protective measures against COVID 19 such as frequent hand washing and cough etiquette. Although, all these precautionary measures might help in reducing the overall size of COVID 19 pandemic outbreak, but will still be insufficient to achieve outbreak control of COVID 19 unless targeted medicines, treatment/prevention/vaccines is not investigated and comes in market for human use". [2, 3]

Looking to the current situation, there is a need for better treatment design. Hence, the present review of N-acetylcysteine (NAC), a Glutathione (GSH) precursor was undertaken to review its potential use as antiviral agent, as adjuvant therapy in COVID 19, provided further research in the area intervenes. NAC provides an important GSH precursor, cysteine. N-acetylcysteine (NAC), the acetylated variant of the amino acid L-cysteine is already been used for decades as an adjuvant therapy for several respiratory conditions; and its multifactorial functions also include the NAC role as antiviral drug, anti-infective and mucolytic, which shows that it has potential to use as antiviral agent and in COVID 19.[5, 6, 7] Moreover, it has been shown that most of the protective effects of NAC are attributable to the incorporation of cysteine into GSH by Glutamate Cysteine Ligase (GCL). They have antioxidant property also. The lungs are exposed to high oxygen levels and also to inhaled toxins. Alveolar macrophages provide an additional ROS source in this tissue. Hence, there are multiple reasons for maintaining adequate GSH levels in lungs.[5, 6]

ROLE OF GLUTATHIONE

Glutathione, a thiol tripeptide, present in most mammalian tissue, acts as an antioxidant, a free radical scavenger and a detoxifying agent. It participates in a number of critically important cellular processes. Intracellularly > 98% of the tripeptide is kept in its reduced state by glutathione reductase (GR) enzyme with the remainder as glutathione disulfide and glutathione conjugates (GS-R). GSH is known to be involved either directly or indirectly in a number of biological phenomena and is mainly responsible for maintaining cellular redox status in endothelial cells. GSH scavenges free radicals and other reactive oxygen species (ROS), and neutralizes toxic metabolites by condensing with them both enzymatically and non-enzymatically.[8, 9]

Glutathione is important as a cofactor for the enzyme glutathione peroxidase, in the uptake of amino acids, and in the synthesis of leukotrienes. Glutathione is also involved in the formation and maintenance of disulfide bonds in proteins and in the transport of amino acids across cell membranes. Also, cells may lose GSH due to transfer of its reduced, oxidized or conjugated forms. As, GSH participates in many metabolic processes, its role cannot be overrated. In phagocytes, a major part of the mechanism of killing microorganisms involves

production of reactive oxygen species.[8, 9] Researchers in their studies have shown that, in some extracellular spaces such as the lining fluid of the lung, a thin layer of fluid covering the air spaces where gas exchange occurs, there is high concentration of GSH that is secreted by epithelial cells. [10, 11] Glutathione has a multiple role in cells, such as in mitochondria it plays a key role in determining apoptosis versus necrosis; it is a key regulator of cellular division in nucleus; while lungs are clearly adversely affected by lowered intracellular and extracellular GSH. Moreover, it has been demonstrated that in people who smoke or inhale particles or other oxidants, there is potential inflammation that involves invasion of neutrophils from the blood through the endothelial and epithelial cells into the air spaces. As these neutrophils squeeze between the cells, they release Hypochlorous acid (HOCl), which can react with GSH secreted from the epithelial cells that normally protects the epithelial cells.[12, 13]

The lungs are exposed to high oxygen levels and also to inhaled toxins. Alveolar macrophages provide an additional ROS source in this tissue. Hence, there are multiple reasons for maintaining adequate GSH levels in lungs.[14, 15] Other pathological conditions of lungs, such as chronic pulmonary disease, acute respiratory distress syndrome, neonatal lung damage, and asthma; and of the immune system are also associated with a compromised mitochondrial GSH system.[14, 15, 16]

POTENTIAL ROLE OF N-ACETYLCYSTEINE: FOCUS AS ANTIVIRAL AGENT

N-acetylcysteine (NAC) was found since 1960s, included in the World Health Organization's 21st List, 2019 (WHO) Model List of Essential Medicines is available as an inexpensive generic drug. N-Acetyl-L-cysteine is the N-acetyl derivative of Cysteine, is also known as N-Acetyl-L-cysteine; Acetylcysteine; N-Acetylcysteine.[6, 17] Acetylcysteine, also known as N-acetylcysteine (NAC), is a modified amino acid, i.e. Cysteine. Acetylcysteine is the nonproprietary name for the N-acetyl derivative of the naturally occurring amino acid, L-cysteine (N-acetyl-L-cysteine). Acetylcysteine regenerates liver stores of glutathione. This agent also reduces disulfide bonds in mucoproteins, resulting in liquefaction of mucus. Studies also suggest that acetylcysteine may exert an anti-apoptotic effect due to its antioxidant activity, possibly preventing cancer cell development or growth. Moreover, acetylcysteine has inhibited viral stimulation by reactive oxygen intermediates, thereby producing antiviral activity in HIV patients.[6] N-acetylcysteine (NAC), has been used for decades as an adjuvant therapy for several respiratory conditions. Also, NAC has role as antiviral drug, anti-infective and mucolytic.[5, 6]

It has been classically used in paracetamol overdose and as well as to combat the toxicity of various substances.[18, 19, 20] Cysteine is present in most high-protein food. Acetylcysteine, precursor in the formation of the antioxidant glutathione (GSH) in the body; is the N-acetyl derivative of the amino-acid L-cysteine. GSH is a cofactor for glutathione S-transferase–mediated xenobiotic conjugation and is an important determinant of cellular redox status in endothelial cells, in maintaining intracellular GSH/Glutathione oxidized (GSSG) homeostasis, hence represents one of the most important antioxidant defense systems in lung cells. Formation of GSH is depicted in Figure 1.[8]

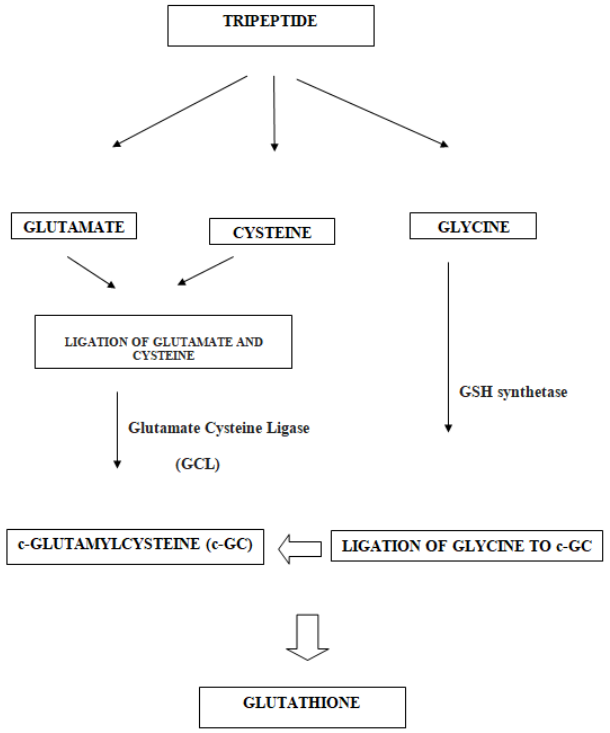

Figure 1: Steps involved in the formation of antioxidant sulfhydryl tripeptide GSH

It has been shown that most of the protective effects of NAC are attributable to the incorporation of cysteine into GSH by GCL. NAC is a thiol, having free –SH (sulfhydryl) group through which it reduces disulfide bonds present in mucoproteins, which are thought to be at least partly responsible for the particularly viscoid nature of respiratory mucus. Thus, reducing the elasticity and viscosity of mucus.[5, 6] Hence, as a mucolytic agent, NAC commonly used as an adjuvant therapy in patients with respiratory conditions associated with excessive mucus production reduces the viscosity of both purulent and nonpurulent secretions. There are numerous other uses or proposed uses in medicine that are still in preclinical and clinical investigations.[4] NAC is used in the prophylaxis or therapy of Virus Diseases. Some of the ways they may act include preventing viral replication by inhibiting viral

DNA polymerase; binding to specific cell-surface receptors and inhibiting viral penetration or uncoating; inhibiting viral protein synthesis; or blocking late stages of virus assembly.[5, 6]

Researchers Oriol Mitjà *et al*, have also recorded the use of antiviral drugs to reduce COVID-19 transmission.[21] Further, N-Acetylcysteine (NAC) is reported through various studies for its therapeutic use to treat chronic lung disorders,, also studies have showed its anti-AIDS virus activity in vitro.[22] Researchers have demonstrated that, immunomodulatory agents that have increased survival in combination with influenza antivirals in murine models include N-acetylcysteine.[23, 24] Geiler J *et al*, have shown NAC to inhibit replication of seasonal human influenza A viruses, through their studies in virus-induced apoptosis that was conducted in H5N1-infected lung epithelial (A549) cells. The effects of NAC on virus replication, virus-induced pro-inflammatory responses was reported.[25] These antiviral and anti-inflammatory actions of NAC included inhibition of activation of oxidant sensitive pathways including transcription factor NF-kappaB and mitogen activated protein kinase p38. The researchers have shown that the NAC inhibits H5N1 replication and H5N1-induced production of pro-inflammatory molecules.[25] Therefore, antioxidants like NAC represent a potential additional treatment option that could be considered in the case of influenza, a virus pandemic. Owing to its antioxidant properties, NAC is able to maintain the redox-dependent cell-signaling and transcription, in particular the Nuclear Factor–kB (NF-kB).[26, 27]

Further, in recent studies NAC was shown to limit lung inflammation, damage associated with the virus, and viral growth, at least in vitro. However, the anti-viral activity was highly variable depending on the influenza A strain. These anti-oxidant capacities of NAC are mostly indirect, via a pro-glutathione effect where NAC provides L-cysteine residues required for glutathione synthesis.[26, 27] Besides NAC can be administered as inhalation, injection or by oral routes.[6]

According to the latest study by Jason Kim *et al*, March 2020, it is indicated glutathione, as top hits and highly ranked for potential benefit against SARS-CoV-2; and have also warranted further investigation for potential benefit against SARS-CoV-2.[28] Glutathione has also been evaluated as an adjunct in patients receiving certain chemotherapy agents following lung transplantation, and for management of HIV and Parkinson's disease with mixed results and also in herpes simplex virus type 1 replication. [29, 30] Top results included several ACE inhibitors, a betalactam antibiotic, two antiviral agents (Fosamprenavir and Emricasan) and glutathione.[28] Hence, the present review article shows the potential of use of NAC, as it has been shown that most of the protective effects of NAC are attributable to the incorporation of cysteine into GSH by GCL.

Conclusions and Prospects

As nowadays corona virus is the most current topic of research as the treatment is not available or is still under investigational stages, and because in most of the world country like China, USA & Italy the death rate is high due to corona virus, today's most important task is to treat the corona patients by giving antiviral tablets along with some adjuvants as a therapy. Looking to this current scenario, the present article indicated the potential use of NAC as antiviral agent, as it has multifactorial actions.

In the past years, various researchers have shown different uses of N-Acetylcysteine, either alone or as adjuvant, which includes its antioxidant, anti-inflammatory, antimicrobial, anticarcinogenic, & antiviral activities. Still, its clinical effectiveness needs further investigations, since most of the results in this area of research are derived from in vitro and in vivo studies. Further research of NAC as alternative options (Adjuvant) for the treatment or prevention; as well as in new drug designs in COVID 19 patients as antiviral agent may provide a new therapeutic strategy for the treatment of viral infections.

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