BioLogical NEWS

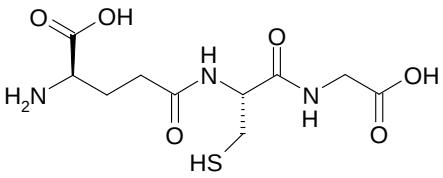
A Biological Therapies Newsletter.

Glutathione – its forms, functions and clinical uses

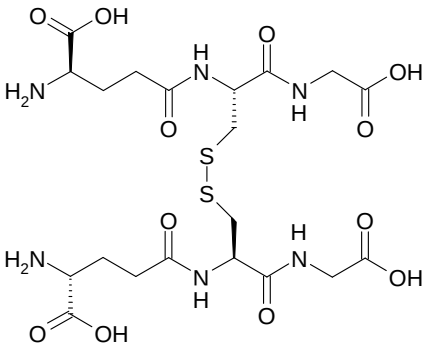
1. Glutathione – What is it?

In 1888 J. de Rey-Pailhade described his discovery of a substance he found widely distributed in nature having the ability to hydrogenate sulphur. He named the substance philothion based on it's reactivity towards sulphur. In 1921 F.G.Hopkins was able to crystallize the substance from yeast extracts and initially characterized it as a dipeptide of glutamic acid and cysteine. Based on this structure Hopkins suggested the name "glutathione" Later work by Hunter and Eagles called the dipeptide structure into question. Hopkins used improved purification techniques to establish the fact that glutathione was in fact a tripeptide containing cysteine, glutamic acid and glycine. In 1929 Pirie and Pinhey deduced the tripeptide sequence based on titration data and this was later confirmed by the synthetic work of Harington and Mead in 1935 [1].

Glutathione contains a thiol (-SH) group which can be readily oxidized and reduced giving glutathione two distinct forms, the "oxidized" and "reduced" form. The "oxidized" form has two glutathione molecules joined together. This can lead to a lot of confusion, so the accepted method of describing these molecules is **GSH** and **GSSG**:



GSH ("reduced glutathione" -the sulphur is reduced)



GSSG ("oxidized glutathione" – the sulphurs are oxidized – really a "di-glutathione")

2. Where is it found in the body?

GSH is made extensively in almost *all* animal cells (including tumor cells). It is suspected that GSH synthesis developed early in the evolution of aerobic eukaryotic organisms, primarily to protect cells against the toxicity of oxygen[2]. Isolated cells that are deprived of GSH typically suffer severe oxidative damage associated with mitochondrial degeneration[3]. It is fairly clear that the highest concentrations of GSH in humans are associated with tissues that undergo maximum oxidative stress, i.e. those tissues exposed to molecular oxygen and its immediate breakdown products, such as red blood cells, brain and lung tissues. GSH is also critical for the liver phase II synthesis of mercapturic acids (one of the phase II drug metabolism pathways), so high levels of GSH are consequently found in the liver. The high concentrations of GSH in these tissues do not belittle the importance of GSH in other tissues - all cells require GSH to some extent.

Correspondence to: Dr. Ian Dettman. PO Box 702, Braeside VIC 3195 Australia. 61 3 9587 3948
biol@biol.com.au

3. How does it work?

In biology, many molecules "work" in cooperation with enzymes. Although GSH itself can act directly as an antioxidant against free radicals, reactive oxygen species and reactive nitrogen species, *mostly* GSH and GSSG act as substrates for enzymes produced in the tissues that GSH is found in. The principal reactions of glutathione are controlled by multiple enzymes in three families called *glutathione peroxidases, glutathione reductases* and *glutathione S-transferases*, as well as *glutathione dehydrogenase (ascorbate)*. Note that these enzymes do *not* contain glutathione; rather they *use* glutathione as a substrate.

GSH also binds to proteins, a reversible process called *glutathionylation*. It is emerging that glutathionylation is a major form of protein regulation and cell signaling[4]. Glutathionylation is involved in the stabilization of extracellular proteins and enzymes, protection of proteins against oxidation of critical cysteine residues, regulation of enzyme activity (including coenzyme A), and transcription. Excessive glutathionylation can lead to dysfunction and glutathionylated haemoglobin is a marker of oxidative stress.

GSH provides the reducing power for many other reactions, including the formation of deoxyribonucleotides by ribonucleotide reductase and the reduction of dehydroascorbate to ascorbate.

GSH contains sulphur, nitrogen and oxygen, which means that GSH acts as an effective *chelating agent* for various metal ions, including mercury. The chelating properties of GSH do *not* depend on enzymes, i.e. like EDTA, GSH will chelate a metal simply if it encounters it.

3.1. Glutathione peroxidases - antioxidants

Glutathione peroxidases (GPx) catalyse the destruction of *some* of the hydrogen peroxide (H_2O_2) and organic peroxides (hydroperoxides) which are produced in kilos every day in our cells via normal oxygen metabolism. GPx enzymes are selenium dependent and as such are known as *selenoproteins*. There are at present four classes of GPx selenoproteins known in humans, with various tissue distributions[5]. The expression of GPx enzymes in cells is dependent on selenium concentration in the cell, so essentially the capacity of GSH to function with GPx enzymes is dependent on selenium availability.

GPx enzymes remove hydroperoxides; GSH does not do this by itself to any great extent. GSH and the peroxide are both *substrates* for the GPx enzyme. In destruction of the peroxide, the GPx enzyme converts 2GSH to GSSG.

The role of GSH is to provide the "reducing power" for the enzyme to do its job; i.e. GSH is the "battery" for GPx enzymes. At least one GPx enzyme (GPX4) can use reducing molecules other than GSH to power it[6].

GSSG may be likened to a "spent battery" and in order to function again must be reduced to 2GSH molecules. The conversion of GSSG to 2GSH again requires an enzyme (one of the various *glutathione reductases*) and a supply of NADPH (a reduced form of vitamin B3). In this reaction, NADPH is the "battery" to power the glutathione reductase enzyme to regenerate GSH.

The most common of the GPx selenoproteins found in all cells is ***cellular glutathione peroxidase*** (GPX1 or cGPx). Unlike most hydroperoxides, phospholipid hydroperoxides (damaged biological membrane fragments) do *not* bind as a substrate for GPX1[5], which means that GSH-GPX1 does not work on them. *Other GPX enzymes and other ubiquitous antioxidants, (e.g. ascorbate, vitamin E, which do not depend on enzymes to act as antioxidants) are required for protection of most biological membranes in the body.*

In mammals, deficiency of GPX1 (congenital or under expressed) does *not* tend to produce catastrophic degradation of cells under *normal* oxidative stress. This is surprising considering the suspected evolutionary role of the enzyme. Mice bred without GPX1 (GPX1 knockout) display normal growth and phenotype, however these mice display various pathologies related to oxidative damage when placed under increased oxidative stress (e.g. compared to normal mice they have increased myocardial ischaemia reperfusion injury, sensitivity to various drugs and viruses)[6]. It is clear that GPX1 (and therefore GSH) has a role in protection from oxidative damage.

In humans GPX1 deficiency is suspected to be the cause of a form of haemolytic anaemia and is associated *strongly* with risk for cardiovascular disease [7]. Red blood cells undergo lysis if they are not protected from oxidative stress. Without adequate GSH or GPX1, haemolysis may occur rapidly. This also occurs in Glucose -6-phosphate dehydrogenase (G6PD) deficiency where the G6PD enzyme is required to reduce NADP to NADPH via the pentose phosphate pathway. Without NADPH, GSSG cannot be reduced to GSH. Without GSH the cells are not protected, so the damage done by G6PD deficiency has essentially the same mechanism as occurs with GSH deficiency or GPX1 deficiency (Other GPx enzyme deficiencies may cause the same problem). Other antioxidants such as vitamin C and vitamin E may well have an increased importance in GPx deficiency or G6PD deficiency.

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Gastrointestinal glutathione peroxidase (GPx-GI or GPX2) is distributed in the epithelium of the gastrointestinal tract. The enzyme is preserved preferentially compared to other GPx enzymes in the face of selenium deficiency (with severe Se deficiency, GPX1 can drop to 1% of normal levels while GPX2 remains normal). It is proposed that GPX2 protects the GIT from food hydroperoxides and hydroperoxides excreted in bile, thus it has a critical role in the prevention of cell changes due to hydroperoxide damage in the GIT.

Extracellular glutathione peroxidase (eGPx or GPX3) is manufactured in lung, heart, breast, placenta and liver, but principally the kidneys. It is distributed widely in plasma and extracellular fluids. Patients with renal diseases (including those on dialysis) have very low plasma GPX3 activity[6]. It is thought that its main function is to protect cell membranes, since unlike GPX1, GPX3 can use phospholipid hydroperoxides as a substrate.

Phospholipid hydroperoxide glutathione peroxidase (PHGPx or GPX4) as its name suggests, metabolizes phospholipid hydroperoxides. GPX4 represents at least 50% of the capsule material that embeds the helix of sperm mitochondria. It is most likely that the function of GPX4 in sperm is as a structural protein (selenium deficiency produces abnormal sperm structure) as well as a sperm antioxidant system. It is also thought that GPX4 has a role in other tissues for the appropriate synthesis of leukotrienes from arachidonic acid.

3.2. Glutathione dehydrogenase (ascorbate)

This enzyme has several names, including *dehydroascorbate reductase*. Essentially the function of this enzyme is to use 2GSH to reduce dehydroascorbate to ascorbate and in the process will produce GSSG (The GSSG is then reduced back to 2GSH by glutathione reductases using NADPH). Ascorbate and GSH are both reducing agents; therefore they do *not* interact negatively with each other and do *not* "cancel each other out".

While GSH is able to regenerate ascorbate through the dehydrogenase enzyme, it is clear from animal studies that giving ascorbate *spares* GSH in tissues and mitochondria. In guinea pigs, tissue damage and early mortality due to GSH deficiency are greatly reduced or prevented by administration of ascorbate[2] – *ascorbate spares GSH*. Guinea pigs given a diet deficient in ascorbate will develop scurvy. Giving glutathione (as the monethyl ester) doubled the amount of time it took ascorbate deficient guinea pigs to develop scurvy[2] – *GSH spares ascorbate*. That GSH/GSSG and ascorbate/dehydroascorbate are linked together in an enzyme system makes nonsense of comments that GSH and ascorbate should not be given together. Most animals manufacture enormous amounts of both *at the same time in the same tissue compartments*.

There is no pharmacological or clinical reason why ascorbate and GSH cannot be given together. GSH and ascorbate mixed together in a pre-injection solution do not break down – the solution is stable for several hours.

3.3. Glutathione S- transferases

Glutathione S-transferases (GST) catalyse the conjugation of GSH with target compounds to form GSH conjugates called *mercapturic acids*; the products of phase II conjugation reactions of drugs with GSH in the liver and other tissues. There are four classes of GST enzymes coded by several genes, producing at least 17 known enzymes. GSTs are heavily expressed in the liver and form about 4% of all the soluble protein in the liver[8]. They are vital for the detoxification of many drugs and endogenous compounds in the liver; however various GSTs have a wide tissue distribution including testis, kidney, brain, lung heart, pancreas, small intestine, spleen and placenta. GSH conjugates are excreted in the bile and the urine.

There is no pharmacological or clinical reason why ascorbate and GSH cannot be given together. GSH and ascorbate mixed together in a pre-injection solution do not break down – the solution is stable for several hours.

GSTs are involved in the detoxification of many environmental toxins, including multiple natural and industrial *carcinogens* (e.g. benzopyrene epoxides, styrenes, acrolein, trichloroethylene), *pesticides* (e.g. lindane, DDT, methyl parathion) and *drugs*(e.g. cisplatin, cyclophosphamide, nitroglycerine, menadione, adriamycin and acetaminophen).

GST polymorphisms and/or depletions are associated with the development of some cancers.

Unlike the GPx enzymes, the product of GST activity is not GSSG, rather it is a drug-GSH conjugate. *This means that GSH is not preserved and large amounts of it are excreted requiring it to be replaced.* GSH is synthesized in the body from its component parts by *glutathione synthetase* enzymes requiring adequate amounts of the component cysteine, glutamate and glycine. Sulphur in the diet as cysteine is the limiting factor in the production of GSH (old medical wisdom has it that sulphur is good for your liver and for detoxification – eat your broccoli).

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Sulphur given as N-acetyl cysteine upregulates the synthetase enzymes that make GSH[9]. Of course, GSH can also be given directly as an injection.

4. GSH Deficiency

"In humans, GSH depletion is linked to a number of disease states, such as in the inherited deficiencies of the GSH-synthesising enzymes, where individuals exhibit limited or generalised GSH deficiency with haemolytic anaemia, spinocerebellar degeneration, peripheral neuropathy, myophaty and aminoaciduria, and often develop severe neurological complications in the fourth decade of life. These conditions are not necessarily lethal because of their incomplete penetrance; in some tissues, GSH can attain 50% of normal. In addition, some GSH is obtained from the diet. Low erythrocyte GSH content also manifests in hereditary nonspherocytic lymphocytic leukaemia, and in glucose-6-phosphate dehydrogenase deficiency. In HIV infection, oxidative stress is elevated at all stages of the disease and low GSH concentration is found in plasma, erythrocytes, T cells and other lymphocytes and monocytes. Even children with HIV demonstrate low plasma GSH level. The cachexia and wasting of AIDS may be amenable to GSH repletion. HIV depletion of lung epithelial lining fluid glutathione (ELF) may predispose to opportunistic infections, and may be repleted using aerosolized GSH. Plasma and erythrocyte GSH concentration can be low in patients with cirrhosis or result from acute or chronic alcohol intake. In nonalcoholic liver disease, liver GSH can be abnormally low and GSSG high. Acetaminophen and other pharmaceutical or environmental xenobiotics can deplete liver GSH. Viral hepatitis can deplete GSH, and in hepatitis C patient's monocyte, GSH has been found to be depleted."[4]

It is perfectly reasonable to expect that GSH given IV in typical doses will indeed cross the BBB and enter the brain intact. It is not necessary to give GSH precursors to have substantial impacts on GSH concentrations in the brain.

GSH deficiency or altered GSH/GSSG ratios are evident in a variety of lung diseases, such as cystic fibrosis, COPD, ARDS and asthma. GSH is lowered in Chron's disease and gastritis and ulcer due to *Helicobacter pylori*. GSH levels also decrease dramatically in sepsis in response to invasive bacterial lipopolysaccharides (endotoxin)[9].

"The cells of the adult human brain consume ~20% of the oxygen utilized by the body although the brain comprises only 2% of the body weight. Reactive oxygen species, which are produced continuously during oxidative metabolism, are generated at high rates within the brain. Therefore, the defense against the toxic effects of reactive oxygen species is an essential task within the brain. An important component of the cellular detoxification of reactive oxygen species is the antioxidant glutathione."[10] GSH deficiency is associated with oxidative stress in the brain.

4.1 GSH, Parkinson's disease and the BBB

In Parkinson's disease (PD), a decrease of GSH concentrations (the reasons for GSH depletion are not clear) has been observed in the substantia nigra in preclinical stages of the disease[11], and this deficiency persists throughout the disease. Although intracellular GSH is depleted, GSSG is not elevated indicating that the mechanism of *removal* of GSH in neurons in the substantia nigra is something other that oxidative stress. It is possible of course, that something is removing GSSG. Even so, with reduced intracellular GSH levels the antioxidant GPx enzymes can't do their job leading to decreased antioxidant defense and subsequent damage.

Because of the depletion of GSH in PD it is a fairly obvious step to trial the effects of GSH clinically. One would reasonably expect that GSH administered IV could enter the brain and restore levels of GSH in depleted areas of the brain. That IV GSH does in fact have *considerable* impact on the symptomatology of PD[12] attests to this as being probable. However there is an historical understanding that GSH does *not* significantly cross the blood-brain barrier (BBB). Numerous papers in the GSH literature refer to the apparent inability of GSH to cross the BBB[11]. The original research that shows that GSH does not cross the BBB comes from the 1970s[13]. Because of this problem researchers in the area of brain GSH deficiencies have typically used GSH precursors, such as N-acetyl cysteine to influence brain GSH levels. Also, because of the BBB problem, research into how GSH does appear in such high levels in the brain has focused entirely on the routes of synthesis of GSH in various brain compartments and cell types.

GSH cannot enter *neurons* directly; it is synthesized in the neurons by glutathione synthetase enzymes[11]. Extracellular GSH is broken down by membrane γ-glutamyltranspeptidase enzymes (γ-GT) to provide the precursors for neurons to synthesize GSH de novo. Research shows that the GSH presented to neurons is synthesized largely in astrocytes, and that the proximity of astrocytes to neurons is the critical factor for neuronal GSH synthesis[14]. Essentially, astrocytes deliver either intact GSH or CysGly to neurons as the substrates for further GSH synthesis in neurons.

In Parkinson's disease, GSH synthesis in neurons in the substantia nigra is normal but there is an increase in the activity of the γ-GT enzymes. This suggests an increased demand for GSH precursors in the neurons for GSH synthesis; however why GSH levels are not able to be maintained in the neurons is unclear.

GSH is not normally consumed to any significant extent in the diet. Because of this, plasma GSH concentration is maintained by GSH synthesis. Plasma GSH comes primarily from the liver and is held at a fairly constant level, however *measured* GSH levels in plasma and other tissues vary widely between individuals. The GSH concentrations in plasma, brain endothelium and astroglia are in the order of millimoles, i.e. roughly similar concentrations on both sides of the BBB. This fact led Kannan et al.[15] to investigate the probable uptake of GSH from the plasma across the BBB in rats. They point out that the previous studies[12] that showed that GSH does not cross the BBB did not differentiate between GSH and GSSG. GSH is rapidly oxidized to GSSG in most tissues, and GSSG does *not* cross the BBB (it is literally twice the size of GSH and the sulphur groups are not available to transporters). When they looked at GSH vs. GSSG, they found that GSH most definitely *does* cross the BBB. The most efficient uptake occurs at low millimolar concentrations, precisely the concentrations normally found in plasma. They also found that GSH uptake is saturable, i.e. at GSH concentrations of 40 mM (millimolar) and above there was no increase in the rate of uptake. The upshot of this is that extremely high concentrations of GSH do not cross the BBB any better than "normal" concentrations.

An obvious question then is to ask what sort of plasma concentrations IV GSH therapy provides. GSH has a molecular weight of 307.43, or put another way a solution with a 1 mM concentration of GSH contains 307.43 mg of GSH per litre. A person with ~5L of blood will have ~1.5L of plasma, a 1 mM concentration of GSH represents approximately 460 mg of GSH in their plasma. A typical IV dose of GSH is 600 mg BD, or 1200 mg per day. This dose is well within the limits that will provide a low millimolar peak blood concentration of GSH. GSH uptake across the BBB in the Kannan study[15] begins to saturate at approximately 20-30 mM, a concentration well beyond what is achievable with 1200 mg/day doses.

Because of this information, it is perfectly reasonable to expect that GSH given IV in typical doses will indeed cross the BBB and enter the brain intact. It is not necessary to give GSH precursors to have substantial impacts on GSH concentrations in the brain.

In normal individuals, GSH deficiency or GSH/GSSG imbalance is likely to occur in areas of high oxidative stress (for whatever reason), with sulphur or selenium deficiency in the diet, or with increased demands on detoxification.

5. GSH and Chemotherapy

The combination of GSH with chemotherapy is a controversial topic, similar to the debate that surrounds vitamin C. With vitamin C, there is a preponderance of literature that shows that vitamin C decreases the amount of "gene damage" (clastogenenic effect) that various chemotherapeutics cause, because it is an antioxidant mopping up the free radicals that cause the damage in the first place. Despite this apparently negative interaction (from the point of view of killing cancer cells), it is evident from many clinical trials and case reports that vitamin C *increases* the effectiveness of several chemotherapeutics while *decreasing* the side effects, *despite it being anti-clastogenic*. This is largely thought to be because vitamin C "turns on" apoptotic mechanisms in cancer cells, typically by increasing the expression of apoptotic genes (mechanism unknown). Thus, although vitamin C pharmacologically has a negative interaction in *some* ways with *some* chemotherapeutics, the clinical outcomes and toxicology are inevitably improved. *Ultimately it is the clinical outcomes and toxicology that determine whether or not a drug should be used.*

An overriding problem with "negative" research published about nutrient/chemotherapeutic interactions is that the *research question* posed in these papers is extremely narrow (e.g. "is vitamin C anticlastogenic in such and such conditions" – these conditions usually being in vitro studies). A narrow research question necessarily answers *that question only*, and extrapolation and interpretation of this data to wider systems and particularly *clinical outcomes* is meaningless (e.g. "yes, vitamin C is anticlastogenic in such and such conditions *therefore* this means that chemotherapy will be less effective*." THIS IS NOT TRUE!*). Unfortunately it is exactly this kind of extrapolation which is published in the discussions and conclusions of the papers.

The data from some studies shows that GSH can *decrease* the apoptotic response of various cancer cell lines to cisplatin and other chemotherapeutics[16]. GSH itself is involved with GST enzymes that detoxify cisplatin in the liver; therefore *on face value* it is a natural response to assume that GSH interacts negatively

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with chemotherapy and should not be used in conjunction. We should however be at pains to look at clinical and toxicological data about GSH with chemotherapy before we can make any statements about it. *We cannot predict the clinical effects of combinations by extrapolating from data derived from narrow research questions*.

Bohm et al[17] conducted a clinical study looking at the feasibility of conducting a high dose cisplatin with carboplatin approach with glutathione to treat advanced ovarian cancer: "Based on previous clinical experience indicating the tolerability and efficacy of high-dose cisplatin with glutathione protection in the treatment of advanced ovarian cancer, this study was undertaken to explore the efficacy and feasibility of an alternative high-dose, platinum-based approach including a combination of high-dose cisplatin plus carboplatin as induction chemotherapy of advanced ovarian carcinoma and intervention surgery…The impressive efficacy suggests a possible contribution of reduced glutathione itself in improving the outcome, as supported by preclinical studies. The results of this study should be placed in context with current platinum-based therapy including paclitaxel." They go on to say that "The efficacy and tolerability of this high-dose regimen further support the clinical interest of GSH as a safe and selective protector against organ-specific toxicities of cisplatin (*Nephrotoxicity, ototoxicity and neurotoxicity*)."

Another platinum drug, oxaliplatin was used in a clinical trial with glutathione by Cascinu et al[18]. "Fifty-two patients treated with a bimonthly oxaliplatin-based regimen were randomized to receive GSH *(1,500 mg/m^2 over a 15-minute infusion period before oxaliplatin)* or normal saline solution. Clinical neurologic evaluation and electrophysiologic investigations were performed at baseline and after four (oxaliplatin dose, 400 mg/m(2)), eight (oxaliplatin dose, 800 mg/m(2)), and 12 (oxaliplatin dose, 1,200 mg/m(2)) cycles of treatment. At the fourth cycle, seven patients showed clinically evident neuropathy in the GSH arm, whereas 11 patients in the placebo arm did. After the eighth cycle, nine of 21 assessable patients in the GSH arm suffered from neurotoxicity compared with 15 of 19 in the placebo arm. This study provides evidence that GSH is a promising drug for the prevention of oxaliplatin-induced neuropathy, and that it does not reduce the clinical activity of oxaliplatin."

"Theoretically, the timing of the GSH infusion before the cisplatin might be critical because, like WR-2721 (amifostine), GSH can form a complex with cisplatin. When cisplatin is given 30-60 min after the GSH infusion in a ratio of GSH:CDDP of 30:1 there is a protection without interfering with therapeutic activity. In order to maximize the cytoprotective effect of GSH, short cisplatin infusions should be given. In the clinical setting *1500-3000 mg m^{-2}* GSH is administered IVapproximately 15 min before chemotherapy… GSH itself produces no toxicity."[19]

> *GSH is a systemic antioxidant, thus it is no surprise that elevated GSH levels, like high ascorbate levels, should protect undamaged cells from the toxicity of chemotherapy.*

That cancer cells themselves are protected by GSH does not appear to be demonstrated in clinical studies – GSH in combination with chemotherapy (platinum drugs) does not decrease the effectiveness of the drugs, but GSH does decrease the side effects and allows an elevation of dosage.

6. GSH in Therapy

GSH is used successfully in chemotherapy to manage the toxic side effects of platinum drugs. GSH independently is also a systemic antioxidant (via enzymes or directly). GSH has received significant press for its use in Parkinson's disease, Irritable Bowel Syndrome (and Chron's disease), fibromyalgia, chronic fatigue syndrome and cystic fibrosis. The initial justification for this is essentially that GSH levels are severely depleted systemically or in the affected tissues in all these diseases[4]. We know however that GSH is depleted (or GSH:GSSG ratios are reduced) in practically *any* tissue exposed to oxidative stress, including brain and inflamed tissues. At this stage, large random controlled trials have not been conducted into all these areas, however there is a wealth of case report information to support the use of GSH. Also, GSH is cheap and is essentially without toxicity so there is no pressing reason *not* to use it in cases where it has been demonstrated to have beneficial effect.

It is under debate whether or not GSH given intravenously crosses the blood-brain barrier or enters the liver *intact*. Normally, GSH is synthesized de novo in these tissues. The GSH normally found in plasma, kidney, lung and intestine predominately originates from liver synthesis[20]. Clearly IV GSH will reach blood cells, kidney, lung and intestinal intact. Glutathione transporters act to take extracellular GSH into the cells in these tissues. It is most likely that some IV GSH is degraded to γ-Glu-$(Cys)_2$ or some similar molecule before uptake into the CNS, whereupon GSH is resynthesised by GSH synthetase enzymes, *as well as directly crossing the BBB* (we have discussed this with

reference to Parkinson's disease). There is a clear consensus in the scientific literature that GSH itself is a sink or "storage form" of cysteine. In this context, exogenous GSH can act either directly as a GSH supply or as a precursor to GSH synthesis in various tissues.

Cysteine availability is the limiting factor for the rate of de novo GSH synthesis. Cysteine may be manufactured in cells by the *transulfuration pathway* by ensuring adequate B_{12}, B_6 folate and methionine (necessary for SAMe synthesis) are present. The same pathway can manufacture cysteine from exogenous SAMe, however the majority of large doses of exogenous SAMe may break down to homocysteine. Homocysteine is a known toxin in high concentrations and is implicated in cardiovascular disease and oxidative damage itself. Side effects such as insomnia and headaches have been reported with high doses of SAMe. It is preferable to use cysteine directly, as N-acetyl cysteine (NAC) or as GSH, the "storage form" of cysteine.

7. GSH or NAC?

The effects of NAC are essentially due to the increased production of GSH; however NAC given orally in large doses may cause nausea and gastrointestinal discomfort. IV NAC, like IV GSH, is largely non-toxic, however there are some reports of adverse effects of NAC at high doses.

NAC (Parvolex) has been typically used to treat acetaminophen (paracetamol) poisoning. It works by increasing GSH concentrations in the liver, since paracetamol rapidly depletes liver GSH. It is recommended to be cautious using NAC with asthma patients. Rash, bronchospasm and anaphylactic reactions have been reported with NAC.

Patients suffering from debility or malnutrition (e.g. protein deficiency or malabsorption) may have difficulty absorbing or providing the component parts for GSH synthesis in the tissues. Congenital aberrations of glutathione synthetase activity will lead to difficulty in manufacturing GSH effectively from its component parts. *In all these cases, IV GSH may be distinctly preferable to NAC.* Since GSH essentially acts as a reservoir of cysteine, giving GSH should theoretically provide any activity that NAC may have independently of GSH.

Normal levels of GSH in the epithelial lining fluid (ELF) of the lungs are 150 times higher than in other tissues. Here it serves as an essential antioxidant and protects the lungs from inhaled toxins. The presence of GSH is essential for normal surfactant production and inflammatory control. In cystic fibrosis there is a systemic GSH deficiency that progresses over time. In cystic fibrosis the manufacture of GSH is normal; however efflux from cells to the ELF is compromised. *This means that it is preferable to give GSH over NAC.* GSH may be given IV, however inhalation of aerosolized GSH has proved very effective in trials[21].

GSH given orally rapidly breaks down in the GIT. GSH given IV is 100% bioavailable, non-toxic and an effective method of GSH delivery, either directly or indirectly, into all tissues.

8. GSH doses

GSH is administered IV or as an aerosol at various doses, depending on the indication.

As an adjunct to chemotherapy (platinum drugs) treatment, the dose is typically 1500-3000 mg m^{-2} administered IV approximately 15 minutes before therapy.

In Parkinson's disease, the dose is typically 1200 mg per day administered IV in divided doses, i.e. 600 mg BD. This dose allows passage of the injected GSH across the BBB, even at its peak concentration.

In cystic fibrosis, GSH is usually given as an aerosol, although some IV GSH will reach lung alveoli. As an aerosol, GSH is typically delivered in a 600 mg dose every 12 hours, for 6 doses[22].

Summary

GSH and GSSG act as a redox couple in all human cells, having developed early in the evolution of eukaryotic cells to protect them from ROS, RNS, free radicals and various hydroperoxides. GSH can act directly on these substances but typically in complex systems is controlled by various enzymes. The glutathione antioxidant enzymes are the glutathione peroxidases (GPx). The expression of GPx enzymes is dependent on selenium concentration (i.e., dietary selenium).

GSH is synthesized in cells by glutathione synthetase enzymes. Availability of cysteine is the limiting factor for the production of GSH. GSH produced in the liver is the primary source of kidney, plasma, lung and intestine GSH. Plasma GSH most likely *does* enter the brain across the BBB; however GSH is also readily degraded into precursor molecules that can enter brain and liver to promote GSH synthesis in these tissues.

GSH is deficient either systemically or in specific affected tissues in a variety of diseases, including AIDS, Parkinson's disease, cystic fibrosis, inflammatory bowel

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disorders, fibromyalgia and chronic fatigue syndrome. Exogenous GSH or GSH precursors such as NAC and SAMe are being used to attempt to redress these deficiencies. Clinical effects are reported with IV GSH therapy, particularly in Parkinson's disease, leading to calls for randomised controlled trials. IV GSH is used effectively to increase the dose and effects of platinum based chemotherapy drugs[17,18,19], as well as reducing the toxic effects of these drugs on other organs, particularly the nervous system and kidney.

References:

[1] Conti, A. and Bickel, M.H.; History of drug metabolism: Discoveries of the major pathways in the 19th century. Drug Metabolism Reviews,6:1-50(1977).

[2] Meister A.; Glutathione, ascorbate, and cellular protection. Cancer Res. 1994 Apr 1;54(7 Suppl):1969s-1975s.

[3] Meister A.; Mitochondrial changes associated with glutathione deficiency. Biochim Biophys Acta. 1995 May 24;1271(1):35-42.

[4] Pastore A, Federici G, Bertini E, Piemonte F. Analysis of glutathione: implication in redox and detoxification. Clin Chim Acta. 2003 Jul 1;333(1):19-39.

[5] Himeno S, Imura N; New aspects of physiological and pharmacological roles of selenium. Journal of health science, 2000 46(6) 393-398

[6] Arthur JR. The glutathione peroxidases. Cell Mol Life Sci. 2000 Dec;57(13-14):1825-35

[7] GLUTATHIONE PEROXIDASE DEFICIENCY, HEMOLYTIC ANEMIA POSSIBLY DUE TO, INCLUDED. Online Mendelian Inheritance in Man, OMIM (TM). Johns Hopkins University, Baltimore, MD. MIM Number: 138320: Mar 17, 2004: . World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/

[8] Eaton DL, Bammler TK. Concise review of the glutathione S-transferases and their significance to toxicology. Toxicol Sci. 1999 Jun;49(2):156-64.

[9] Macdonald J, Galley HF, Webster NR. Oxidative stress and gene expression in sepsis. Br J Anaesth. 2003 Feb;90(2):221-32.

[10] Dringen R, Gutterer JM, Hirrlinger J. Glutathione metabolism in brain metabolic interaction between astrocytes and neurons in the defense against reactive oxygen species. Eur J Biochem. 2000 Aug;267(16):4912-6.

[11] Schulz JB, Lindenau J, Seyfried J, Dichgans J.Glutathione, oxidative stress and neurodegeneration. Eur J Biochem. 2000 Aug;267(16):4904-11.

[12] Sechi G, et al.: Reduced intravenous glutathione in the treatment of early Parkinson;s Disease. Prog. Neuro-Psychopharmacol & Biol. Psychiat. 1996, Vol. 20, pp 1159-1170

[13] Cornford EM, et al.: Blood-brain barrier restriction of peptides and the low uptake of enkephalins. Endocrinology. 1978 Oct;103(4) : 1297-303

[14] Dringen R et al.; Synthesis of the antioxidant glutathione in neurons: Supple by astrocytes of CysGly as precursor for neuronal glutathione. J. Neuroscience. 1999, Jan 15, 19(1):562-569

[15] Kannan R, et al.: Evidence for carrier-mediated transport of glutathione across the blood-brain barrier in the rat. J. Clin. Invest. June, 1990. 85:2009-2013

[16] Tormos C, Javier Chaves F, Garcia MJ, Garrido F, Jover R, O'Connor JE, Iradi A, Oltra A, Oliva MR, Saez GT. Role of glutathione in the induction of apoptosis and c-fos and c-jun mRNAs by oxidative stress in tumor cells. Cancer Lett. 2004 May 10;208(1):103-13.

[17] Bohm S, Oriana S, Spatti G, Di Re F, Breasciani G, Pirovano C, Grosso I, Martini C, Caraceni A, Pilotti S, Zunino F. Dose intensification of platinum compounds with glutathione protection as induction chemotherapy for advanced ovarian carcinoma. Oncology. 1999;57(2):115-20.

[18] Cascinu S, Catalano V, Cordella L, Labianca R, Giordani P, Baldelli AM, Beretta GD, Ubiali E, Catalano G. Neuroprotective effect of reduced glutathione on oxaliplatin-based chemotherapy in advanced colorectal cancer: a randomized, double-blind, placebo-controlled trial. J Clin Oncol. 2002 Aug 15;20(16):3478-83.

[19] Hospers GA, Eisenhauer EA, de Vries EG. The sulfhydryl containing compounds WR-2721 and glutathione as radio- and chemoprotective agents. A review, indications for use and prospects. Br J Cancer. 1999 May;80(5-6):629-38.

[20] Wu G, Fang YZ, Yang S, Lupton JR, Turner ND.Glutathione metabolism and its implications for health. J Nutr. 2004 Mar;134(3):489-92.

[21] Townsend DM, Tew KD, Tapiero H. The importance of glutathione in human disease. Biomed Pharmacother. 2003 May-Jun;57(3-4):145-55.

[22] Roum J, et al.: Glutathione aerosol suppresses lung epithelial surface inflammatory cell-derived oxidants in cystic fibrosis. J. Appl. Physiol. 87(1): 438-443, 1999

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