COVID-19: can glutathione (GSH) help to reduce severe symptoms?

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Abstract

Currently, the prevalent approach to decrease the number of deaths by SARS-CoV-2 is to contain the spread of the virus by reducing interpersonal contacts and, hence, the number of infected people. An alternative strategy could involve the understanding of the molecular mechanisms underlying the known risk factors (old age, sex, diabetes, hypertension, cardiovascular diseases, or cancer, former smokers, etc) and to correct them, when possible, as a prevention/treatment of the disease.
The decrease of glutathione (GSH) is one of the biochemical alterations identified up to now as being linked to the risk factors.
Here, we discuss how GSH can mitigate the inflammatory response of the host, specific for the SARS-CoV-2 and dependent on its binding to its receptor ACE2, and we also suggest how to increase GSH levels to prevent and subdue the disease.

Keywords: SARS-CoV-2; ACE; ACE2; glutathione; inflammation; n-acetylcysteine; glycine; chloroquine; paracetamol

Introduction

Many recent clinical papers report that severe SARS-CoV-2 symptoms can be associated with different factors: advanced age, gender, air pollution, and comorbidities like hypertension, diabetes, cardiovascular diseases [1,2]. Most of these diseases were compensated with standard treatments (usually not recorded in clinical reports) but nevertheless, the patients were hyperresponsive to the SARS-CoV-2 infection. Lung inflammation is the main cause of life-threatening respiratory disorders at the severe stage, characterized by the so-called "cytokine release syndrome (CRS)".
The key to fight this harmful inflammatory response resides in: (i) addressing the mechanism of the virus penetration into the cell, mediated by binding to and inactivation of the ACE2 protein; (ii) in contrasting the exacerbation of the inflammatory response. The standard pharmacological approach would suggest either the use of an antiviral drug with the aim of blocking viral replication, or the exploitation of drugs previously validated as inhibitors of some inflammatory pathway in other chronic diseases. Unfortunately, these drugs are ineffective in healing the most severe cases of SARS-CoV-2 and, additionally, they have several side effects.
The scientific community is trying to find alternative therapies to treat SARS-CoV-2. This is a novel virus that is supposed to have originated from an animal reservoir and acquired the ability to infect human cells using the SARS-CoV cell receptor ACE2.

ACE2 is a protease that, with its companion, the angiotensin-converting enzyme ACE, takes part in the Renin-Angiotensin System. They are localized at the cell surface and compete for the same substrate, Angiotensin I. ACE2 counters the activity of ACE by reducing the amount of angiotensin-II and increasing Ang (1-7).
ACE downstream effects lead to Vasoconstriction, Oxidative stress, Inflammation, Apoptosis.
ACE2 downstream effects are Vasodilation, Angiogenesis, Anti-inflammatory, Anti-oxidative, and Anti-apoptotic.
The balance between ACE and ACE2 expression and activity is regulated by a set of interactions between dehydroepiandrosterone (DHEA), Cortisol, 25-$(OH)_2$-Vitamin D, and Glutathione (GSH). Each person can have a different balance between ACE and ACE2, and this can explain the different responses to the infection caused by the same virus.
Reducing the oxidative stress secondary to the imbalance between ACE and ACE2 could be the best approach for the prevention and treatment of COVID-19.
As most of the cofactors (aging, diabetes, hypertension, cardiovascular diseases, air pollution) of COVID-19 are associated with low levels of GSH, we propose the increase of body GSH as a new anti-inflammatory approach.

1. Glutathione (GSH) and its functions

Glutathione (GSH) is an antioxidant ubiquitous in most living organisms. Glutathione is capable of preventing damage to important cellular components caused by Reactive Oxygen Species (ROS) such as free radicals, peroxides, lipid peroxides, organic pollutants, and heavy metals.

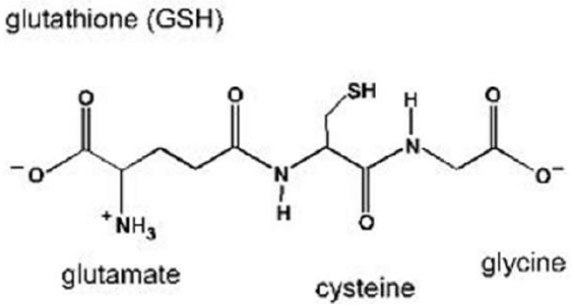

Fig.1 Glutathione (GSH) formula from [3]

It is a tripeptide with a gamma peptide linkage between the carboxyl group of the glutamate side-chain and cysteine. The carboxyl group of the cysteine residue is attached by a normal peptide linkage to a glycine.
GSH is one of the more represented molecules in our body: its concentration is 2-5 mM according to the tissue, comparable to the very abundant molecular species such as glucose in the blood (5 mM) and intracellular ATP (5-10 mM).
GSH half-life ranges from 3/5 hours to 30/50 hours, depending on the tissue and the environmental stresses.
If we assume 40 kg tissues with an average 2.5 mM GSH (ca 750 mg/l) it means 30 g GSH in the whole body. With a half-life of 48 hours, its life is around 10 days; this means that the tissues loose 3 g of GSH a day, therefore the dietary support for full replacement is approximately 1.5 g glutamate, 0.75 g glycine, 1.20 g cysteine (1,63 g.if assumed as N-Acetyl-L-cysteine) a day.

2. GSH functions

Due to the peculiar reactivity of its -SH group, GSH is involved in a number of chemical reactions, from disulfide bridges formation to conjugation to xenobiotics. As the pool of the available GSH molecules is more or less fixed, any unexpected increase of its utilization leads to a decrease of the free molecules and impairment of the other pathways. This is much meaningful, as in our hypothesis severe symptoms depend on GSH low levels.
Listing all the pathways depending on GSH will allow a better comprehension of the interactions between the metabolic pathways sharing this molecule [4].

In short, GSH is involved in the following pathways:

a. Protection against Reactive Oxygen Species (ROS)
b. Protecting Proteins -SH groups
c. Detoxification of methylglyoxal, formaldehyde, and many other environmental aldehydes
d. Binding to heavy metals
e. Conjugation to xenobiotics

a. Protection against Reactive Oxygen Species (ROS)

GSH protects cells by neutralizing (i.e., reducing) Reactive Oxygen Species (ROS).
ROS are key signaling molecules that play an important role in the progression of inflammatory disorders. An enhanced ROS generation by polymorphonuclear neutrophils (PMNs) at the site of inflammation causes endothelial dysfunction and tissue injury [5].
This conversion is illustrated by the reduction of peroxides:
$2\ GSH + R_2O_2 \rightarrow GSSG + 2\ ROH$ (R = H, alkyl)
and of free radicals:
$GSH + R^{\cdot} \rightarrow 0.5\ GSSG + RH$
GSSG is subsequently reduced by reactions involving NADPH.

b. Protecting Proteins -SH groups

The reaction involves the formation of an unsymmetrical disulfide from the protein (RSH) and GSH:
$RSH + GSH + [O] \rightarrow GSSR + H_2O$
Disulfides are subsequently reduced by reactions involving NADPH.

c. Detoxification of methylglyoxal, formaldehyde

GSH reacts with the reactive endogenous carbonyls methylglyoxal and formaldehyde to intermediates which are substrates of detoxifying enzymes [6].

d. Binding to heavy metals

Mercury, Lead, and other metals are toxic as they can irreversibly bind to SH groups of many enzymes, including many membrane ATPase. They are eliminated as GSH conjugates and modify the activity of glutathione-related enzymes: decrease of glucose-6-phosphate dehydrogenase and glutathione-S-transferase, increase of glutathione reductase [7,8].

e. Conjugation to xenobiotics

Glutathione S-transferase (GST) enzymes catalyze GSH conjugation to lipophilic xenobiotics, facilitating their excretion or further metabolism. The conjugation process is illustrated by the metabolism of N-acetyl-p-benzoquinone imine (NAPQI). NAPQI is a reactive metabolite formed by the action of cytochrome P450 on paracetamol (acetaminophen). Glutathione conjugates to NAPQI and the resulting product is excreted [9].
GSSG and GSH conjugates are exported from the cell by GSH transporters, which requires ATP for active pumping [10].
In particular, the multidrug resistance-associated proteins (Mrp/Abcc) appear to mediate GSH export and homeostasis. The Mrp proteins mediate not only GSH efflux, but they also export oxidized glutathione derivatives (e.g. glutathione disulfide (GSSG), S-nitrosoglutathione *(*GS-NO*)*, and glutathione-metal complexes), as well as other glutathione S-conjugates [11].

The GSH decrease due to the activation of the previous pathways will heavily impact the activity of the enzymes using GSH as a cofactor or substrate.

Reactions requiring GSH as a cofactor or substrate are:

- Prostaglandin H synthase (PGHS) is a rate-limiting enzyme in the production of prostaglandins and thromboxane, which are important regulators of vascular function [12], and GSH is a key cofactor [13].
- Leukotriene C(4) synthase. LTC(4) synthase conjugates LTA(4) with glutathione (GSH) to form LTC(4), the parent compound of the cysteinyl leukotrienes [14].
    - The cysteinyl leukotrienes are potent mediators of airway narrowing, derived from the lipoxygenation of arachidonic acid and the adduction of glutathione to this eicosanoid backbone [15].
- S-Nitrosoglutathione (GSNO) is an endogenous S-nitrosothiol (SNO) that plays a critical role in nitric oxide (NO) signaling and is a source of bioavailable NO.

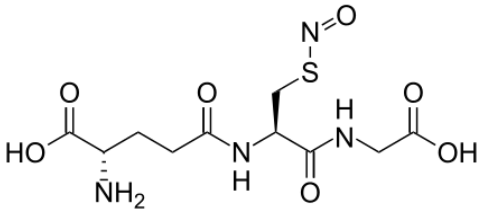

Fig. 2 S-Nitrosoglutathione (GSNO) formula from Wikipedia (https://en.wikipedia.org/wiki/S-Nitrosoglutathione)

The generation of GSNO can serve as a stable and mobile NO pool which can properly transduce NO signaling [16]. NO produced by eNOS and nNOS, in the presence of GSH, can effectively modulate vessels and neuronal functions, regulating the blood flow according to the local calcium influx.

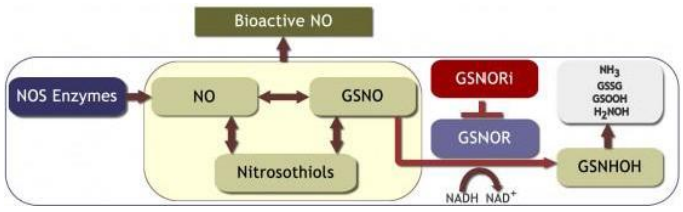

Fig. 3 The enzyme GSNO reductase (GSNOR) from Wikipedia (https://en.wikipedia.org/wiki/S-Nitrosoglutathione)

NO produced by iNOS (inducible by glucocorticoids) is a hallmark of inflammation and can lead to excess ROS and proteins S-nitrosation with their reversible inactivation.

S-nitrosation has been recognized as an important mechanism of protein posttranslational regulations, based on the attachment of a nitroso group to cysteine thiols. Reversible S-nitrosation, similarly to other redox-based modifications of protein thiols, has a profound effect on protein structure and activity and is regulated by the availability of GSH [17].

3. Conditions associated with low GSH

Our working hypothesis is that the severity of symptoms in COVID-19 patients does not depend on virus variants but on specific host conditions, namely a low level of GSH, required to counteract the ROS mediated inflammation elicited by SARS-CoV-2 infection.

Let's examine in detail the conditions clinically associated with severe disease, as far as the GSH is involved.

a. Age
b. Diet
c. Sex
d. Diabetes
e. Hypertension
f. Drugs
g. Air pollution

a. Age

In laboratory animals, it is possible to test the age-related changes in GSH content in different tissues. Old mice had lower GSH than young mice in organs. The difference in GSH decrease among different tissues may depend on the local environmental stress (lymph nodes: infectious agents, lung: pollutants). The sharp decrease in the lung is highly remarkable because the sum of aging and air pollution can lead to very low GSH [18].

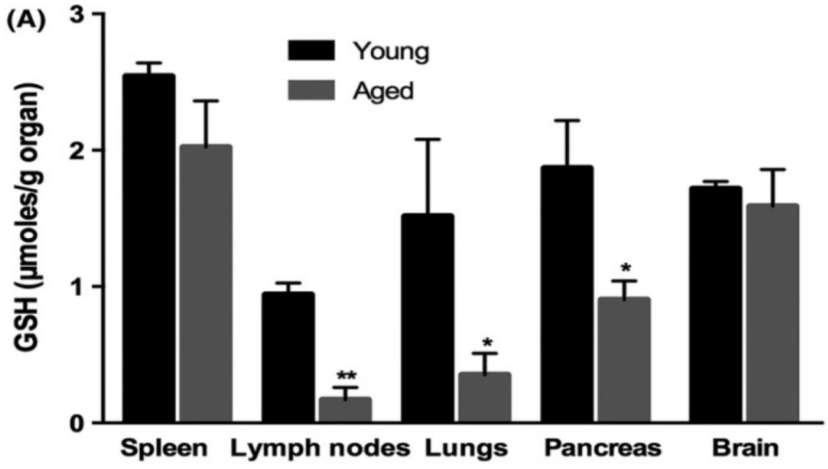

Fig 4 Reduced Glutathione(GSH) content in organs of young (8 week-old) and aged (15-month-old) mice, from [18]

As it is not possible to test GSH in human tissues, usually in humans GSH and its related enzymes are tested in serum, red blood cells, lymphocytes. Similar to the data obtained in animals, several studies have reported that also in human subjects the concentration of GSH declines with aging [19–24].
Some observations are particularly relevant:

- Comparing a 60- to 79-year-old group. with a group of 20- to 39-year-old subjects, their GSH was 17% lower than the reference group ($p < 0.001$) [22]
- A recent study carried out on red blood cells of young and old human subjects has demonstrated that the rate of intracellular GSH synthesis and GSH concentrations were markedly lower in the elderly than in the young humans. This GSH deficiency was associated with increased oxidant stress, Interestingly, replenishing the supply of cysteine and glycine by oral supplementation was effective at restoring GSH concentrations and reducing oxidative stress to levels observed in young healthy humans [25]. The age range of the elderly subjects was 60–75 y, and that of the young control subjects was 20–40 y. The young subjects had a lower body mass index than did the elderly subjects. No differences in hematocrit, hemoglobin concentrations, renal function, or liver enzymes were found between the 2 groups. Younger subjects were euglycemic, but the elderly subjects had impaired glucose tolerance and higher concentrations of fasting glucose and glycated hemoglobin [25]
- GSH reductions apparently participate in aging-induced blood pressure elevations, most strongly in males, and correlate to BMI [26]

b. Diet

Higher GSH levels have been correlated to a diet with abundant whey proteins, rich in cysteine [27].
Similar data have been obtained correlating the average daily dairy servings with the GSH concentrations in the brain, measured by using a unique, noninvasive magnetic resonance chemical shift imaging technique at 3 T. The concentrations in the frontal, and frontoparietal regions were correlated with average daily dairy servings [28].

c. Sex

As the severity of COVID-19 is strictly correlated to the sex, we accurately looked for the sex-linked differences in GSH content.
There are no differences between sexes in GSH content in different tissues while there are significant differences in the drug-metabolizing enzymes.
The half-life of glutathione in the kidney was significantly shorter in males (29 min) than in females (57 min). The specific activity of the catabolic enzyme gamma-glutamyltranspeptidase in female mice was 73% of that in male mice. These results suggest that the faster glutathione turnover in males could account for the higher susceptibility to oxidative injury [29]. Glutathione peroxidase (GSH-Px) activity of the male adult was 61% of the female adult [30].
A time-dependent depletion of hepatic and renal cortical glutathione was observed in both male and female mice following a dose of acetaminophen. At the level of renal cortical glutathione depletion in male mice was significantly greater than that in the females [31].
In conclusion, even if in basal conditions there are no significant differences in GSH level, after challenges with drugs or pollutants the decrease of GSH is higher in males than in females.

d. Diabetes

Correlation between low GSH and diabetes is well established.
Glutathione is considered a marker for human disease, due to its function of "master antioxidant" [32]. A study from Samiec and coll. showed that the levels of total glutathione and its reduced form were lower in plasma of older subjects and even lower in diabetic patients [19].
Diabetes has been widely associated with oxidative damage, increased GSSG/GSH ratio, and decreased GSH in different tissues. Decreased GSH is, in most cases, associated with increased activity of NF-kappaB [33–35].
Atorvastatin and alcohol decreased the GSH in hepatic tissues and induced insulin resistance [36].
Type II diabetes depends mostly on muscle insulin resistance.
Insulin resistance is negatively related to the activity of endothelial NO synthase (eNOS), thus creating a link between metabolic and cardiovascular diseases [37–39]. Lower NO production induces both insulin resistance and hypertension. The observation that low levels of GSH are associated with insulin resistance points to the fact that the increase of insulin sensitivity in muscle, adipocytes, and liver depends more on S-Nitrosoglutathione (GSNO) than on pure NO [40–42].

e. Hypertension

Diabetes and hypertension seem to be the most frequent comorbidity in patients dying [2].
Hypertension may depend on multiple factors:

- activation of the Renin-Angiotensin System (RAS) mainly by Renin overexpression secondary to low (1,25-)OH2-Vit D3
- Decreased activity of eNOS
- Increased production of Cortisol
- Decreased levels of GSH

As already described for diabetes the combination low GSH/low NO leads to a higher calcium influx into vessel wall smooth muscle with vasoconstriction as demonstrated also in animal models [43].
Since in hypertensive patients the low levels of GSH are often accompanied by a decrease of companion enzymes (Catalase *CAT*, GSH-Px, and *GST*.) and increased lipid peroxides; the prevailing hypothesis is that the GSH decrease is due to a burst of ROS production, secondary to an inflammatory process [44,45].

f. Drugs

Glutathione S-transferase (GST) enzymes catalyze its conjugation to lipophilic xenobiotics, which includes most of our drugs.
Acetaminophen (paracetamol) is the best-known drug affecting GSH levels (more than 600 entries in PubMed on this interaction).
Others drugs as well have the same effect: doxorubicin (adriamycin), isoproterenol, antimalarial drugs, chloroquine (CQ), etoposide, opiates, ethanol [46], antidepressants [47]. These effects are also dependent on the diet and modulate the absorption of the nutrients [48].
The use of CQ deserves special caution because of its chemical properties.
In addition to its prooxidant activity leading to GSH depletion [49,50], chloroquine accumulates into lysosomes leading to their alkalinization. Lysosomes alkalinization impairs the uptake of many nutrients from the blood, including transferrin-bound iron. Iron deficiency in the nerve reduces cytochrome C synthesis, respiratory chain activity, and ATP synthesis. This toxicity is untreatable and can progress to blindness [51,52].
As symptomatic herpes infection is strictly correlated with the iron stores of the host [53] the inhibition of iron absorption induced by CQ can lead to severe HSV reactivation.

Opinions about Ibuprofen are controversial. Based on data published in a study (2008, some years ago), it should be beneficial in COVID-19, as Ibuprofen through the combined inhibition of COX-1 and COX-2 is able to attenuate glutathione depletion, TNF-alpha secretion, mitochondrial alterations, and hepatic apoptosis [54].
Recently the use of anti-inflammatory drugs in people with suspected COVID-19 was questioned. The French minister, Oliver Veran, tweeted on Saturday 14 March 2020 that people with suspected COVID-19 should avoid anti-inflammatory drugs. "Taking anti-inflammatory drugs (ibuprofen, cortisone...) could be an aggravating factor for the infection. If you have a fever, take paracetamol," he said. The choice of the best anti-inflammatory to be used is currently under examination by different Institutions [55].

g. Air pollution

Previous research suggests that exposure to persistent organic pollutants (POPs) increases the risk of chronic diseases such as hypertension [56,57] and diabetes [58,59]. As conjugate derivatives of GSH are major excretory products of POPs, we can expect that long-term exposure to POPs can continuously consume GSH.

4. Renin–Angiotensin System (RAS) and COVID-19

ACE2 is the receptor of SARS-Cov-2 [60] and it is inactivated when bound to the virus. ACE2 is mainly expressed in lung AT2 cells, liver cholangiocytes, colon colonocytes, esophagus keratinocytes, ileum endothelial cells (ECs), rectum ECs, stomach epithelial cells, and kidney proximal tubules [61], all possible localizations with associated symptoms.

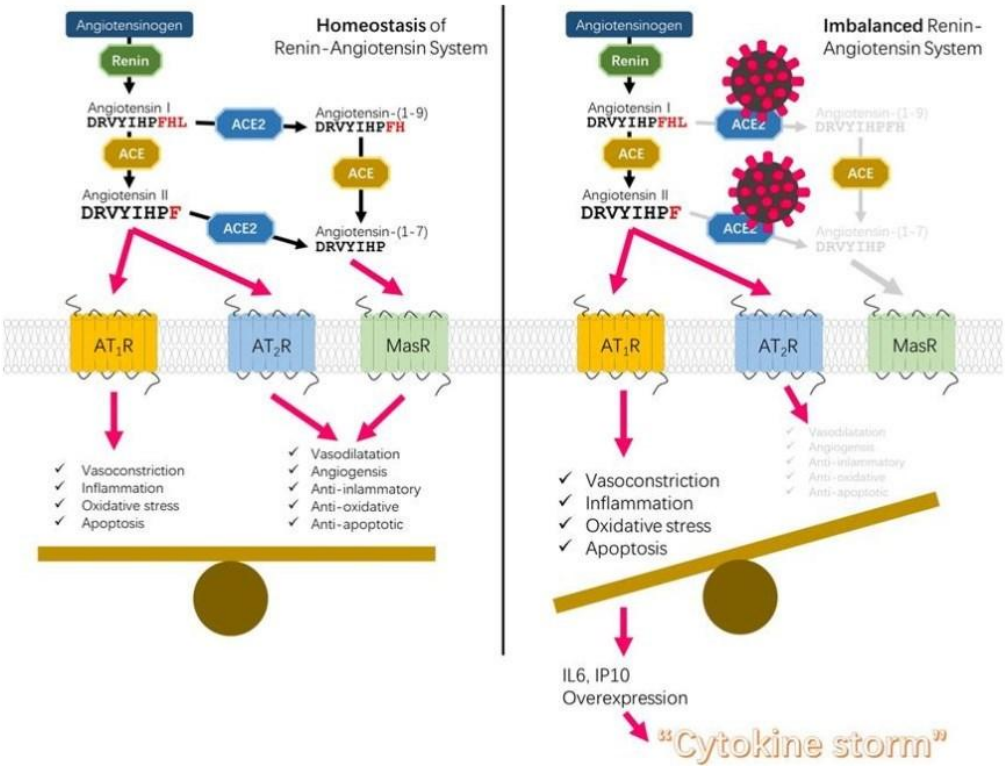

Fig 5 A comprehensive scheme of the interactions between the molecules involved in the Renin–Angiotensin System (RAS), from [62]

Inactivation of ACE2 by the virus leads to an imbalance between the number of molecules of Angiotensin II (ANGII) and Angiotensin (1-7).
ANGII, through binding to AT1R activates NADPH oxidases that transfers an electron from NADPH to O2 generating $O^{\cdot}-2$ and downstream peroxynitrite, hydroxyl radical and H2O2, which can be scavenged by GSH. ROS-mediated oxidation can, in turn, alter gene expression through signaling cascades induction, or interaction with transcription factors [63].

Among these factors, a prominent role is played by NF-kB, whose role in inflammation in severe acute respiratory syndrome (SARS) has been demonstrated in both SARS-CoV-infected cultured cells and mice [64]. Drugs that inhibited NF-κB activation led to a reduction in inflammation and lung pathology.
NF-kB is involved in inflammation with multiple mechanisms: high levels of interleukin-6 (IL-6) in the acute stage associated with lung lesions were found in SARS patients. In vitro, the viral nucleocapsid (N) protein activated IL-6 expression in a concentration-dependent manner. Promoter analyses suggested that NF-kB binding element was required for IL-6 expression regulated by N protein [65].
The oxidant/antioxidant imbalance is not peculiar to SARS, but it is shared by all inflammatory lung diseases, and activates redox-sensitive transcription factors such as NF-kB. This activation is reverted by GSH [66].
In an animal model of oxidative stress, NFκB binding activity was inversely related to liver glutathione and was further suppressed by oral administration of green tea extract [67].

The increase of number of molecules of ANGII, responsible for the ROS production, is dependent on many factors:

- increased Renin Activity
- increased ACE expression and activity. GSSG showed activatory effect on ACE activity whereas GSH provided inhibitory effect [68]
- decreased ACE2 expression and activity

Among them, only ACE activity is modulated by GSH redox state.

5. GSH and SARS-CoV-2 replication

There are no data of a direct effect of GSH on the replication of the SARS-CoV-2 but measurement of GSH antiviral activity in PEDV-infected Vero cells demonstrated that the effective concentration of GSH in inhibiting PEDV replication was 1.5 mg/mL (ca. 5 mM) [69], suggesting a possible direct effect also on the new virus SARS-CoV-2 replication.

6. GSH therapeutic use

On the basis of our previous considerations, pointing to a possible role of low GSH level in the pathogenesis of respiratory symptoms of COVID-19, we propose possible therapies aimed to increase the local (lung) or the whole body GSH concentration.

6.1 Local use

GSH has been used locally in the treatment of Emphysema because the tissue damage in emphysema is thought to be mediated by an oxidative down-regulation of the activity of α-1-proteinase inhibitor.1 This down-regulation has been shown in vitro to be slowed by glutathione, and the authors suggest this treatment can be considered an option for acute respiratory crises due to

Chronic obstructive pulmonary disease (COPD). Previous clinical trials of nebulized reduced glutathione have demonstrated the bioavailability and safety of up to 600 mg twice daily [70].
As an increased ROS production in COVID-19 is the currently prevailing hypothesis, this approach should be suitable in this case as well.

6.2 Oral supplementation

Body GSH concentration may be increased with oral supplementation of :

a. GSH
b. the two limiting amino acids Cysteine and Glycine, as the body availability of glutamate is usually not limiting
c. Whey proteins

a. GSH supplementation

Oral GSH can be assumed as non-liposomal or liposomal glutathione [71,72], but is more expensive than supplementations with cysteine and glycine, and therefore less suitable for use on a large population. Moreover, the systemic bioavailability of orally consumed glutathione may be poor because the tripeptide is the substrate of proteases (peptidases) of the gut and that explains the lack of results [73].

b. NAC (*n*-acetylcysteine) and Glycine supplementation

Dietary supplementation with the glutathione precursors cysteine and glycine, in proper conditions, fully restores glutathione synthesis and concentrations. These findings suggest a practical and effective approach to decreasing oxidative stress in aging and diseases associated with low GSH concentration [74]. Compared with younger control subjects, elderly subjects had markedly lower RBC concentrations of GSH (53%). After oral treatment of 14 d with 0.81 (132 mg NAC) mmol cysteine · kg−1 · d−1 (as *n*-acetylcysteine) and 1.33 (100 mg) mmol glycine · kg−1 · d−1 they reached the GSH concentration of the younger controls [25].
This dosage corresponds to 9.10 g.· d−1 NAC, and 7 g.· d−1glycine for 70 kg weight.
NAC has been largely used in the past as a treatment for bronchitis. A meta-analysis evaluating 13 studies and a total of 4,155 people with COPD, concluded that the standard dose of 1,200 milligrams of N-acetylcysteine per day reduces the incidence and severity of flares (known as exacerbations) compared to a placebo [75]. The stoichiometric dose of glycine should be ca. 1000 mg (exactly 938). This amount roughly corresponds to the daily turnover in healthy people and seems a reasonable dosage free of side effects, either for a preventive or therapeutic approach.

c. Whey proteins

Clinical evidence for nutritional strategies that could be used to improve GSH status has been extensively reviewed and focus on supplementation with whey proteins, green tea and green tea extracts [27].
The effect of short term and controlled administration of whey proteins on plasma GSH has also been evaluated. After two weeks of 45 g daily whey protein supplementation, plasma total GSH levels, in patients with low plasma GSH, reached normal values [76]. Similar effects were obtained within 12 weeks of 20 g daily [77]. Results may vary on the basis of commercial products used and therefore this approach requires a complex standardization of the response to the different preparation, making NAC (*n*-acetylcysteine) and Glycine supplementation an obligatory choice.

Conclusions

The outlined hypothesis that SARS-CoV-2 can unbalance a high activity of the Renin–Angiotensin System in the lung via ACE2 downregulation, which is followed by free radicals mediated inflammation, needs to be further investigated for the precise understanding of the involved molecular mechanisms.
However, based on this approach, we hypothesize that the low levels of GSH are one of the major causes of the excessive inflammatory response leading to severe COVID-19. Increasing body GSH by the supplementation of NAC and glycine hopefully will reduce the number of symptomatic patients.
This approach certainly awaits further refinement (patient selection, comorbidities, dosage), but it should not prevent us from using it immediately for saving lives and reducing the health care system burden.

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