Glutathione: pharmacological aspects and implications for clinical use

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Abstract

Glutathione is a tripeptide found in many tissues which plays a pivotal role in critical physiological processes such as maintenance of redox balance, reduction of oxidative stress by enhancement of metabolic detoxification of both xenobiotic and endogenous compounds, and regulation of immune system function.

Glutathione depletion is associated with many chronic degenerative diseases and loss of function with aging and altered glutathione metabolism has been implicated in central nervous system diseases, frailty and sarcopenia, infected state, chronic liver diseases, metabolic diseases, pulmonary and cardiovascular diseases.

Therefore, the glutathione status may be an important biomarker and treatment target in various chronic, age-related diseases.

Here we describe the main pharmacological aspects of glutathione, focusing on its synthesis and role in several vital functions including antioxidant defense, detoxification of xenobiotics and modulation of immune function and fibrogenesis and the clinical implications of its depletion and we discuss the different strategies for increasing glutathione cellular levels either by providing specific precursors and cofactors or directly administering the tripeptide.

Introduction

Glutathione is a tripeptide found in many tissues at relatively high concentrations (1-10 mM in cells, similarly to glucose, potassium, and cholesterol), which plays a fundamental role in critical physiological processes such as maintenance redox balance, reduction of oxidative stress through the enhancement of the metabolic detoxification of both xenobiotic and endogenous compounds, and the regulation of immune system function.[1]

It has been widely recognized that glutathione depletion is associated with many chronic degenerative diseases and loss of function with aging.

In particular, impaired glutathione metabolism has been implicated in central nervous system diseases (such as Alzheimer's disease and Parkinson's disease), chronic conditions associated with aging (such as frailty and sarcopenia),[2] infected state, chronic liver diseases, metabolic diseases (such as diabetes mellitus), pulmonary and cardiovascular diseases.[3]

Therefore, the glutathione status may be an important biomarker and treatment target in various chronic, age-related diseases.

In some cases, there may be useful to straighten low levels of glutathione to restore a proper balance and optimizing glutathione levels has been proposed as a strategy for health promotion and disease prevention. An obvious strategy is to directly administer glutathione, although oral administration is controversial as in many experiences orally administered glutathione has not resulted in elevate cellular levels and the possible clinical benefits needs to be clarified.[1,4] A different approach to promoting glutathione production could be to provide precursors, cofactors or specific foods and nutrients that con increase or maintain optimal levels of glutathione, such as supplemental cysteine in the form of whey or *N*-acetylcysteine (NAC), antioxidant vitamins (B,C,E), alpha-lipoic acid, selenium or phytonutrients (*i.e.*, Brassica vegetables and green tea).[1,4] Data are scant and often controversial leading to some ambiguity in the efforts to highlight the ability of nutritional interventions to enhance glutathione status. More research is absolutely needed to clarify optimal dose and delivery forms and one mandatory target should be the identification of sub-groups of individuals most likely to respond to particular supplements, nutrients or foods.

Pharmacological aspects

Synthesis

Glutathione, as a tripeptide (γ-L-glutamyl-L-cysteinylglycine), consists of a glutamate residue linked to cysteine with an atypical bond via its γ-carboxyl rather than the α-carboxyl group and followed by a conventional peptide bond between cysteine and glycine. Glutathione exists in cells in 2 states: thiol-reduced (GSH) and disulfide-oxidized (GSSG). GSH is the predominant form and accounts for >98% of total GSH. Eukaryotic cells have three major reservoirs of GSH. Most (80-85%) of the cellular GSH are in the cytosol; 10–15% is in the mitochondria (where its concentration in the matrix equals that of the cytosol, thus requiring specific transport systems) and a small percentage is in the endoplasmic reticulum (ER).[1,5,6]

The synthesis of GSH from its constituent amino acids occurs exclusively in cytosol, where glutamate cysteine ligase (GCL) and glutathione synthetase (GS) reside. GSH is synthesized in two steps from the amino acid precursors, and it involves two ATP-requiring enzymatic steps. The first step, in which glutamic acid and cysteine are joined, is catalyzed by GCL, previously known as γ-glutamylcysteine synthetase (γ-GCS), to form glutamylcysteine. L-glutamate is phosphorylated by MgATP to form the enzyme-bound intermediate γ-glutamylphosphate, which subsequently reacts with the amino group of cysteine. De novo glutathione synthesis is primarily controlled

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by the cellular level of the amino acid cysteine, the availability of which is the rate limiting step and GCL is the enzyme involved in the rate-limiting step.[1,5,6]

The second step, in which addition of glycine takes place, is catalyzed by GS, a homodimeric enzyme, which is also a potent antioxidant. It is a member of the ATP-grasp superfamily and rapidly catalyzes the ligation of γ-glutamylcysteine and glycine to generate glutathione[7,8] (Figure 1). Unlike GCL, GS is not feedback-inhibited by GSH, and it is not associated with a regulatory subunit. Thus, GS activity appears to be mainly controlled by substrate availability.[1,5-7]

Cysteine is derived normally from the diet, protein breakdown and in the liver, from methionine via trans-sulphuration pathway, which allows the utilization of methionine for GSH synthesis. The first step is represented by the generation of S-adenosylmethionine (SAMe), the principal biological methyl donor, catalyzed by methionine adenosyl transferase (MAT). This pathway is highly active in hepatocytes but, outside of the liver, it is almost absent, and it is markedly impaired or absent in the fetus and newborn infant as well as in cirrhotic patients.[9]

In addition, cirrhotic patients also have decreased MAT activity and diminished SAMe biosynthesis, which further contribute to decreased GSH levels.[5,6,9]

Cysteine is unstable extracellularly where it readily autoxidizes to cystine, which is the oxidized dimer form of the amino acid cysteine and is both a site of redox reactions and a mechanical linkage that allows proteins to retain their three-dimensional structure. Thus, extracellular concentrations of GSH (with the exception of bile acid, which may contain up to 10 mmol/L), are relatively low (*i.e.,* 2-25 μmol/L in plasma), compared with that of cystine (50-150 mmol/L). Cysteine and cystine are transported by distinct membrane carriers, and the latter is taken up by some cells (*i.e.*, endothelial cells) and is rapidly reduced to cysteine intracellularly where it is the prevailing form due to the highly reducing conditions.[10,11] The hepatocytes have little or no capacity for direct transport of extracellular cystine. However, GSH that effluxes from the liver can reduce cystine to cysteine on the outer cell membrane and the resulting cysteine is taken up by hepatocytes.[11]

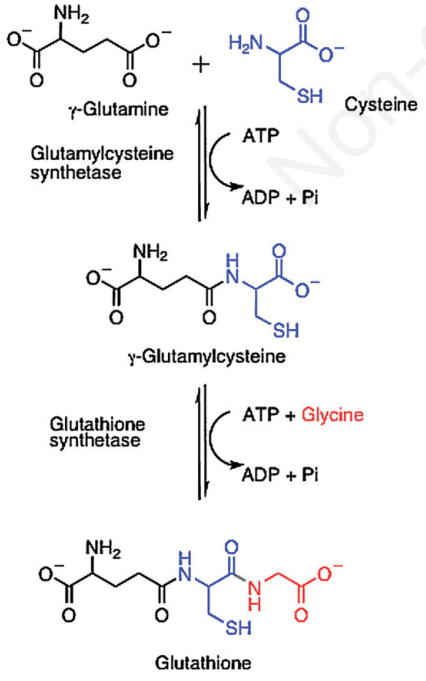

Figure 1. Synthesis of glutathione.[8]

Intracellular conversion of L-cystine into L-cysteine has been considered to be a key process to mediate extracellular L-cysteine/L-cystine redox, as well as the synthesis of protein and glutathione (GSH).[10] Although L-cysteine and L-cystine metabolism *via* multiple ways have not been fully explored in all tissues, results obtained with different studies indicate that the balance between extracellular and intracellular L-cysteine/L-cystine is largely regulated by transportation.

Imbalance of extracellular L-cysteine/L-cystine is associated with oxidative stress and other pathological disorders.

GSH is resistant to intracellular degradation and is only metabolized extracellularly by cells that express γ-glutamyl transpeptidase (GGT), throughout the hepatobiliary tree and in other organs such as the heart, kidney, lungs, pancreas and seminal vesicles. This allows for released GSH to be broken down and its constituent amino acids taken up by cells and reincorporated into GSH (so called γ-glutamyl cycle). The bulk of plasma GSH originates from the liver, which exports nearly all the synthetized GSH into plasma and bile. Thus, dysregulation of hepatic GSH synthesis has impact on GSH homeostasis systemically.[1,5,6,11]

In summary GSH is made available in cells in 3 ways:

i) *De novo* synthesis *via* a 2-step process catalyzed by GCL and GS (requiring ATP) which is primarily controlled by the cellular levels of cysteine. Moreover, GCL activity is in part regulated by GSH feedback inhibition.

ii) Recycling of cysteine from conjugated glutathione via GGT (requiring NADPH) in the γ-glutamyl cycle, where the γ-glutamyl moiety of GSH is transferred to an amino acid and once transported back into the cell, can be further metabolized to release the amino acid and 5-oxoproline, which can be converted to glutamate and used for GSH synthesis.

iii) Regeneration of the oxidized dimer GSSG to two reduced GSH molecules in cells by glutathione reductase (GR), a ubiquitous enzyme of the family of disulfide reductases, in the presence of NADPH and FAD, a derivative of the water-soluble vitamin riboflavin, as the reducing cofactors, forming a redox cycle. GR is found in cytoplasm and within organelles including the nucleus and the mitochondria. Oxidoreductase activity was also found in the endoplasmic reticulum and in the lysosomes. GR has a key role in the cellular redox homoeostasis, since it is responsible for maintaining the supply of reduced glutathione[1,5,6,11,12] (Figure 2).

Role

Glutathione is implicated in several vital functions including antioxidant defense with reduction of oxidative stress, metabolic detoxification of xenobiotics, regulation of cell cycle progression and apoptosis, storage of cysteine, maintenance of redox balance, modulation of immune function and fibrogenesis. It catalytically detoxifies hydroperoxides, peroxynitrites, and lipid peroxides and directly scavenges diverse oxidants such as superoxide anion, hydroxyl radical, nitric oxide, and carbon radicals.

Glutathione shields cellular macromolecules from endogenous and exogenous reactive oxygen (ROS) and nitrogen (RNS) species and deals directly with the causes of oxidative stress such as heavy metals and persistent organic pollutants (POPs). Among many conjugators, GSH conjugate derivatives are major excretion products of POPs. This mechanism is extremely important for the health state since exposure to POPs is linked to many chronic diseases, including diabetes and cardiovascular diseases.[1,13]

Glutathione directly neutralizes singlet oxygen, hydroxyl radicals, and superoxide radicals. ROS can be generated in several intracellular sites, including cytosol, peroxisomes, plasma membrane, and ER. However, in most cell types of mitochondria appear as the main source of superoxide generation and the mitochondrial electron transport chain (ETC) is the main cellular process of ROS generation in physiological circumstances.[14] The first line of defense against superoxide is the presence of a specific member of the family of metalloenzymes called superoxidedismutases (SODs), specifically located in the mitochondrial matrix, which catalyzes the dismutation of superoxide anion into H_2O_2. The detoxification against H_2O_2 in mitochon-

dria occurs mainly through the GSH redox system, including the glutathione peroxidases(Gpxs) and GSH reductases, as well as the presence of peroxiredoxins (Prxs) a family of thiol-specific peroxidases that rely on thioredoxins (Trx) as the hydrogen donor for the reduction of H_2O_2 and lipid hydroperoxides. Both Gpxs and thioredoxin reductases (TrxR) contain selenocysteine (SeCys), with Se, as an anion at biological pH, carrying out biological redox reactions, and are among the constituents of the human selenoproteome, which comprises 25 selenoproteins with several functions ranging from protection against oxidative stress, to Se storage and transport, from SeCys synthesis to redox signaling.[15]

So far, eight isoforms of Gpx have been identified in humans, which vary in cellular location and substrate specificity.[16] Gpx1 is the major isoform localized in various cellular compartments, including the mitochondria.[17,18] which in the liver account for about one third of the total Gpx activity.[19] Gpx has been considered to be the major H_2O_2 reducing enzyme. Due to their high rate constant and high abundance, Prxs are thought to be responsible for scavenging nanomolar concentrations of H_2O_2 associated with redox signaling, while Gpxs are likely important at higher intracellular concentrations, buffering high ROS levels to avoid cell damage and stress signaling response.[20]

The antioxidant function of GSH is accomplished largely by GPx-catalyzed reactions, which reduce hydrogen peroxide and lipid peroxide as GSH is oxidized to GSSG. This function is determined by the redox- active thiol (-SH) of cysteine that becomes oxidized when GSH reduces target molecules.[21] Upon reaction with ROS or electrophiles, GSH becomes oxidized to GSSG, which can be reduced to GSH by GR. GSSG is potentially toxic to the cells by inducing apoptosis by activation of the SAPK/MAPK pathway.[22] However, cells normally contain high GR activity, which maintain most of the GSH in the reduced form. Some GSSG is also secreted from cells. During oxidative stress, GSSG could react by disulfide exchange with a protein thiol to produce a protein mixed disulfide (PSSG), which can further exchange with another protein thiol to a protein disulfide.[23] This reversible formation of mixed disulfides between GSH and low-pKa cysteinyl residues of proteins is the so called *S*-glutathionylation.[3] This is an important mechanism for dynamic, posttranslational regulation of a variety of regulatory, structural, and metabolic proteins, and for the regulation of signaling and metabolic pathways in intact cell systems.[3] Since GSSG is not readily exported out of mitochondria, the activity of GR is an important mechanism to control the level of GSSG in mitochondria, and the uncontrolled generation of GSSG during oxidative stress can contribute to mitochondrial dysfunction by glutathionylation of target proteins.[24,25]

Thus, the GSH/GSSG ratio reflects the oxidative state and can interact with redox couples to maintain appropriate redox balance in the cell.

In addition to the defense against oxidants and ROS, GSH also plays an important role in the protection against electrophiles by glutathione-*S*-transferases (GSTs), generated as a consequence of metabolic processes involving both endogenous compounds and xenobiotics. GSTs exhibit a wide intracellular distribution, being localized in mitochondria, cytosol and membrane-bound isoforms.[26,27] Mitochondrial GSTs display both GSH transferase and peroxidase activities that detoxify harmful by products through GSH conjugation or GSH-mediated peroxide reduction. Thus, glutathione neutralizes free radicals produced by Phase I liver metabolism of chemical toxins.

GSH facilitates the plasma membrane transport of toxins by different mechanisms, the most important of which is formation of glutathione S-conjugates of the activated intermediates produced to make them water soluble for excretion by the kidneys.

Besides their fundamental role in energy generation, mitochondria also play a strategic role in the regulation of cell death, including apoptosis (caspase-dependent and independent) and necrosis. Therefore, mitochondrial GSH has been shown to critically regulate the level of sensitization to secondary hits that induce mitochondrial membrane permeabilization and release of proteins confined in the intermembrane space that once in the cytosol engage the molecular machinery of cell death.

Glutathione is also a cofactor for several antioxidant enzymes.

Among small molecular weight antioxidants, GSH is the most important one produced in the cells, while there are others obtained from the diet such as vitamins E and C. Interestingly, the oxidized vitamin E is reduced by vitamin C in a non-enzymatic but rapid reaction, while the oxidized vitamin C can then be restored to the reduced form by GSH as substrate.[4] Thus, another way glutathione protects cells from oxidants is through recycling of vitamins C and E.[28]

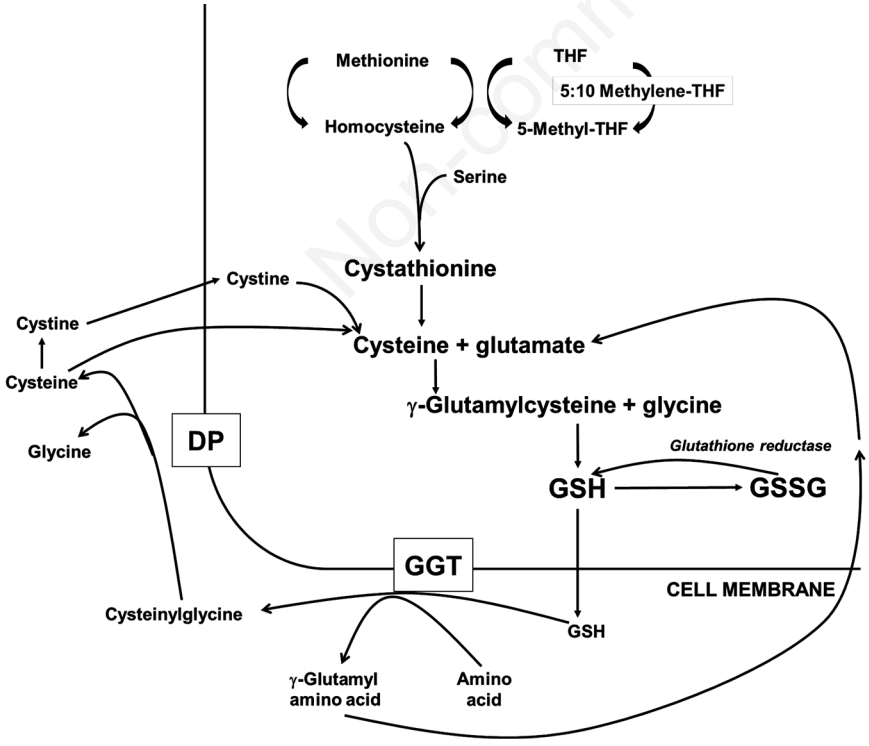

Figure 2. Glutathione network. DP, dipeptidases; GGT, γ-glutamyl transpeptidase; GSH, glutathione; GSSG, glutathione disulfide.

Clinical aspects

Depletion of glutathione and clinical implications

Low levels of glutathione and/or transferase activity are associated with chronic

exposure to chemical toxins, alcohol and cadmium exposure. Based on the pivotal role of GSH in many vital functions it follows that depletion of GSH is implicated in multiple conditions and chronic degenerative diseases, including neurodegenerative disorders as either Alzheimer or Parkinson (Table 1).[1,4,6,11]

However, given the many roles played by GSH, it is difficult to ascribe causal relationships between changes in GSH levels or redox state and development of specific diseases.[1,4,29]

While oxidative injury plays a dominant role in GSH depletion in many of these disorders, some are related to reduced expression of GSH synthetic enzymes.[30] GCL polymorphisms have been reported in many disorders, including schizophrenia, cardiovascular diseases, stroke, and asthma.[31,32] In the most severe cases, polymorphisms with significantly reduced GCL expression and activity can lead to severe phenotypes including hemolytic anemia, aminoaciduria and spinocerebellar degeneration.[5,31]

Outside of polymorphism, decreased GSH synthesis occurs in fibrotic diseases (including cystic fibrosis and pulmonary fibrosis), several hepatic disorders such as cholestatic and alcoholic liver injury, diabetes mellitus and endotoxemia.[30,33] GSH synthesis and metabolism are altered during hyperglycemia and diabetes. Lower levels of GSH in the erythrocytes of diabetic patients are concomitant to an increased susceptibility to oxidative stress of these.[34] Since liver clears gut-derived lipopolysaccharide (LPS, synonymous as endotoxin), a major constituent of the outer cell wall of all Gram-negative bacteria that can trigger the synthesis and release of pro-inflammatory cytokines and inducible nitric oxide synthase (iNOS), it explains why endotoxemia occurs in cirrhotic patients.[35] and It also participates in worsening of alcoholic liver disease and non-alcoholic steatohepatitis.[35,36]

Endotoxemia lowers GSH levels in the liver[37,38] peritoneal macrophages and lymphocytes.[39] Thus, Septic patients have lower blood GSH:GSSG ratios.[3] GSH level is an important variable that determines susceptibility to LPS-induced injury in multiple tissues.[40] This may be related to GSH's ability to influence toll like receptor 4 (TLR4) signaling. Specifically, LPS-induced mortality and TNFα secretion were higher when GSH level was reduced.[41]

Mounting attention is being given to the role thiol status plays during the onset and progression of inflammatory and autoimmune states. In smokers glutathione depletion accelerates cigarette smoke-induced inflammation and airspace enlargement, since a reduced GSH adaptive response leads to an increase in pro-inflammatory cytokines present in the lung.[29] Increased levels of these pro-inflammatory cytokines such as IL-1, TNF-α, IL-6, decrease intracellular GSH and the mechanism may be in response to increased levels of free radicals.[42] Elevated levels of IL-1 may also contribute to the depletion of intracellular GSH facilitating the depletion of intracellular cysteine.

In general, high oxidant burdens are a common feature in many immune dysfunctions and GSH plays a major role in quenching these oxidant species, and hence protecting the cell from damage.

GSH has a significant impact on the immune system's ability to activate the appropriate Th response, altering its levels may have significant implications in Th1/Th2-related diseases.

Therefore, any decrease in GSH may influence the immune system through perpetuating reactive species signaling events and increasing ROS-related damage. Interestingly, low GSH levels are associated with many autoimmune, and inflammatory diseases, including rheumatoid arthritis,[43] systemic lupus erythematous,[44] Crohn's disease,[45] multiple sclerosis,[46] psoriasis[47] and contact dermatitis.[48]

In addition, there is evidence that GSH deficiency enhances the signal transduction pathways associated with HIV expression. Decreased GSH levels are known to activate NFκB, which has been shown to bind and activate genes controlled by the HIV long terminal repeat and thus may affect viral replication. In summary, GSH works to modulate the behavior of the cells of the immune system, augmenting the innate and the adaptive immunity as well as conferring protection against microbial, viral and parasitic infections.[3,42]

Not surprisingly, there have been interesting connections between GSH levels and cell signaling pathways involved in immune responses.

With the aging process there is a gradual lowering of GSH levels and of general antioxidant defenses, as well as a decline in the ability of these systems to be induced by exogenous stimuli,[49,50] and this decline is associated with a higher incidence of age-related chronic illnesses.[3,51]

Oxidative stress is also thought to be involved in the pathogenesis of osteoporosis.[52,53] Glutathione peroxidase and superoxide dismutase activity is significantly lower in osteoporotic women, suggesting that compromised antioxidant defenses play an important role in the development of osteoporosis.

Glutathione depletion and aging

As previously described the association between low levels of glutathione and specific chronic conditions is described in various studies. From a general point of view, geriatric syndromes, such as frailty, sarcopenia and multimorbidity, are characterized by physical vulnerability to stress and a lack of physiological reserve.[2] Given the multiple biological mechanisms of glutathione (antioxidant activity, detoxification of endogenous compounds and xenobiotics; modulation of immune function, DNA synthesis, repair, and expression) it is hypothesized that glutathione plays a role in the aging process itself.[54-56]

GSH depletion is also manifested by

Table 1. Clinical conditions and diseases associated with a reduction in glutathione levels.[4]

• Aging and related disorders
• Brain disorders (autism, Alzheimer's disease, Parkinson's disease, bipolar disorders and schizophrenia, amyotrophic lateral sclerosis, Huntington's disease, multiple sclerosis)
• Cancer
• Chronic liver disease
• Cystic fibrosis
• Diabetes, especially uncontrolled diabetes
• Human immunodeficiency virus (HIV)/acquired immune deficiency syndrome (AIDS)
• Infertility in both men and women
• Blood hypertension

progressive loss of mitochondrial function due to the accumulation of damage to mtDNA and GSH status has been found in parallel with telomerase activity, an important indicator of lifespan.[54,55]

The best indicator of the importance of glutathione is that its cellular and mitochondrial levels directly are highly associated with health and longevity and the ability of animal species to protect their mtDNA is directly proportional to longevity.[1,49]

GSH also plays a central role in cell death, including apoptotic cell death.[18]

GSH depletion has been strongly associated with the progressive loss of function with aging. Glutathione depletion triggers apoptosis, although it is unclear whether it is mitochondrial or cytosol pools of GSH that are the determining factor.[18]

The opposite situation, namely increased GSH synthesis, plays an important role in conferring drug and/or radiation resistance to many different cancers.[3,30]

A representative study of community-dwelling elderly found that higher glutathione levels were associated with higher levels of physical health, fewer illnesses, and higher levels of self-rated health.[3]

A recent study explored the hypothesis that glutathione plays a role in the development of multimorbidity (*i.e.*, the coexistence of multiple chronic conditions in a single individual.[57] Multimorbidity is a clinical condition linked to aging; it affects more than 80% of people aged 65 years or older and leads to functional and cognitive decline, avoidable hospitalizations, decreased quality of life, and increased mortality.[58] In the study serum levels of GSH were inversely associated with multimorbidity development, independently from the link with specific chronic conditions, supporting the hypothesis that GSH is a biomarker of multisystem dysregulation. A study showed that higher circulating levels of inflammatory markers are cross-sectionally associated with multimorbidity and predict steeper rates of disease accumulation over time.[59]

Sarcopenia (the syndrome of progressive and generalized loss of muscle mass and strength) and frailty (a clinically recognizable state of reduced homeostatic reserves and higher vulnerability) are two major geriatric syndromes closely related to the aging process.[60] The development of one or both of them is linked to progressive functional disability, loss of quality of life and death.[61] Their prevalence in elderly populations approximates 10% and 15%, respectively; however, in the presence of chronic conditions and multimorbidity, this prevalence can raise to 20% and 60%, respectively.[62] Sarcopenia and frailty may be considered as part of the same phenomena: sarcopenia is a frequent cause of physical frailty, and the two syndromes share clinical manifestations, the role of nutrition and physical activity, and pathophysiological pathways and biological mechanisms.[61] In particular, both syndromes have been associated to chronic inflammation and oxidative stress.[63]

The relation between frailty and sarcopenia is particularly close in patients with multimorbidity, in which aging and chronic conditions may trigger more oxidative stress, telomere shortening, and apoptosis.[62]

The observation of the association between low GSH levels, common features of aging and a wide range of pathological conditions, have made the use of glutathione as an anti-aging drug very popular, although large clinical studies are not available.[64]

Glutathione and neurodegenerative diseases

GSH imbalance or depletion has been reported to be involved in many brain disorders such as autism, Alzheimer's disease (AD), Parkinson's disease (PD), bipolar disorder, schizophrenia, amyotrophic lateral sclerosis, Huntington's disease, and multiple sclerosis[65] (Table 1).

GSH detoxifying role in the brain is critically important because neurons are at extremely high risk of excessive ROS generation and oxidative damage since they show high oxygen consumption and energy production, as it utilizes 20% of the O_2 consumed by the body whereas constitutes only 2% of body weight.[66] The vulnerability of neurons to the oxidative damage stems also from a relatively large amount of redox active metals, promoting ROS formation, high content of polyunsaturated fatty acids (PUFA), which are more sensitive to oxidation, and from comparatively modest levels of antioxidant enzymes.[67]

The impairment of physiological glutathione's levels and the alterations in the activities of its related enzymes in neuronal cells are increasingly suggested to be implicated in the initiation and progression of neurodegenerative diseases.[68]

GSH redox imbalance has been observed during the onset and progression of AD and decreased GSH levels have been reported in blood and brain samples of AD subjects.[69]

The development of AD pathology (in particular the accumulation and abnormal aggregation of amyloid β-peptide) is linked to oxidative stress in the brain.[68]

Neuropathological studies showed decreased levels of GSH in AD brain in comparison to health control, in particular in cingulate cortex and in prefrontal cortex, and similar data were obtained also in post-mortem autopsy brain of MCI patients.[70]

Cellular depletion of GSH has been linked to cognitive decline and memory deficits in AD and in MCI.

Compelling evidence presenting disrupted oxidant/antioxidant balance in AD led to the formulation of the hypothesis that compounds scavenging free radicals, and/or boosting oxidative stress defense mechanisms might provide therapeutic benefits in AD. Therefore, several antioxidants, such as N-acetylcysteine, curcumin, resveratrol, vitamin E, ferulic acid, coenzyme Q, selenium, and melatonin, have been tested for their potential to preserve or improve cognitive performance in healthy individuals, MCI, and AD.[71,72] A recent preclinical study demonstrates that the administration of a precursor of GSH in an animal model of AD (APP/PS1 mice) lead to a reduction of the levels of brain lipid peroxidation, protein carbonyls and apoptosis, an increase both total GSH and the glutathione/glutathione disulphide (GSH/GSSG) ratio, with lowering brain Aβ load and an improvement of acetylcholinesterase activity, and spatial memory was also improved.[72] These data suggest that GSH supplementation could represent a strategy for the treatment or prevention of cognitive impairment and neurodegeneration.

PD is characterized by intracellular accumulation of misfolded a-synuclein (Lewy bodies) and loss of dopaminergic neurons in the midbrain substantia nigra pars compacta. Several studies show that brains from PD subjects present low levels of endogenous antioxidants, such as GSH and coenzyme Q10, increased oxidation of dopamine, and high levels of iron, suggesting that oxidative stress plays an important crucial role in the pathology of the disease.[73]

The production of oxygen-reactive species damages the substantia nigra through lipid peroxidation, protein oxidation, and DNA oxidation and a loss of nigral GSH is a characteristic change occurring in the earliest stages if PD.[74] These changes cause the formation of endotoxins in pigmented SN cells that contribute to the degeneration of these neurons observed in PD. GSH depletion is also associated with the presence of Lewy bodies in SN, a characteristic hallmark of PD.

Numerous studies have suggested the potential beneficial effects of antioxidant supplementation in the treatment of PD and so far several approaches have been attempted, from traditional antioxidant approaches, such as vitamin E, C, and β-carotene supplementation, to more innovative and bold approaches, like the use of nanoparticles to deliver antioxidant mole-

cules.[74] Moreover, a recent review showed that GSH supplementation may improve motor scores in PD.[75]

Glutathione and infectious disease: possible role in COVID-19 treatment

Antiviral activity of glutathione is well known; changes in redox homeostasis, including intracellular GSH depletion, are one of the key events that favor virus replication and contribute to the pathogenesis of virus-induced disease.[76] Redox homeostasis has an important role in maintaining an appropriate Th1/Th2 balance, which is necessary to mount an effective immune response against viral infection and to avoid excessive inflammatory responses.[77]

The antiviral activity of pro-GSH molecules was demonstrated against different viruses, such HIV, influenza, Herpes-virus type 1, tuberculosis.[76]

Based on these data, the possible use of GSH as an adjunct to current treatment modalities in COVID-19 patients has been hypothesized.[78] Morbidity and mortality of SARS-CoV-2 infection are due in large part to severe cytokine storm and hypercoagulable state brought on by dysregulated host-inflammatory immune response, ultimately leading to multi-organ failure. Exacerbated oxidative stress caused by increased levels of interleukin (IL)-6 and tumor necrosis factor (TNF-) along with decrease levels of interferon and interferon) are mainly believed to drive the disease process. It has been hypothesized that endogenous glutathione deficiency may contribute to the pathogenesis of severe COVID-19 disease.[79]

Therefore, restoration of glutathione levels in COVID-19 patients would be a promising approach for the management of the novel coronavirus SARS-CoV-2, although clinical data in this regard are preliminary and limited.[80]

Strategies for increasing glutathione cellular levels

Considering how important glutathione is to health many researchers have looked for ways to increase intracellular and intramitochondrial levels.

Dietary Protein or omega-3 fatty acids supplementation are the most popular among the different strategies to improve glutathione levels. Basically, since the precursors of glutathione are amino acids, intake of dietary protein may influence the amino acid pool from which to draw to synthesize glutathione. One potential beneficial source might be whey protein, likely due to its higher cysteine content. The daily supplementation with whey protein may lead to an increase in lymphocyte glutathione levels. Moreover, even serine may be helpful in positively influencing glutathione production, potentially through increased cysteine availability or by increasing glutathione levels through its metabolism into glycine.[1,4,81-84]

Chronic inflammation can contribute to oxidative stress and deplete glutathione supply. Therefore, omega-3 fatty acids, due to their involvement in inflammation may lead to positive effects on glutathione levels. Promising results have been observed after a 3 month treatment in older patients at high risk of developing depression, in patients with Parkinson's disease and in women with genetic polymorphisms for reduced GST activity.[1,4,85-87]

However, a recent meta-analysis of the effect of the co-supplementation of omega-3 fatty acids with vitamin E on the oxidative stress, failed to demonstrate a significant change in glutathione concentrations in the treatment group compared to another study.[88]

For many years one of the most used strategies has been based on providing specific nutrients to promote glutathione production. As noted above, cysteine availability is the rate-limiting step in the de novo production of glutathione. While oral cysteine does not make it through the digestive track, supplemental cysteine in the form of whey or *N*-acetylcysteine (NAC) may be effective at raising levels.

While there is substantial variation, 1000 mg/d of NAC will substantially increase glutathione in virtually all patients.

Yet a review of the data indicates its use may be inconclusive or equivocal. A systematic review that included twelve clinical trials utilizing NAC supplementation with a specific focus on cognitive markers indicated that there may be some benefit to using NAC in certain populations; however, the studies were too variable in design and outcome to make any definitive conclusion.[4,89,90] Although NAC is promising as a supplement to both boost glutathione levels and potentially mitigate some of the issues related to oxidative stress, the research is not conclusive, and some of the findings are disease specific. There have also been studies with no significant impact by taking NAC.[91-96]

In addition, it is important to note that NAC has antioxidant properties besides to being able to provide cysteine for glutathione synthesis. Thus, it is unclear if the effects of NAC on oxidative stress are due to these antioxidant properties or due to increased glutathione synthesis.

For the rare patient who reacts to NAC, S-adenosyl-methionine (SAMe) can be used.[1,4]

On the other hand, the obvious strategy to increase glutathione levels is to directly administer it.

This can be done orally, topically, intravenously, intranasally, or in nebulized form. Glutathione administered intravenously, inhaled, and ingested intranasally increases systemic levels. IV glutathione has a short half-life but has shown at least short-term efficacy in several diseases. Limited data using intravenous glutathione has been documented in patients with Parkinson's disease. Sechi *et al*. administered glutathione intravenously (600 mg twice daily for 30 days) to subjects with Parkinson's disease, and reported significant improvements, which lasted for 2-4 months even after ceasing the therapy.[97] In a case report a 61 year old man with Parkinson's disease treated with a complex protocol consisting of a gluten-free diet and dietary supplements (such as N-acetylcysteine and Silybum), and glutathione injections (1400 mg) administered twice, or three times weekly also reported symptom improvement.[98]

Finally, preliminary cellular research using glutathione monoesters has been shown to be an effective delivery agent of glutathione due to improved bioavailability.[99]

Oral administration is controversial; while most research shows that oral glutathione does not increase RBC glutathione, there are a few studies that show efficacy. However, unmodified oral glutathione is unlikely to consistently elevate cellular levels since it would be degraded by digestive peptidases.[4] Some studies have shown no change in glutathione levels or in parameters of oxidative stress despite acute or chronic oral supplementation. Nevertheless, there is also some evidence to the contrary. For example, a randomized, double-blinded, placebo-controlled trial found that taking oral glutathione at either 250 or 1000 mg/day led to significant increases in the body stores of glutathione in non-smoking adults in a dose-dependent manner.[100] There was also a decrease in the markers for oxidative stress at six months as indicated through an improvement in the oxidized (GSSG) to reduced (GSH) glutathione ratio in whole blood, in conjunction with favorable increases in natural killer cell cytotoxicity.

While the data on providing oral glutathione are mixed and inconclusive, recent research suggests that when glutathione is administered in liposomal or sublingual forms it may be made more bioavailable and favorably impact systemic glutathione levels.[101,102] Even the transdermal administration of liposomal glutathione show promise, but research is early.[101]

There continues to be debate as to the best delivery system, whether oral, sublin-

gual, liposomal, or intravenous. Intravenous, liposomal, and sublingual delivery can bypass the breakdown that may occur in digestion, and thus may be superior to oral supplementation. Since GSH is very well absorbed through mucosa, the sublingual delivery may represent a safe and reliable way to administer glutathione. It is also well-known that sublingual route allows to by-pass the effect of hepatic first-pass metabolism.[102] A novel food-grade sublingual dosage form of GSH has been designed and tested, comparing the bioavailability, the effect on oxidative stress markers and the safety with NAC and oral GSH in a three-week randomized crossover trial in volunteers with metabolic syndrome.[103] The sublingual form proved to be superior to both the oral GSH form and NAC in terms of GSH supplementation.[103]

Recently an optimized orobuccal fast-slow release formulation tablet containing pure stabilized GSH, capable of immediately dissolving and releasing the active ingredient upon contact with the oral mucosa, has been developed in combination with vitamin C, L-cystin and selenium (GLU-9599, Named Research Co., Lesmo, Italy). The formulation has been tested *in vitro* and *in vivo* in healthy volunteers, demonstrating a high, fast and time-dependent absorption through oral mucosa, raising GSH blood concentration.[104]

This formulation has been successfully evaluated in an experimental rat model of liver injury compared to silymarin extract and YHK (an herbal Japanese compound with putative hepatoprotective effects), with both histological and transmission electric microscopy (TEM) confirmation.[105]

Finally, Makida and co-workers tested GLU-9599 in 75 poor detoxifier subjects (GSTM-1null) regarding their oxidative stress, ammonia metabolism and heavy metal clearance, confirming an efficient restore of antioxidant defenses and hypothesized in a potential wider application of the combination as an integrative adjuvant co-treatment in patients environmentally exposed and also under pharmacological burden.[106]

Conclusions

This review deals with the pharmacological aspects and clinical data that support the importance of glutathione, the most important antioxidant substance present in the human body, for the control of numerous chronic diseases and its correlation with the mechanisms that lead to a successful aging. Many studies have focused on ways to increase intracellular and intra-mitochondrial levels of glutathione, either through changes in dietary habits or through the use of specific supplements. There is data indicating how antioxidant supplementation, and in particular the use of glutathione, may be beneficial in different chronic diseases and may positively alter the aging mechanisms. The use of substances with antioxidant activity cannot be considered the 'panacea' for every disease and the cure for every chronic condition, but an integrated approach is essential to face the problems related to aging, where lifestyles, nutrition, adequate physical and mental exercise is associated with timely, careful treatment of diseases. From this point of view, it is essential to pay attention to the nutritional principles which today have a consolidated protective action.

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