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Research Article,

The role of Glutathione as an adjunct therapy in the treatment of patients with COVID-19-Related Acute Respiratory Syndrome

Taís Mazzini Setti (TM), MD[1], Thiago Setti (TS), MD[2], Lucas Furtado da Fonseca (LF), MD[3], Stephany Cares Huber, PhD[4], Gabriel Silva Santos (GS)*[5], José Fábio Santos Duarte Lana (JL), MD[6]

1. Anesthesiology, Indolor - Centro Intervencionista de Controle da Dor 583 Sul Brasil Avenue – room #406 – Centro – Zip code 89814-210 Maravilha – SC, Brazil

2. Orthopedics – Sports Medicine – Pain Physician, Indolor - Centro Intervencionista de Controle da Dor 583 Sul Brasil Avenue – room #406 – Centro – Zip code 89814-210 Maravilha – SC, Brazil

3. Orthopedics – Sports Medicine – Pain Physician, Orthopaedic Department – UNIFESP/EPM 715 Napoleão de Barros St – Vila Clementino – Zip code 04024-002 São Paulo – SP, Brazil

4. Biomedical Scientist, Universidade Estadual de Campinas (UNICAMP) / The University of Campinas Cidade Universitária Zeferino Vaz Campinas – SP, Brazil

5. Biomedical Scientist, IOC – Instituto do Osso e da Cartilagem / The Bone and Cartilage Institute 1386 Presidente Kennedy Avenue – Cidade Nova I – Zip code 13334-170 Indaiatuba – SP, Brazil

6. Orthopedics – Sports Medicine – Pain Physician, IOC – Instituto do Osso e da Cartilagem / The Bone and Cartilage Institute 1386 Presidente Kennedy Avenue – Cidade Nova I – Zip code 13334-170 Indaiatuba – SP, Brazil

Correspondence: Gabriel Silva Santos

IOC – Instituto do Osso e da Cartilagem / The Bone and Cartilage Institute Presidente Kennedy Avenue, 1386 – 2nd floor, Room #29 – Cidade Nova I Indaiatuba – São Paulo, Brazil. ZIP: 13334-170 Phone: +551930174366 Mobile: +5519989283863

Abstract:

Covid-19 is a novel coronavirus disease that has been (SARS-CoV-2) responsible for a worldwide pandemic of infectious pneumonia associated with severe acute respiratory syndrome. Although in most cases the disease can be resolved on its own, in severe or critical cases, patients can ultimately pass away, mainly due to the diffuse and massive alveolar damage associated with disease progression. One in four patients will be admitted to the Intensive Care Unit (ICU). A constant characteristic in severely affected patients is the exacerbated systemic inflammatory response. This is attributed to the excessive immune response mediated by cytokine secretion, which therefore causes acute lung injury, acute respiratory distress syndrome, multiple organ failure and even death. Currently, there are no effective antiviral agents and there are no fully elucidated or validated therapeutic options that can halt disease progression in some patients. Therefore, there is an urgent need for new treatments to delay the excessive inflammatory response and accelerate the repair of functional lung tissue in these patients. Glutathione may fit these criteria because it has some properties which can be associated with antiviral effects and it also participates in immune responses with the ability to balance oxidative stress.

Key words: acute respiratory syndrome, COVID-19, glutathione.

INTRODUCTION

1.1 The Respiratory Tract

The respiratory tract is complex and contains several different types of epithelial cells, distributed in several different regional microenvironments along its path. The trachea is composed of ducts and submucosal glands, where different stem and progenitor cells are found. In the bronchi there is a predominance of basal cells in the intercartilaginous zones, where the prominent feature is the high proliferative rate. In the bronchi, the main cell type is the clear cell, responsible for secreting and absorbing glycoproteins, in addition to assisting in the degradation of toxic substances. Type 1 and 2 alveolar cells (or pneumocytes) are located in the alveoli and become responsible for the production of surfactant substances, which reduce the surface tension at the interface between the liquid present in the alveolar cavity and the air (1).

1.2 Acute Respiratory Syndrome (SARS)

The acute respiratory syndrome is a progressive inflammatory lung process characterized by an alveolar lesion with diffuse alveolar damage. The first cases of SARS were described about 50 years ago by Ashbaugh and collaborators, who reported twelve patients with symptoms such as tachypnea, refractory hypoxemia and diffuse opacity in the lung on radiographs (2). Population estimates of SARS range from 10 to 86 cases per 100,000, with the highest rates reported in Australia and the United States. Since the diagnosis is based on imaging exams, SARS is likely to be underreported in low-income countries, where the resources to obtain chest radiographs and measure arterial blood gases are limited (3). Four major definitions of SARS have evolved over the years and all have retained the central characteristics of Ashbaugh's initial description. As pulmonary permeability, edema and inflammation are not routinely measured in clinical care and a validated diagnostic biomarker is not yet available, these definitions are based on clinical features and chest radiographs. In 2012, the Berlin definition was proposed, which establishes three levels of risk based on the degree of hypoxemia, assessed in minimum positive final expiratory pressure (4). The new proposal makes the radiographic criteria more explicit and allows the use of computed tomography (CT) to detect qualified opacities, often heterogeneous (table 1).

Table 1: Definition of Acute Respiratory Syndrome (Fergunson et al., 2012) (4)

	Acute Respiratory Distress Syndrome
Timing	Within 1 week of a known clinical insult or new or worsening respiratory symptoms
Chest imaging[a]	Bilateral opacities—not fully explained by effusions, lobar/lung collapse, or nodules
Origin of edema	Respiratory failure not fully explained by cardiac failure or fluid overload Need objective assessment (eg, echocardiography) to exclude hydrostatic edema if no risk factor present
Oxygenation[b]	
Mild	200 mm Hg < PaO_2/FiO_2 ≤ 300 mm Hg with PEEP or CPAP ≥5 cm H_2O[c]
Moderate	100 mm Hg < PaO_2/FiO_2 ≤ 200 mm Hg with PEEP ≥5 cm H_2O
Severe	PaO_2/FiO_2 ≤ 100 mm Hg with PEEP ≥5 cm H_2O

Abbreviations: CPAP, continuous positive airway pressure; FiO_2, fraction of inspired oxygen; PaO_2, partial pressure of arterial oxygen; PEEP, positive end-expiratory pressure.
[a]Chest radiograph or computed tomography scan.
[b]If altitude is higher than 1000 m, the correction factor should be calculated as follows: [PaO_2/FiO_2 × (barometric pressure/760)].
[c]This may be delivered noninvasively in the mild acute respiratory distress syndrome group.

In addition, it is also acknowledged that SARS usually develops within seven days after clinical recognition of the known risk factor, most commonly pneumonia or sepsis. The factors are divided into direct ones, such as aspiration of gastric contents, pulmonary contusion, inhalation injury or drowning, and indirect ones, such as trauma or non-thoracic shock, pancreatitis, burns, overdose by illicit drugs, and the presence of edema after lung transplantation (4). For patients with SARS with a more indolent onset or in the absence of identifiable risk factors, the so-called "SARS mimics" should be taken into account, which comprises a large number of diseases or syndromes that may require specific treatments, such as interstitial lung disease, polymyositis , diffuse alveolar hemorrhage, cancer and lung diseases caused by drugs (5). Previous definitions

excluded volume overload or heart failure, but recent evidence suggests that these problems can coexist in up to a third of patients with SARS.

The histological finding of SARS is "diffuse alveolar lesion", a term described by Katzenstein and colleagues almost a decade after Ashbaugh's report. Katzenstein et al described the rapid development of capillary congestion, atelectasis, hemorrhage and alveolar edema, followed by formation of hyaline membrane, epithelial cell hyperplasia and interstitial edema (6). Animal models of SARS have been developed to recap these histological findings. However, the Berlin definition (as well as the definition of the 1994 American-European Consensus Conference) (7) has little specificity for diffuse alveolar damage. In cadaver exams, 40% to 58% of patients with a moderate to severe clinical diagnosis of SARS have diffuse alveolar damage. Pulmonary edema and pneumonia without hyaline membranes are other common findings, although 14% of patients do not have lung injuries, probably due to atelectasis disguised as SARS (8, 9).

More than 40 genes have been associated with the development of SARS, including genes encoding the angiotensin converting enzyme (ACE), interleukin 10 (IL-10), tumor necrosis factor (TNF) and vascular endothelial growth factor (VEGF), as well as SOD3, MYLK, NFE2L2, NAMPT and SFTPB. In the only genomic association study in SARS associated with trauma, no polymorphism was significant (10). Impaired fatty acid oxidation causes increased apoptosis and altered function of alveolar epithelial cells. These data suggest that increased fatty acid oxidation in the epithelial and endothelial cells of the lung may mitigate lung injury. The rupture of the alveolar-capillary membrane is a registered trademark of SARS (11, 12).Increased levels of plasma biomarkers, including markers of systemic inflammation (interleukin-6 and interleukin-8), epithelial injury (receptor for advanced glycation end products and surfactant protein D) and endothelial injury (angiopoietin 2), as well as dysregulated coagulation markers (low levels of protein C and high levels of plasminogen activator inhibitor 1), have been associated with adverse SARS results. These biomarkers provide information on the pathogenesis of the disease and can assist in monitoring the response to treatment (13). The pathogenesis of SARS begins with the exudative phase, and is characterized by a response mediated by innate immune cells of the alveolar endothelial and epithelial barriers, as well as the accumulation of protein-rich fluid in the interstitium and alveolus. The resident alveolar macrophages are activated, leading to the release of potent pro-inflammatory mediators and chemokines that promote the accumulation of neutrophils and monocytes. Activated neutrophils further contribute to the injury by releasing toxic mediators. The resulting injury leads to loss of barrier function, in addition to increased interstitial and intra-alveolar fluid. The mediated expression of tumor necrosis factor (TNF) promotes the aggregation of platelets and the formation of microthrombi, as well as intra-alveolar coagulation and the formation of hyaline membrane. Endothelial activation and microvascular injury also contribute to the rupture of the barrier in SARS and are aggravated by mechanical stretching (13, 14). The repair processes initiated during the second phase, called the proliferative phase, are essential for the patient's survival. The proliferative phase aims to restore tissue homeostasis and is characterized by the transient expansion of fibroblasts and the formation of a temporary matrix, as well as the proliferation of airway progenitor cells and type II alveolar epithelial cells (AECII), with differentiation into cells type I alveolar epithelial cells (AECI) (15).

The final phase, referred to as the fibrotic phase, is strongly associated with mechanical ventilation and extensive damage to the basement membrane and inadequate or delayed re-epithelialization can lead to the development of changes such as interstitial fibrosis. Once the epithelial integrity is restored, the resorption of the alveolar edema and

the provisional matrix restore the alveolar architecture and function (13).

1.2.1 SARS-CoV infection-related Acute Respiratory Syndrome

In the case of severe coronavirus-related respiratory syndrome, which is the scope of this project, it is known that the first reports originated in China between the years 2002 and 2003 after the evolution of this viral species in bats (16, 17). This virus was responsible for the infection of more than eight thousand people in the world, with a mortality rate of approximately 10% (18). Almost a decade after this outbreak, a new species of coronavirus emerged in the Middle East causing the Middle East Respiratory Syndrome (MERS). The reported consequences are severe pneumonia and kidney failure with an even higher mortality rate, reaching 55% in documented cases (19). The severity of the disease is strongly associated with advanced age and other existing comorbidities in patients, resulting in mortality rates above 50% in individuals over 65 years of age. Regarding the pathophysiological mechanism of SARS-CoV, apparently, there is an infection of type 2 pneumocytes. These cells are responsible for the production of pulmonary surfactants and also act as progenitor cells for type 1 pneumocytes (20). In the acute phase of SARS-CoV infection, there is an erosion of the epithelial cells that line the airways, in addition to the accumulation of debris and particles that obstruct the regular breathing pattern (21, 22). There is also a risk of progression to more severe complications such as acute lung injury (ALI) and the subsequent Acute Respiratory Distress Syndrome (ARDS), which cover alveolar damage (20).

Inflammation from such an infection can stimulate an excess of secretions in the lung, including fibrin compounds and proteinaceous materials, preventing the essential gas exchange for the maintenance of vital organs (23). If this secretion is not removed, the pathophysiology progresses to the state of pulmonary fibrosis, established by misplaced collagen deposition as well as the conversion of mucus into fibrous tissue (23). Therefore, acute injury and alveolar damage can be interpreted as an exacerbated reaction in response to microbiological agent exposure, in this case, the SARS-CoV family. Sequencing of the complete genome and phylogenic analysis indicated that the coronavirus strain responsible for COVID-19 is a beta-coronavirus in the same subgenus as the severe acute respiratory syndrome virus (SARS), but in a different clade. The structure of the receptor-binding genetic region is very similar to that of the SARS coronavirus and the virus uses the same receptor, the angiotensin-converting enzyme 2 (ACE2), for cell entry (24). The Coronavirus Study Group of the International Virus Taxonomy Committee has proposed that this virus be called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The spectrum of symptomatic infection varies from mild to critical; most infections are not serious, no pneumonia was found in 81% of cases; severe disease (with dyspnea, hypoxia or> 50% of lung involvement in images within 24 to 48 hours) was reported in 14%; and critical illness with respiratory failure, shock or multi-organ dysfunction was reported in 5% (25). Global mortality rates of 5% • 7% converge with current WHO estimates. Some patients with initially mild symptoms may progress over the course of a week. The disease usually develops five days after the onset of symptoms, and hospitalization may occur after an average of seven days of symptoms. Acute respiratory distress syndrome is a serious complication in patients with severe illness and can manifest itself soon after the onset of the illness. In the study described above, ARDS was established eight days after the onset of symptoms by 20%; mechanical ventilation was implemented in 12.3% of cases (26). In another study of 201 patients hospitalized with COVID-19 in Wuhan, 41% developed SARS; constraints such as advanced age (65 and above), diabetes mellitus and hypertension were each associated with SARS (27). Some patients with severe COVID-19 have laboratory evidence of an exuberant inflammatory

response, similar to the cytokine release syndrome, with persistent fever, elevated inflammatory markers (eg, D-dimer, ferritin) and elevated pro-inflammatory cytokines; these laboratory abnormalities have been associated with critical and fatal diseases. Initial plasma IL-1B, IL-1RA, IL-7, IL-8, IL-9, IL-10, basic FGF, GCSF, GMCSF, IFNγ, IP10, MCP1, MIP1A, MIP1B, PDGF, TNFα and VEGF were higher in ICU and non-ICU patients than in healthy adults. Plasma levels of IL-5, IL12p70, IL-15, Eotaxin and RANTES were similar between healthy adults and patients infected with 2019-nCoV. The comparison between ICU and non-ICU patients showed that plasma concentrations of IL-2, IL-7, IL-10, GCSF, IP10, MCP1, MIP1A and TNFα were higher in ICU patients than in non-ICU patients (28). In addition, other studies indicate that the development of ALI and alveolar damage can also occur with other types of respiratory infections, such as the influenza virus H1N1, respiratory syncytial virus (RSV), again highlighting a greater risk in the elderly population (29– 31).

1.2.2 Lung Oxidative Stress

Oxidative stress is defined as the disproportion between the presence of antioxidants and free radicals/pro-oxidants, in a biological system. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are the by-products of various cellular processes, including aerobic metabolism (32). However, when the redox balance is disrupted, these free radicals can have harmful impacts and are commonly linked to the appearance of various lifestyle-related diseases. These effects are ultimately caused by unregulated free radicals containing oxygen and nitrogen, which attack different cells and damage their DNA, proteins and lipids (32).

The redox environment in the pulmonary lining fluid is an important factor in determining the lung's innate and adaptive immune system. The normal lung has extremely high levels of extracellular antioxidant to maintain the extracellular space in a highly reduced state and facilitate the maintenance of the immune response. The balance between antioxidants and oxidants is sufficient in the normal lung. In oxidized states, a hyper-responsive immune system is present. The increase or decrease in antioxidants can disturb this balance, known as "oxidative state", which is associated with several pulmonary pathologies. This facilitates the binding of pathogens or antigens to effector cells, leading to a greater release of oxidants, such as superoxide and nitric oxide, causing activation nuclear factor kappa β (NF-kβ) and increasing cytokine production, including TNF-α, interleukin-1β, (IL-1β), IL-12. The creation of a markedly reduced environment by the addition of antioxidants dulls all primary responses of the innate immune system (33).

1.2.3 Oxidative Stress in Critical Illness

Oxidative stress has been implicated in the manifestation of critical illnesses, including ischemia and reperfusion injury and systemic inflammatory states. Excessive production of ROS can lead to tissue injury. ROS causes direct cell damage to cell proteins and nucleic acids and induces lipid peroxidation, leading to cell membrane destruction (34). ROS also plays a role as a second messenger in inflammatory cell intracellular signaling pathways. In particular, the activation of NF-kβ induced by hydrogen peroxide. NF-kβ resides in the cytoplasm as an inactive complex linked to its inhibitor I-kβ. After stimulation with various agents, including cytokines, viruses and ROS, mitogens cause dissociation of the NF-kβ-Ik-β complex and translocation of NF-kβ to the nucleus with its high-binding affinity to specific sites in the promoter region of target genes stimulating their transcription. NFkB is involved in many cellular events, such as the regulation of several pro-inflammatory genes, including many cytokines (TNF-α, IL-1, IL-6, IL-8, IL-2), hematopoietic growth factors such as the granulocyte colony-stimulating factor (G-CSF), cell adhesion

molecules-1 (CAM-1), endothelial leukocyte adhesion molecule (ELAM-1), vascular CAM-1 (VCAM-1), and synthesis of nitric oxide. A second messenger transcription factor activator -1 (APC-1), which also appears to be regulated by changes in the redox states of the cell, may contribute to the continuous production of inflammatory cytokines and progression of systemic inflammation leading to damage to vital organs, manifested by the development of ALI / ARDS or multiple organ failure syndrome. In addition, ischemia / reperfusion can lead to significant ROS production by increasing xanthine oxidase activity and increasing hypoxanthine production due to the reintroduction of both (33).

1.2.4 Oxidative Stress and COVID-19

The angiotensin-converting enzyme 2 (ACE2) is a protease that, along with the angiotensin-converting enzyme (ACE), participates in the renin-angiotensin system (RAS). They are present on the cell surface and compete for the same substrates, angiotensin I and II. ACE2 neutralizes ACE activity, the amount of angiotensin-II (ANGII) and increased ANG peptide (1-7, 34-35).

The downstream effects of the two enzymes are opposite: ACE activity leads to vasoconstriction, oxidative stress, inflammation and apoptosis, whereas ACE2 causes vasodilation, angiogenesis, anti-inflammatory, antioxidant and anti-apoptotic effects (35). The oxidative stress generated by ACE activity is due to the effects of its product, ANGII, which increases the production of reactive oxygen species (ROS) through the activation of NADPH oxidase and the generation of peroxynitrite anions. In contrast, the ANG (1-7) peptide synthesized by ACE2 activity leads to a negative reduction in pro-oxidant pathways, which prevent or attenuate cell damage induced by oxidative stress. Every individual has a unique ACE/ACE2 balance and may be more prone to inflammation if ACE prevails. When this happens, especially in coronavirus-related cases, SARS-CoV-2 infection negatively regulates the abundance of ACE2 on cell surfaces, as suggested by evidence (36). The result is a toxic over-accumulation of ANGII, exacerbated inflammation and, finally, acute respiratory distress syndrome and fulminant myocarditis. Suboptimal balance of ACE / ACE2 may explain the heterogeneous responses to the viral infection. The link between deregulation of the RAS cascade and probability and severity of SARS-CoV-2 infection has been discussed in recent investigations (37).Several studies have suggested that a delicate disulfide-thiol balance is crucial for viral entry and fusion in the host cell; oxidative stress generated by free radicals can affect this balance (38). Particular focus is shifted towards the impact of antioxidants, such as NADPH and glutathione, and redox proteins, such as thioredoxin and protein disulfide isomerase, which maintain the disulfide-thiol balance in the cell (38). The possible influence of these biomolecules on the binding of the viral protein to the host cell's ACE2 receptor protein is discussed, as well as on the severity of COVID-19 infection (38).

Several studies have suggested that excessive ROS production and a disproportionate cellular antioxidant/oxidant balance play an important role in the pathogenesis of respiratory viral infections, such as SARS-CoV infections. In addition, recent work has suggested that individuals with pre-existing conditions, such as diabetes, hypertension and lung, heart and kidney disease are at a higher risk of developing a serious infection. In addition, pathologies like cancer, diabetes mellitus, cardiovascular diseases, chronic kidney disease etc. can cause an increase in oxidative stress. Recent studies have also highlighted the importance of the disulfide-thiol balance in the viral entry of the SARS-CoV and SARS-CoV-2 coronaviruses. Similar to the HIV gp120 protein, experimental data showed that as the thiol content in SARS-CoV S1 (the receptor-binding subunit) increased, its ability to bind to target cells decreased, suggesting that both viruses require a specific thiol content for fusion and entry to occur (38).

1.3. Treatments

The first priority in the care of patients with SARS is the identification and treatment of the underlying cause. In patients with sepsis-associated SARS, good results may arise from early resuscitation experimentation, combined antibiotics and source control (13). Supportive therapy for SARS is focused on limiting more pulmonary actions through a combination of pulmonary protection to prevent ventilator-associated lung injuries and conservative fluid therapy to prevent the formation of pulmonary edema and promote resorption of pulmonary edema. The ideal parameters for pulmonary protection are unknown. As current evidence, there may be no safe level of tidal volume or airway pressure in patients with acute acute injury. As the volume of aerated lung is reduced in patients with SARS, even the normal volumes delivered with airway pressures surveyed for the uninjured lung can cause regional distention (called volumetric trauma), activating or further damaging the epithelium and, with that, amplify the inflammation. The repetitive opening and closing of the lung units (atelectrauma) amplifies the regional pulmonary tension and denatures the surfactant. Finally, epithelial and endothelial damage results in the translocation of pro-inflammatory mediators and bacterial products, leading to a worsening of systemic inflammation (biotrauma) (13, 39). There is no pharmacological therapy for SARS that has reduced mortality in the short or long term. Inhaled nitric oxide greatly improves oxygenation and can improve long-term lung function among patients who survive, but it does not reduce mortality and is associated with acute kidney injury (40). The use of glucocorticoids can improve oxygenation pressures and airways and, in patients with pneumonia, can accelerate radiographic improvement. These agents are not associated with consistent benefit from concordants and are harmful if initiated 14 days or more after the diagnosis of SARS (41).

Surfactant substitution, neutrophil elastase inhibition and anticoagulation have not shown good results in clinical trials, neither have non-steroidal anti-inflammatory agents (ketoconazole and lysophylline), statins, aluterol and antioxidants (procystein [l-2- oxothiazolidine-4-carboxylic acid]) (42). Although not proven to be fully effective in relation to its use, researchers recently tested the effect of hydroxychloroquine, an immunomodulatory drug (43). Despite limitations, especially the number of participants, Gautret and collaborators report a significant reduction in viral load in patients affected by COVID-19. In addition, the researchers claim that its effect is enhanced when combined with azithromycin. Chloroquine is traditionally used to fight malaria, but apparently it showed signs of possible reduction in viral replication in cases of infections resulting from SARS-CoV and MERS-CoV (44,45). This drug has been used for more than 70 years worldwide. It is listed as one of the essential drugs on the World Health Organization (WHO) list, in addition to being cheap and clinically safe (45). Regarding the treatment of SARS-CoV-2, however, its effectiveness and safety it remains to be established.

1.4. Glutathione

Glutathione (GSH - y-glutamylcysteineaglycine) is found in the cytosol of most cells in the body (46). It is a tripeptide that has glycine, cysteine and glutamate. GSH acts on several enzymatic systems in the body that help eliminate free radicals and detoxify fat-soluble components (47). Furthermore, it has a metabolic role in various biochemical processes, such as amino acid transport, deoxyribonucleic acid synthesis and immune system improvement (48). Glutathione in the epithelial lining fluid of the lower respiratory tract is the first line of defense against oxidative stress (48). In the epithelial lining fluid, GSH concentrations are 140 times higher than in serum, and changes in GSH metabolism in the lungs are considered central in the context of inflammatory lung diseases (49-51). In inflammatory lung

diseases, supplementation with exogenous sources of GSH may help to reduce the oxidant content. Few clinical studies have demonstrated that oral administration of GSH was ineffective in increasing plasma levels of healthy controls (52), while its intravenous administration increases its levels in the pulmonary epithelial lining fluid, albeit for a short period of time (53). Based on these preliminary studies, it appears that inhalation is one of the methods that can significantly increase the levels of GSH in the pulmonary epithelial lining fluid (54). Studies on GSH inhalation have revealed positive results for cystic fibrosis, chronic otitis, HIV positive patients, idiopathic pulmonary fibrosis and chronic rhinitis (54). However, practitioners should be vigilant about the dispersion of particles in patients undergoing nebulization, which is why this route of administration should only be considered if the patient does not respond to adjunctive therapy intravenously. Regarding the mechanism of action, studies have shown that there is no change in the plasma concentration of GSH, only locally, with its effects on the upper and lower respiratory tract. The predominant mechanism is attributed to its antioxidant properties that confer protection against oxidative damage, in an attempt to reestablish equilibrium in the respiratory tract. Most studies were unable to demonstrate changes in oxidation markers with the use of GSH. Additional explanations for the effects of GSH may include improved host defenses (increased cytotoxic lymphocytes) and better oxygenation. GSH inhalation produced clinically significant results for most of the diseases studied. Specifically, GSH inhalation has shown improvement in respiratory function markers that impact quality of life and disease progression (54).

1.5. Clinical evidence for the use of glutathione

1.5.1. Glutathione Clinical Trials

Glutathione has been explored in clinical and preclinical studies for some time now. In 1995 Meyer and colleagues published a study on the intravenous use of a glutathione precursor for patients with idiopathic cystic fibrosis. 14 individuals were recruited: 8 patients assigned to treatment and 6 healthy controls. 1800 mg or 4800 mg of the glutathione precursor were administered to all included subjects. Bronchoalveolar lavage fluid was obtained 3 hours after administration. As a result, it was found that the lower dose of 1800 mg showed greater benefits by significantly increasing the concentration of glutathione in the lavage of bronchoalveolar fluid, as well as in the pulmonary epithelial lining fluid. No adverse events were reported. In conclusion, the glutathione precursor administered intravenously increases pulmonary glutathione in patients with pulmonary fibrosis, but it did not show the same significant response on pulmonary glutathione in control subjects (55).

Intravenous glutathione for skin whitening was attempted in a 2005 study, which included 7 patients with an administration dose of 50 mg per kilogram of weight for 10 days. No adverse events were reported in this study (56). Other studies have addressed the intravenous use of glutathione for neuroprotection due to chemotherapy. In 1995 Cascinu and collaborators recruited 50 patients for a double blind trial comparing glutathione versus placebo (saline solution) for the prevention of cisplatin toxicity in gastric cancer. As a result, it was verified that until the fifteenth week, the glutathione group showed a reduction in the amount of neuropathy. Adverse events were not reported (57). In 2009, Milla and collaborators evaluated the use of intravenous glutathione in neurotoxicity caused by the infusion of oxaliplatin for the treatment of colorectal cancer. 27 patients were evaluated after receiving an infusion of 1500 mg / mL in saline solution after treatment cycles. As a result, there was a significant reduction in neurotoxicity compared to placebo. No adverse events were reported (58).

Some studies have evaluated the use of intravenous glutathione in neurodegenerative diseases. Secchi et al. published a study evaluating

9 patients with Parkinson's disease, who received 600 mg of glutathione twice a day for 30 days. As a result, there was a 42% decrease in the disability of these patients. The therapeutic effect lasted between 2 to 4 months and there were no reports of adverse events (59). In 2009, Hauser and colleagues published a pilot randomized controlled trial with 21 patients attempting to assess the safety and preliminary efficacy in Parkinson's disease. These patients received 1400 mg of glutathione 3 times a week for 4 weeks, with a 3 month follow-up. As a result, there was an improvement in motor scores in the glutathione group when compared to the placebo group. There were no reports of adverse events associated with the use of glutathione in this study (60). In 2020, Horowitz and collaborators published a study evaluating the use of oral / intravenous glutathione in the treatment of patients with dyspnea associated with COVID-19-related pneumonia. Two patients living in New York City with a history of Lyme and tick-borne co-infections had cough and dyspnea and their radiological findings were consistent with pneumonia caused by the new coronavirus. The study implemented 2 g of oral or intravenous glutathione. In both patients, dyspnea improved within 1 h of use. The repeated use of 2000 mg of oral and intravenous glutathione was effective in providing additional relief of respiratory symptoms. The authors concluded that oral and intravenous glutathione may represent a new treatment approach to block NF-κB and control “cytokine storm syndrome” in patients with COVID-19-derived pneumonia (61).Therefore, the use of intravenous glutathione presents encouraging results for its clinical use in the context of COVID-19, showing efficacy in the studies evaluated, as well as the absence of adverse events related to its use, which led us to propose it in this study.

2. HYPOTHESES

The respiratory tract of patients affected by COVID-19 ends up presenting acute inflammation with escalated immune response and cytokine production, which are responsible for injury, multiple organ failure and death. The use of glutathione in acute respiratory syndrome due to COVID-19 can help control pulmonary epithelial oxidants. Improvement in lung function is expected, with increased oxygen saturation as well as control of pulmonary inflammation. Lower glutathione levels increase cellular oxidative stress and are associated with several disease states and immune disorders that lead to greater susceptibility to viral infections, including uncontrolled SARS-CoV-2 infection [71]. Uncontrolled replication leads to oxidative damage to the lungs, increasing viral load, thus increasing the severity of the virus infection [72]. On the other hand, high levels of GSH can prevent the virus from replicating efficiently, producing lower viral loads and, therefore, milder symptoms. A doctor at Kursk State Medical University investigated the effects of GSH levels on an individual's ability to recover from COVID-19 infection and found that high ratios of ROS to GSH appeared to be strongly related to aggravated symptoms and longer recovery times (62).GSH deficiency can interfere with the body's ability to detoxify the cellular microenvironment, fold proteins, regenerate antioxidants, maintain healthy immune responses and even modulate apoptosis events - this promotes suboptimal cellular function and leads to disease (50). Age has a major impact on the severity of COVID-19 symptoms, where older individuals are at greater risk compared to younger individuals. Age is also associated with GSH levels, where GSH concentrations decrease with advanced age in mammalian erythrocytes [73]. GSH deficiency is known to cause a variety of adverse physiological effects and can alter genes that work together to synthesize vitamin D and is linked to an increase in oxidative stress. (63) Despite a series of publications reporting the beneficial effects of glutathione on human health, the main role of this powerful antioxidant in human physiology and pathology and in clinical applications remains

underestimated. Our objective is to justify the different roles of glutathione in determining individual responsiveness to COVID-19 infection and disease pathogenesis and the feasibility of using glutathione and its precursors as a means for the treatment and prevention of complications related to COVID-19. Numerous studies report that the deficiency of endogenous glutathione attributed to decreased biosynthesis and / or increased depletion of GSH represents a significant factor for the pathogenesis of various diseases through mechanisms involving oxidative stress and inflammation and that the main risk factors for the more aggressive forms and lethal manifestations of COVID-19 appear exactly in the population that naturally presents a natural or pathological depletion of GSH. Treatment with GSH is a promising approach, but high doses of GSH are necessary to achieve therapeutic effectiveness, due to its poor transport to cells and tissues. GSH has been used locally in the treatment of emphysema, where experimental evidence has shown that the negative oxidative regulation of 1-proteinase inhibitor activity has been reduced by glutathione, and it is suggested that this treatment may be considered an option for acute respiratory crises due to COPD. Previous clinical trials on GSH nebulization have demonstrated the bioavailability and safety of doses up to 600 mg twice daily (64). As an increase in the production of ROS in COVID-19 is currently the prevailing hypothesis, therefore this approach may be appropriate in this case. GSH is one of the most abundant molecules in our body: its concentration is 2 to 5 mM, comparable to other very abundant molecular species, such as blood glucose (5 mM) and intracellular ATP (5–10 mM). If we consider 40 kg of tissue, for instance, with an average of 2.5 mM GSH (about 750 mg / L), this means that there is 30 g of GSH in the whole body. With a half-life of 48 hours, its lasts around 10 days; this means that tissues lose 3 g of GSH per day; therefore, dietary support for complete replacement is approximately 1.5 g of glutamate, 0.75 g of glycine and 1.20 g of cysteine (1.63 g if it is assumed to be N-acetylcysteine) per day. The concentration of body GSH can be increased with the oral intake of GSH or proteins enriched in the constituent amino acids of GSH, or supplementation of the two limiting amino acids cysteine and glycine, since the availability of glutamate in the body is generally not limiting. Oral administration of GSH is more expensive than supplements with cysteine and glycine, and its systemic bioavailability may be poor due to degradation in the intestine; therefore, its suitability for use in a large population could be limited. Interestingly, a case study showed that the repeated use of 2000 mg of oral administration and intravenous injection of glutathione was effective in alleviating the severe respiratory symptoms of COVID-19, showing for the first time the effectiveness of this antioxidant therapy for COVID-19 (61).

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