Review

Glutathione deficiency in human disease

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Glutathione has multiple metabolic actions that are essential for cellular homeostasis. In spite of this important role in cellular physiology, disease states due to glutathione deficiency are not common. The participation of both tissue and circulating glutathione deficiency in disease pathogenesis is likely to be subtle and not easily defined. Despite these difficulties, a number of inherited conditions of glutathione deficiency are known, and acquired ones are being identified. Examples of the acquired deficiency state include idiopathic pulmonary fibrosis, human immunodeficiency virus-related disease, and respiratory distress syndrome. Much of our current understanding of the utility of glutathione supplementation in states of tissue injury is derived from biochemical, animal, and cell culture studies. In this article we will review what is known about glutathione deficiency states and explore strategies by which tissue glutathione levels might be maintained or increased to prevent tissue injury and disease. (J. Nutr. Biochem. 5:218–226, 1994.)

Keywords: glutathione synthesis; glutathione redox cycle; glutathione supplementation; oxidants; glutathione deficiency states

Introduction

Glutathione (GSH) is a simple tripeptide that is present in all eukaryotic cells. It plays an important role in cellular metabolism and protects cells against free-radical induced oxidant injury. Through the action of the GSH-transferase system, GSH inactivates drugs and toxic compounds. It is this ability of GSH to detoxify compounds that underlies the usefulness of N-acetylcysteine in paracetamol overdose. Other functions of GSH include the reduction of disulfide linkages in proteins and the synthesis of precursors of DNA.

An enormous amount of data has been obtained from cell culture systems on the cellular biochemistry of GSH. Recently, we have begun to understand the importance of GSH in the preservation of tissue integrity. The pathways involved in the synthesis, transport, and metabolism of GSH in animals and humans are now more clearly defined. The role that tissue levels of GSH play in the pathogenesis of disease is being explored, and important observations have been made in diseases that appear to be associated with at least localized GSH deficiency, such as pulmonary fibrosis and HIV-related disease. Hereditary conditions associated with deficiency of GSH are rare, but related syndromes have been described and have helped to provide a better understanding of some of the clinical manifestations of GSH deficiency.

The awareness of possible GSH deficiency states and the potential role of GSH supplements in human immunodeficiency virus (HIV)-related disease have provided impetus for establishing strategies for insuring the availability of GSH in vivo.

Cellular regulation of GSH

Synthesis of GSH

The biochemical pathways of the synthesis of intracellular GSH have been extensively reviewed[1–3] and are summarized as follows *(Figure 1)*:

The GSH molecule is synthesized within the cytosol from the amino acids glutamate, cysteine, and glycine by a two-step enzymatic process utilizing ATP as the energy source. Two enzymes are central to the GSH synthetic process. First, γ-glutamyl-cysteine synthetase catalyzes the production of the dipeptide γ-glutamyl-cysteine from cysteine and glutamate. This initial step is rate limiting for GSH synthesis and is inhibited by GSH. A second enzyme, GSH synthetase, completes the synthetic process by adding glycine to the γ-glutamyl-cysteine dipeptide.

Amino acid precursors for GSH are transported into the cell either directly via a series of membrane bound transport proteins or indirectly via a reaction catalyzed by γ-glutamyl transpeptidase, an enzyme present on the cell membrane. Adequate pools of precursor amino acids, particularly cyste-

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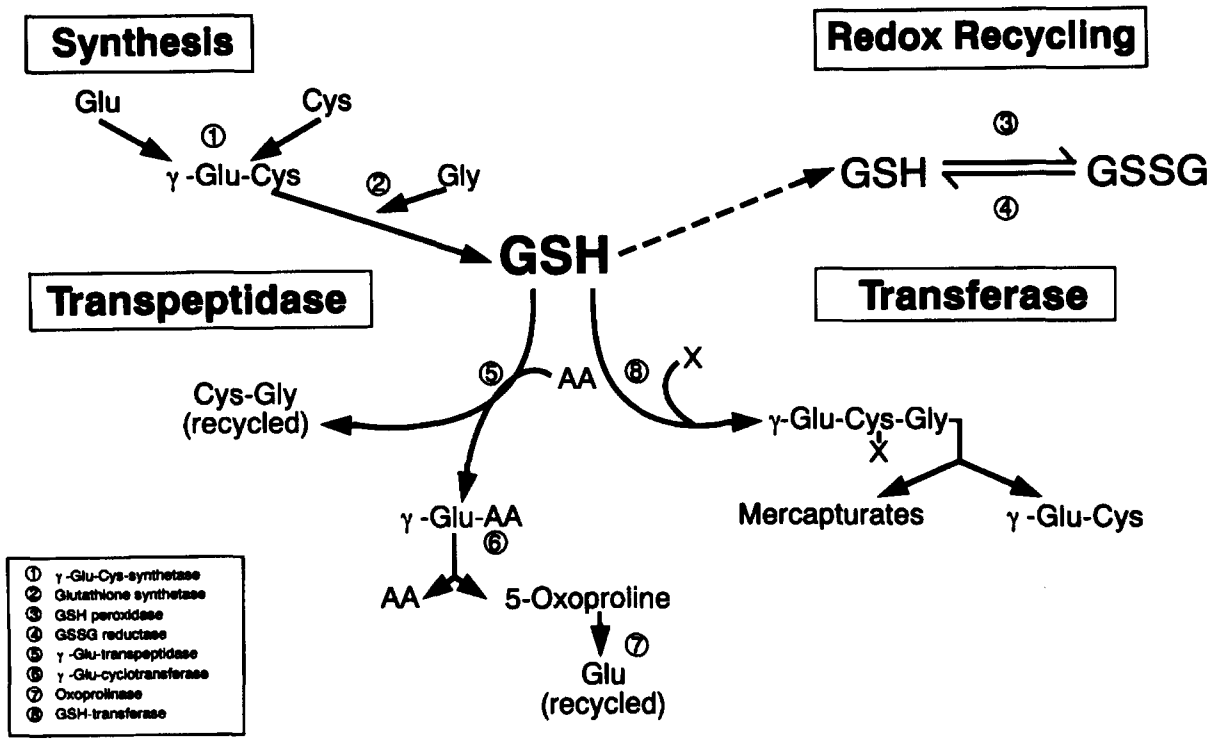

Figure 1 Diagram depicting a summary of glutathione biochemistry (see text for details).

ine, are necessary for GSH synthesis. The cell membrane transport systems for GSH precursor amino acids have been well described for fibroblasts[4] and endothelial cells.[5,6] Nomenclature has been developed for these transport systems based on their amino acid specificity and sodium dependence. Glutamate is transported by two proteins denoted "x_c-" (sodium independent) and "X_{AG}" (sodium dependent); cysteine is transported by the "ASC" protein (sodium dependent); and cystine (the oxidized form of cysteine) shares the "x_c-" system with glutamate. Cystine is reduced to cysteine within the cytoplasm and then used for GSH synthesis.

Precursor amino acids can also be transported into the cell indirectly via the γ-glutamyl transpeptidase reaction, which transfers a γ-glutamyl moiety to another amino acid. This dipeptide complex is then moved intracellularly, and its amino acid components are made available for GSH synthesis. In this way, intact GSH serves as a substrate for γ-glutamyl transpeptidase and its component amino acids are transported intracellularly and used for GSH synthesis. The presence of the γ-glutamyl transpeptidase enzyme on cells lining the intestinal mucosa and in the liver may result in the hydrolysis of orally administered GSH. Some evidence suggests that systemic availability of dietary GSH is suboptimal due to the activity of this enzyme.[7]

Ninety percent of the GSH that is synthesized within the cell is stored in the cytosol with a small amount (<10%) stored in the mitochondria. Mitochondria are unable to synthesize GSH. The small amount of GSH contained in mitochondria appears essential for mitochondrial function, and studies of hepatocytes have demonstrated that GSH moves from the cytoplasm into the mitochondria.[8,9] Under conditions of oxidant stress cytoplasmic GSH is rapidly depleted, whereas the mitochondrial GSH appears to be relatively spared, at least in the short term.[8]

Redox cycling of glutathione

The glutathione molecule exists either in the reduced (GSH) or the oxidized (GSSG) form. GSSG is produced from GSH by the activity of the enzyme glutathione peroxidase (GSHPX), which degrades hydrogen peroxides and lipid hydroperoxides, both of which are capable of oxidant injury. The activity of GSHPX is dependent on adequate cellular GSH and selenium. The cell utilizes a series of enzymes to maintain the cellular level of glutathione predominantly (> 90%) in the reduced state *(Figure 1)*. The enzyme GSSG-reductase reduces GSSG to GSH using NADPH as the reducing agent. NADPH is regenerated from NADP by the pentose-phosphate shunt pathway with the enzyme glucose-6-phosphate dehydrogenase (G6PD) being the first enzyme in this pathway. Exposure of the cell to an "oxidant stress" such as hyperoxia may overwhelm this system, thereby causing GSSG to accumulate. Because GSSG diffuses more easily than GSH from the cell, its formation and subsequent efflux leads to depletion of the intracellular pool of glutathione. GSSG can also react with cellular proteins via mixed disulfide reactions resulting in impaired protein function[10] and depletion of cellular GSH.

Depletion of cellular glutathione by complex formation

The cellular stores of glutathione can also be depleted through the glutathione transferase reaction in which a gluta-

thione molecule is conjugated to foreign compounds *(Figure 1)*. This GSH complex is then excreted, resulting in a loss of cellular GSH. This system is active in hepatocytes, an important source of plasma GSH,[11] and there is evidence that this system is important in the gastrointestinal mucosa as a means to detoxify compounds present in the bowel lumen.[12] It is of interest that areas of the bowel that are more susceptible to malignancy, such as the colon and rectum, also have low levels of glutathione in the mucosal lining cells.[13,14]

Modulation of cellular levels of GSH

Most of the available data concerning the modulation of GSH has been obtained from in vitro studies. Although the precise relevance of these findings to the more complex in vivo situation is unclear, the data provide some guidelines for understanding in vivo events. Regulation of cellular levels of GSH can be broadly divided into three areas: (1) the uptake of precursor amino acids and intact GSH; (2) the regulation of the enzymes necessary for GSH synthesis; and (3) alterations in the cellular redox system.

In cells in culture the uptake of cystine is rate-limiting for glutathione synthesis. Cysteine present in medium is rapidly oxidized to cystine. Under certain conditions cells are able to increase cystine uptake (which is reduced to cysteine within the cell) and thereby increase cellular GSH. It has been reported that a 0.5 to 2.0 mM concentration of cysteine in medium containing cystine will increase intracellular glutathione above control values.[15] Diethylmaleate (DEM), an electrophilic agent that binds GSH at higher concentrations, has been shown at lower concentrations (i.e., approximately 0.25 mM) to increase uptake of cystine and glutamate in a variety of cell types, including fibroblasts and endothelial cells.[16,17] The mechanism of this effect is not currently known. Exposure of endothelial cells to hyperoxia also increases uptake of cystine and glutamate. Current data suggest that lowered cellular levels of GSH stimulate amino acid uptake through another secondary mechanism, possibly via the production of reactive oxygen species.[18] The level of cysteine available to the cell for uptake and subsequent use for GSH synthesis can also be increased in the presence of reducing agents in medium such as N-acetyl cysteine (NAC).[15] In the presence of NAC, extracellular cystine is reduced to cysteine, and cysteine transport is more efficient than that of cystine. These data suggest that under certain conditions cells can regulate cellular GSH levels by altering the uptake of precursor amino acids, presumably at the level of the membrane-bound transport protein. The uptake of glycine does not appear to play a major role in GSH homeostasis, at least in cell culture systems.

Certain cell types (in particular kidney and liver) also have γ-glutamyl transpeptidase activity and use circulating GSH as a source of precursor amino acids. These amino acids are transported across the cell membrane and utilized for intracellular GSH synthesis.[12,19]

As noted above, γ-glutamyl-cysteine synthetase is an important regulating enzyme in the synthesis of GSH. High levels of GSH block further synthesis of GSH by inhibiting the activity of this enzyme. Glutamate is able to bind to the regulatory site, blocking the inhibitory effect of GSH and thus allowing GSH levels to rise to supernormal levels.[20] Buthionine sulfoximine (BSO) is a relatively specific and irreversible inhibitor of γ-glutamyl cysteine synthetase[19] and can be used to deplete cellular GSH. For example, 0.05 mM BSO can deplete endothelial cell (EC) GSH to 10% of control levels in 24 hours without affecting cell growth.[21] Thus, the activity of γ-glutamyl-cysteine synthetase is essential for the cell to maintain cellular levels of GSH.

In addition, alterations in substrates and enzymes that participate in the redox cycle may influence levels of GSH. For example, selenium-dependent glutathione peroxidase activity is reduced in states of selenium deficiency (see the section on "Acquired GSH deficiency").

Interorgan distribution

All eukaryotic cells are capable of synthesizing glutathione. The liver is the major site of GSH synthesis in humans and animals, and, hence, the major source of plasma GSH.[6] This location of GSH synthesis reflects the important role of the liver in the metabolism and excretion of ingested toxic compounds. Through the GSH-transferase systems, GSH plays a role in the excretion of certain compounds. Intact GSH, once synthesized by hepatic cells, is either translocated to plasma or excreted in the bile.[22] Studies in rats estimate the normal rate of synthesis of GSH in the liver to be 12.4 ± 1.4 nmol/min/g of liver. This rate can be increased significantly during fasting to 26.4 ± 1.2 nmol/min/g,[22] but the physiological implications of these changes are not known. GSH efflux from the hepatocyte into the biliary system appears to occur through a carrier-mediated transport system and is inhibited by bilirubin. The transfer of GSH from the cytosol to the plasma appears to be mediated by hormones that may act by altering membrane potential.[23] Hormonally induced efflux of GSH may explain the reduction in liver GSH that has been observed in states of shock.[24]

The bulk of plasma glutathione (85%) is present in the reduced form with the remainder being oxidized (15%). Because reduced glutathione is rapidly converted to the oxidized form, for accurate GSH/GSSG measurements plasma determinations need to be carried out as rapidly as possible after obtaining samples.[25] Analysis of glutathione levels in plasma drawn from different sites has provided some insight into the interorgan transport of GSH in the rat model.[26] The highest level of glutathione is found in the hepatic vein reflecting the major role that the liver plays in glutathione synthesis. Levels in the aorta and inferior vena cava are significantly less than in the hepatic vein, and the lowest venous levels are found in the renal vein. It is unclear how important plasma GSH levels are in maintaining GSH levels in all tissues, but intestinal and lung epithelial cells appear to be able to utilize plasma GSH.

GSH is cleared in the kidney, as evidenced by the low level of GSH in the renal vein. The kidney uses at least one of two systems to clear GSH from the circulation: (1) direct glomerular filtration and (2) a non-filtration mechanism utilizing the γ-glutamyl transpeptidation reaction.[4] A case of a patient with severe γ-glutamyl transpeptidase deficiency has been reported, and lack of the enzyme results in significant glutathionemia and glutathionuria.[27] These data, along with animal studies in which γ-glutamyl transpeptidase was

inhibited, indicate that GSH is an important substrate of this enzyme, which is a major participant in GSH excretion.

Thus, there is significant interorgan flow of glutathione with hepatic production, systemic distribution, and renal excretion.

GSH deficiency state

GSH deficiency states may be inherited or acquired *(Table 1)*. The hereditary causes that have been reported are rare and due to a deficiency in one of the enzymes required for GSH synthesis or maintenance of GSH in the reduced form *(Figure 1)*. Acquired forms of deficiency that occur in association with certain diseases have only recently been described.

Hereditary GSH deficiency

Families with γ-glutamyl-cysteine synthetase deficiency have been described.[28,29] Affected family members have extremely low red blood cell GSH concentrations associated with a nonspherocytic hemolytic anemia. The association of this enzymatic defect with spinocerebellar degeneration, present in two members of the first family described,[28] is unclear because it was not found in any of the affected family members in a subsequent report.[29]

GSH synthetase deficiency is an autosomally inherited trait that produces a mild hemolytic disorder in homozygotes.[30] Some patients with this disease accumulate 5-oxoproline (a metabolite of γ-glutamyl-cysteine) *(Figure 1)*, leading to a life-threatening metabolic acidosis[31] with neurologic defects, including mental retardation, spastic tetraparesis, and ataxia. Although controversial, it has been suggested that this metabolic abnormality is linked to the lowered activity of GSH synthetase in the γ-glutamyl cycle.[32]

GSH reductase deficiency has been reported in a family in which all three children from a consanguineous marriage were found to have a virtual complete absence of erythrocyte GSH reductase activity.[33] The red blood cell GSH concentrations were normal in each case, suggesting that GSH reductase activity is not a major determinant of cellular GSH levels in the absence of oxidative stress, or that an extremely low resting level of enzyme activity is sufficient for maintaining cellular GSH levels under normal conditions. Clinically, two of the children developed cataracts (see the section on "Acquired deficiency"), and one had to be treated for an acute hemolytic crisis thought to be related to ingestion of fava beans.

The enzyme, glucose 6-phosphate dehydrogenase (G6PD), is not directly involved in GSH synthesis, but it catalyses the first step in the hexose monophosphate shunt generating reducing equivalents in the form of NADPH. NADPH is utilized by GSH reductase to reduce GSSG to GSH *(Figure 1)*. Some patients with G6PD deficiency are susceptible to a rapid fall in red blood cell GSH when exposed to oxidant stress resulting in an acute hemolytic crisis. More than 300 variants of G6PD deficiency, regarded as being biochemically distinct, have been reported and disease severity also tends to be highly variable.[34] Most forms of G6PD deficiency are clinically insignificant unless there is an added oxidant stress. It is interesting that patients with G6PD deficiency appear to be protected from falciparum malaria. It appears that the intracellular parasites are deprived of essential GSH (reduced glutathione needed for optimal growth).[35]

Table 1 GSH deficiency states

Inherited	Acquired
γ-Glutamylcysteine synthetase deficiency	Idiopathic pulmonary fibrosis
Glutathione synthetase deficiency	Adult respiratory distress syndrome
Glutathione reductase deficiency	HIV infection
	Hepatic cirrhosis
	Cataract formation
	Advanced age
	? Parenteral nutrition (selenium)

Acquired GSH deficiency

Acquired GSH deficiency states, as determined by measurements in various tissues and body fluids, have been well documented and may be more common than hereditary deficiency states. It is not known whether reduction in GSH is an effect or cause of the disease process.

GSH is present in the epithelial lining fluid (ELF) of the normal respiratory tract, where it participates in the prevention of parenchymal oxidant injury. In one study, normal levels of GSH in the ELF were 429 ± 34 μM.[36] The source of the GSH is not clear but most likely is derived from alveolar lining cells. Deficiency of GSH in the ELF has been demonstrated in adult respiratory distress syndrome (ARDS)[37] and idiopathic pulmonary fibrosis (IPF),[36] both forms of lung disease in which reactive oxygen species are thought to have a pathogenic role. In one study, a four-fold decrease in the total GSH concentration of alveolar lining fluid was found in patients with IPF as compared with healthy controls.[36] A similar deficiency of alveolar fluid GSH has been reported in patients with sepsis and ARDS.[37] This observation may be of particular importance because the lung in ARDS is exposed to a significant oxidant burden from recruited neutrophils.

Studies in humans with HIV infection and a monkey model of HIV infection indicate that the presence of HIV is associated with depletion of GSH.[38] This observation may be of considerable importance because adequate GSH levels appear to be necessary for both T and B lymphocyte function and immune function in general.[39] In asymptomatic HIV-positive individuals, levels of GSH are low in plasma, peripheral blood mononuclear cells, and epithelial lining fluid of the lung.[40] It is important to note that these individuals were not nutritionally deprived, a common finding in the late stage of HIV-related disease. Elevated glutamate levels in serum have also been reported in this population. Glutamate competitively inhibits cystine transport in immunocompetent cells and may further impair cell-mediated immunity.[41]

Cellular GSH may affect HIV replication on a molecular level. Thiols in general and GSH, in particular, contribute to the redox potential of the cell. It now appears that alterations in the redox potential of a cell can modulate gene transcription, including that of the HIV virus.[42] There have

been many observations made with the use of cell culture models of HIV infection indicating that NAC, GSH, and GSH ester inhibit cytokine-stimulated HIV replication.[43] In a small clinical study, oral NAC has been shown to increase the concentration of cysteine and GSH in mononuclear cells of patients with HIV infection.[44] Trials investigating the clinical usefulness of NAC in HIV-related disease are underway.

The liver is the major source of plasma GSH (see the section on "Interorgan distribution"). Studies performed on patients with cirrhosis have demonstrated reduced plasma levels of GSH and cysteine along with elevated methionine levels in these individuals compared with controls.[45] These data suggest that the hepatic transulfuration pathway (by which methionine is converted to cysteine) is impaired in cirrhosis. It is unclear what effect low plasma levels of GSH have on patients with cirrhosis, but there is evidence that hepatocyte GSH levels are also low, and this may play a role in exacerbating hepatic injury. Studies performed using both a cell culture model of ethanol-induced injury and intact animals have identified a block in the ability of hepatocyte mitochondria to take up cytosolic GSH.[46] Furthermore, ethanol-exposed hepatocytes have increased susceptibility to oxidant injury and can be protected against this injury by preincubation with a GSH ester.[47] Therefore, alcohol may deplete hepatocyte mitochondrial GSH, which may predispose the liver to injury from ingested drugs (e.g., therapeutic levels of paracetamol) and other toxins that would normally be cleared by adequate GSH levels in the normal hepatocyte.

The lens of the eye is known to contain a relatively high concentration of GSH in most species. The incidence of lens opacification (i.e., cataracts) increases with age, at the same time lens GSH concentration falls dramatically. Lens GSH may prevent cataract formation by maintaining protein thiols in the reduced state or by preventing oxidant-related lens injury.[48]

Finally, GSH levels appear to decrease in general with increasing age. Erythrocyte GSH and total free thiol concentrations were found to decrease with increasing age in a rodent model,[49] and GSH deficiency observed in the aging mosquito appears to be due to cysteine deficiency.[50] A similar observation of GSH deficiency has recently been made in healthy human subjects.[51] The deficiency of GSH may partly explain the altered immunity (especially cell mediated immunity) and increased risk of neoplasia seen with increasing age.[52,53]

A previous report from our laboratory demonstrated that rats fed a low protein diet had decreased lung GSH levels and a lower tolerance to hyperoxic stress.[54] It has been suggested that GSH deficiency might also predispose the subject to multisystem organ failure in sepsis or shock. In a recent study, rats rendered GSH-deficient by the intraperitoneal administration of DEM developed a higher incidence of liver, kidney, and gut dysfunction when subjected to hemorrhagic shock than control animals.[55]

Models of GSH depletion have been developed using BSO, an agent that depletes cellular GSH. Experiments performed with BSO have shown that severe GSH deficiency leads to marked mitochondrial damage in a number of tissues including muscle,[56] lung,[57] intestine,[58] and brain.[59] This damage appears to be mediated by endogenous production of hydrogen peroxide and other reactive oxygen species produced during normal aerobic metabolism.[60] The depleted levels of mitochondrial GSH are considered inadequate to prevent injury in this setting.

In summary, both hereditary and acquired forms of GSH deficiency have been described. Whether a localized reduction of GSH reflects generalized tissue reduction of GSH is not known and is in need of evaluation. Also, further work is needed to establish the precise role that the lowered GSH levels play in the pathogenesis of the conditions described.

Nutritional aspects of glutathione

Cysteine is classified as a non-essential amino acid[60] because humans can synthesize it from two other amino acids, methionine (essential) and serine (non-essential). It is estimated that an adult requires 10 mg/kg body weight/day of S-containing amino acids (including methionine and cystine), and this is easily provided by a normal balanced diet.[61] Cysteine is found in plasma in one of three forms: free cysteine (11.2 $\pm$ 0.9 μM), cystine (oxidized cysteine) (79.4 $\pm$ 7.6 μM), and protein-bound cysteine (150.6 $\pm$ 5.3 μM). Total plasma cysteine is the sum of these components.[45,62] The formation of cysteine from methionine in healthy adults on a regular diet is regulated by the enzymes cystathione β-synthase and cystathione cysteine-lyase, the activities of which are dependent on adequate levels of vitamin B-6.[63]

In animal models it has been shown that inadequate levels of vitamin B-6 reduce cysteine production from methionine with variable effects on hepatic GSH levels.[63] This metabolic reaction occurs in the liver and results in degradation of methionine and the biosynthesis of a variety of metabolically active compounds that include cysteine and GSH, in addition to taurine, choline, carnitine, and creatinine.[64] In patients with cirrhosis of the liver this pathway is impaired, and levels of free cysteine (not protein bound) and GSH are depleted by about 30%.[65] In this study, enteral feeding was more effective than total parenteral nutrition (TPN) in increasing the plasma concentrations of cysteine and GSH in both normal individuals and cirrhotics, suggesting that the effect of the first pass of methionine through the liver increases the efficiency of the trans-sulfuration pathway. Thus, nutritionally deprived normal patients may become deficient in cysteine and glutathione if they receive TPN alone as opposed to enteral feeding.

Selenium is a trace element and in the form of selenocysteine is essential for the activity of the enzyme glutathione peroxidase (GSHPX). A selenium deficiency state known as Keshan disease has been described in certain parts of China where soil has been found to be low in selenium.[66] Deficiency of selenium has more recently been described in patients receiving home parenteral nutrition and is manifested by cardiomyopathy and skeletal muscle weakness.[65] GSHPX activity is reduced in plasma and red blood cells in these patients, resulting in impaired metabolism of H_2O_2. Cellular activity of GSHPX can be restored with selenium supplements.[65] Other manifestations of selenium deficiency that have been described include macrocytosis and skin abnormalities.[67] It is unclear if GSHPX deficiency is responsible for the clinical manifestations of selenium deficiency.

Little available data exist on the amount of GSH in the

normal human diet. One study estimated the daily intake of GSH to be 150 mg/day.[68] It is felt that this amount of intake is too low to significantly influence fasting plasma levels of GSH. As a result, there has been considerable work done over the past decade to determine if an increase in oral intake of GSH might elevate circulating levels of GSH. This will be discussed further in the following section. In animal models, most of the increases that have been observed in plasma and tissue GSH with administration of GSH by the oral route have been modest.[68,69]

Strategies for replacement or elevation of GSH

Amino acid precursors and N-acetylcysteine

Cysteine in solution is readily oxidized to cystine, which has a low solubility and easily precipitates out in solution. Cystine can also react with glucose to form D-glucosecysteine.[70] Cysteine administration is associated with significant toxicity, including retinal and hypothalamic necrosis, brain atrophy, and convulsions.[71] In addition, cysteine administration has been associated with a reduction in tissue GSH that is thought to be mediated by hydroxyl radical production generated by the auto-oxidation of cysteine to cystine that occurs in vivo following cysteine administration.[72] For these reasons, cysteine is not used in commercially available TPN solutions, instead methionine is used as a source of cysteine. Excess levels of methionine may be atherogenic, and attempts have been made to find other sources of cysteine. N-acetyl cysteine[73] and 2-oxo-thiazolidine-4-carboxylate (OTC) have been used experimentally in rats as alternative sources of cysteine *(Table 2)*.

OTC, a compound in which the thiol group is masked, is converted to cysteine intracellularly by the action of 5-oxoprolinase. It has been used to increase cysteine levels in rat brain[71] and has been shown to increase hepatic GSH levels in protein-deficient rats.[72,74] The effect of OTC on plasma and lymphocyte thiols has been recently studied in human volunteers.[75] OTC at a dose of 0.15 mmol/kg produced a significant increase in plasma cysteine and lymphocyte cysteine and GSH. Higher doses of OTC (0.45 mmol/kg) were associated with side effects (pruritus, flushing, and drowsiness). These data are preliminary, and more studies will be needed to establish the efficacy and safety of OTC in humans.

N-acetyl cysteine (NAC), a derivative of cysteine, has been used to augment GSH levels both in vitro[15,76] and in vivo. In endothelial cells, NAC appears to increase GSH levels by two mechanisms. At low concentrations (1 mM), NAC may increase cellular GSH by increasing cysteine availability through conversion of cystine to cysteine in the medium. At higher NAC concentrations, the increase in cellular GSH is cystine independent and the mechanism is unclear.[15] Intravenous NAC has been used effectively for many years to prevent the hepatotoxicity associated with paracetamol overdose.[77] The hepatotoxicity of this agent is largely due to the production of N-acetyl-p-benzoquinonimine via the cytochrome P-450 oxidase system. A recent study in an animal model suggests that selenium deficiency further increases paracetamol toxicity. Thus, paracetamol toxicity may be moderated in part through the production of free radicals.[78] NAC appears to prevent hepatic injury by enhancing the hepatic GSH level. It can be given either intravenously (300 mg/kg over 20 hours) or orally (140 mg/kg loading dose, followed by 70 mg/kg every 4 hours for a total of 17 doses).[79] NAC is most effective in preventing toxicity if given within 8 hours of ingestion of paracetamol.[79]

Data obtained from human volunteers demonstrate that NAC (30 mg/kg po) can increase circulating cysteine levels significantly.[80] However, an increase in the GSH level was only seen in the presence of paracetamol and not in normal controls. In contrast, increased levels of GSH in plasma and bronchoalveolar lavage (BAL) fluid have been observed following oral administration of 600 mg NAC.[81] These data suggest that it is possible to transiently increase plasma and BAL GSH levels with NAC under certain conditions.

Table 2 Agents used to increase glutathione

Agent	Toxicity	Route
Cysteine	+ + +	po/iv
L-2-oxothiazolidine-4-carboxylate	−	po/iv
N-acetyl cysteine	−	po/iv/inhaled
Diethylmaleate	+ + +	ip
Glutathione	−	po/iv
Glutathione esters	−/+	po/iv/ip

Glutathione and glutathione esters

Oral GSH can be given safely without side effects. Some of the GSH is metabolized by transpeptidases on cells lining the gastrointestinal mucosa,[82] but a significant amount of oral GSH may also move across cell membranes as an intact molecule.[83] Some cell types may utilize this transport system to augment cellular GSH from that circulating in the plasma. Epithelial cells of the intestine and the kidney have been shown to take up intact GSH.[82] Alveolar type-2 cells (alveolar lining cells) may utilize exogenous GSH directly to prevent paraquat-induced injury.[83] In this way, some epithelial cells appear to be able to supplement endogenously produced pools of cellular GSH with exogenously produced GSH. Intravenous GSH has also been used as a means to augment GSH in various tissue compartments. In one study, GSH administered intravenously at a dose of 600 mg only transiently (< 1 hour) increased venous, lymph, and urine GSH with minimal effects on ELF GSH.[84] In contrast, in the same study 600 mg GSH by aerosol dramatically increased ELF GSH for up to 2 hours, with insignificant changes in other compartments. The increases seen with intravenous GSH appear to be short lived, and this study suggests that aerosolized GSH is a more effective way to increase ELF GSH *(Table 2)*.

The GSH esters (monoethyl, methyl, or isopropyl) have also been used to increase cellular GSH. These compounds are made by esterifying the carboxyl group of the glycine residue of GSH. The resulting ester is more lipophilic than GSH and passes more easily across the membrane lipid bilayer. Studies performed with human lymphoid cells and fibroblasts have demonstrated that the monoethyl ester of GSH is readily transported intracellularly and hydrolyzed to

GSH, leading to significant increases in cellular GSH levels.[85] In these experiments the ester protected human lymphoid cells from the lethal effects of radiation. Many cell types appear to possess the esterase needed to convert the ester to GSH. This method for increasing cellular GSH bypasses the normal synthetic process and is not subject to negative feedback. Thus, it has the potential to increase cellular levels of GSH to "supernormal" levels. Intraperitoneally administered GSH monoethyl ester has been shown to protect mice from the toxicity of carmustine (BCNU), monocrotaline, and cyclophosphamide and, in particular, to prevent histologic damage in liver, lung, and spleen.[86] Similar data are available for the isopropyl ester and demonstrate that significant tissue levels of GSH can be achieved with this compound. Pretreatment of animals with the GSH isopropyl ester protected against paracetamol and alcohol-induced liver injury.[87] In an animal model of acute pancreatitis, pretreatment with the GSH monoethyl ester significantly reduced both biochemical and histological evidence of pancreatitis.[88]

GSH and GSH ester have also been tested intravenously to supplement GSH stores. In a recent study on intact animals (not GSH depleted), GSH monoethyl ester given intravenously increased plasma, liver, kidney, and intestinal mucosa GSH. High levels of the GSH ester were associated with evidence of toxicity, possibly mediated by metabolites of the ester, which is cleaved from the GSH molecule and converted to ethanol.[89] Intravenous GSH did not produce a similar increase in the GSH level in this study. However, there is some evidence that intravenous GSH alone augments hepatic and renal GSH levels, possibly by increasing cysteine availability either from the GSH or via GSH-mediated reduction of cystine.[90]

Conclusion

Cellular GSH homeostasis depends on a complex process of precursor amino acid uptake, synthetic enzymatic activity, and redox recycling of the oxidized tripeptide to its reduced state. A small number of inherited enzymatic deficiencies resulting in altered GSH metabolism have been identified, but these seem to predominantly influence only erythrocyte metabolism on a clinical level. Other deficiencies in GSH associated with acquired disease states have been recently described and are in need of further exploration. Animal models of GSH deficiency should allow a better understanding of the effect of GSH deficiency on organ function. Recently developed strategies for GSH supplementation offer new approaches for protection against oxidant injury.

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