Practicalities of glutathione supplementation in nutritional support

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New nutraceutical products for nutritional support and antioxidant therapy such as glutathione require practical advice and information on the indications, methods and routes of administration, dosing (therapeutic drug monitoring), stability and physicochemical compatibility. This review is based on recent clinical and experimental publications in which glutathione has been used as a drug. Curr Opin Clin Nutr Metab Care 5:321–326.

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Abbreviations

GSH	reduced glutathione
GSSG	oxidised glutathione
NAC	*N*-acetylcysteine

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Introduction

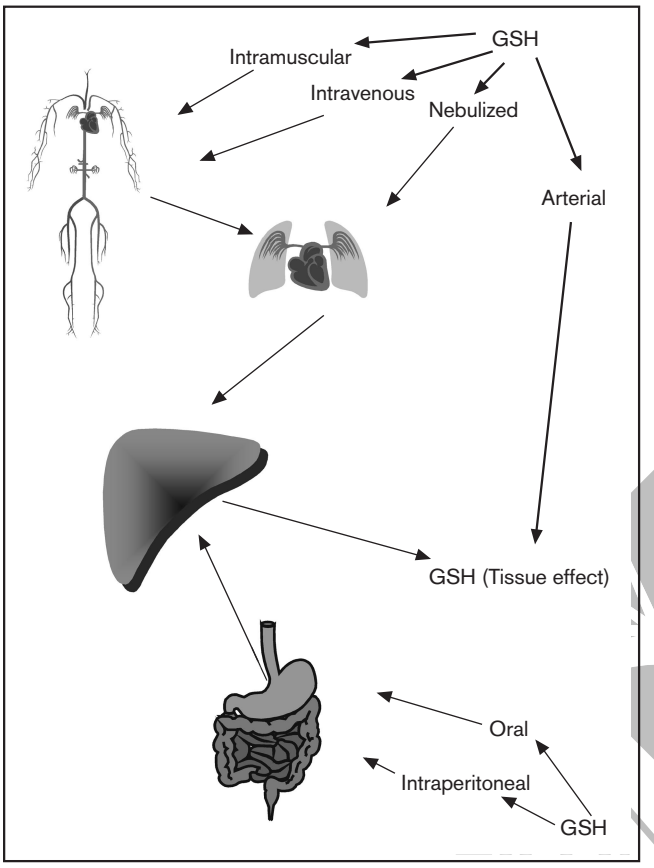
New metabolic and/or biochemical mechanisms [1,2] to explain the pathophysiology of ageing [3], diseases such as cancer [4], sepsis [5,6•,7,8•], trauma [9,10], and ischaemia/reperfusion [11,12•], have created opportunities for the development and use of a wide group of new pharmacologically active nutrients or nutraceuticals. The sequence of development is essentially as follows. First, novel substrates can be isolated from biological organisms or synthesized by new technology. Second, new molecules that produce a pharmacologically active product need to be analysed according to the literature background in order to demonstrate any potential clinical applicability. Third, the new product's development is based on its potential to modify the course of pathophysiological conditions on a specific disease. Finally, a nutraceutical emerges as a new drug looking for an innovative clinical applicability.

Antioxidant defence against free radicals in the body has led to the production of many such new drugs, for example, to reduce lipid peroxidation. Towards the end of the last century, for instance, many studies investigating pharmacological doses of vitamins were published. However, no drug has such a pronounced intracellular antioxidant effect as glutathione (γ-glutamyl-cysteinyl-glycine, GSH). This tripeptide certainly belongs to the group of molecules looking for clinical applicability, not only to act as an extracellular antioxidant but also as the main intracellular antioxidant against protein, lipid or enzymatic oxidation. It should therefore be understood that destructive oxidative reactions inside the cell are more important than just lipid peroxidation [13,14]. The potential clinical and practical applications of GSH are the main objective of this review.

How to increase glutathione concentrations?

The rapidly expanding field of antioxidant pharmacology as a therapeutic option has witnessed a dramatic proliferation of available drugs. These drugs can be administrated as a bolus or over time by various routes. There are many ways to increase plasma and tissue GSH levels in the body: via oral, parenteral, inhalation, intra-arterial, intraperitoneal and/or intramuscular administration (Fig. 1). The current state of the art of GSH requires an intimate familiarity with dosing information, pharmacology and new indications/routes of administration for this nutraceutical. When solutions are administered orally, the liver has direct access to dietary amino acids through the portal circulation. The liver can then alter

Figure 1. Potential routes to increase levels of GSH



the concentration of its amino acid precursors in portal blood before they enter into the peripheral circulation and can supply or utilize these amino acids rapidly in a manner complementary to the needs of other tissues to produce GSH.

Intravenous

A study published by Ortolani *et al.* [15•] evaluated the feasibility and safety of intravenous GSH and *N*-acetylcysteine (NAC) in septic shock. Patients were randomised to receive either; 70 mg of GSH alone or 70 mg of GSH plus 75 mg NAC/kg/day or placebo. Outcomes measured included: mortality at 10 days, plasma concentration of GSH and oxidised glutathione (GSSG), and reduction of four biochemical parameters to indirectly evaluate peroxidative stress, i.e. expired ethane, plasma malondialdehyde, erythrocyte membrane deformability and complement C5 activation. There were no significant changes in the mean arterial pressure, heart rate, or cardiac output of the treated patients, in comparison with those of the control group. All patients showed a marked decrease in their lipoperoxidative index. Mortality was similar in all the groups during the trial, but by day 10 the mortality in the control group was twice that in both test groups ($P<0.01$) suggesting a potentially therapeutic effect of intravenous GSH and/or its precursors.

Recently, Stabler *et al.* [16] showed the effect of GSH infusion on cysteine plasma levels in an animal model of premature birth with respiratory distress. Premature baboons were maintained in neonatal intensive care units for ± 14 days. Parenteral feeding with or without supplemental cysteine (125 μmol/kg/h) and intravenous GSH (24 μmol/kg/h in the first hours of life and titrated up to 48 μmol/kg/h by 24 h) respectively was given. Supplementation with GSH from the first day of life raised plasma total cysteine markedly and the arterial–alveolar oxygen gradient was significantly higher in the 125-day-old animals that received GSH infusions.

The effect of GSH infusion (2.4 g/day) compared to normal saline on plasma and erythrocyte GSH levels and on liver function tests has been evaluated in alcoholic cirrhotic patients and abstainers [17]. Persistent alcohol consumption prolongs antipyrine metabolism which was counteracted by intravenous GSH but GSH blood levels only increased in abstainers. In a more recent study clinical signs and some hepatic functions appeared to improve with a daily infusion of GSH [17].

Oral

GSH is involved in detoxification by binding to fat soluble toxins, such as heavy metals, solvents, and pesticides, and transforms them into a water-soluble form that can be excreted in urine. GSH is present in most plant and animal tissues from which the human diet is derived. Much of GSH is bioavailable because cells of the gastrointestinal tract have Na^+-dependent and Na^+-independent uptake mechanisms, and net absorption of GSH from the lumen into the vascular circulation occurs in the small intestine [18•]. Although knowledge of GSH content in foods is incomplete, an insight into the range of values can provide some useful generalizations. Considerable variations in GSH content occur in different foods, and estimated daily intakes of GSH differ considerably between individuals [18•]. Oral GSH appears to be efficiently absorbed in rats [19,20], however, the same may not necessarily be true for GSH supplements in humans. For example, when seven healthy subjects were given a single dose of up to 3 g of GSH, there was no increase in blood GSH levels [21], the authors of the study concluded 'it is not feasible to increase circulating GSH to a clinically beneficial extent by the oral administration of a single dose of 3000 mg of GSH'. Absorption of GSH may be better in rats because the human gastrointestinal tract contains significant amounts of an enzyme γ-glutamyltranspeptidase that breaks down GSH [18•]. Very preliminary evidence has suggested that absorption of GSH can occur in the

mouth when GSH tablets are placed between the teeth and the inner cheek [22]. Although there have been several anecdotal reports on the possibility that oral administration of GSH may have beneficial effects against specific disease processes, relatively few scientific studies have been published.

Nebulization

An aerosol generator (Ultravent, Mallinckrodt) driven by compressed air, supplies droplets containing GSH with 2.8 μm median aerodynamic diameter. When GSH is atomized in this fashion, it remains in its reduced form. Aerosol delivery of each dose (administered by mouthpiece with the nostrils of spontaneously breathing patients occluded), requires 20–30 min.

Roum *et al.* [23] used this technique to evaluate the feasibility of increasing epithelial lining fluid and enhancing GSH levels in respiratory epithelial surfaces as antioxidant protection in a group of patients with cystic fibrosis. GSH aerosol was delivered (600 mg twice daily for 3 days) to seven patients. Epithelial lining fluid, total, reduced, and oxidized GSH increased ($P<0.05$), compared with values before GSH therapy, suggesting adequate respiratory epithelial surface delivery and utilization of GSH. Superoxide anion (O_2-) release by epithelial lining fluid inflammatory cells decreased after GSH therapy ($P<0.002$). No adverse effects were noted during treatment. Together, these observations demonstrate the feasibility of using a GSH aerosol to restore respiratory epithelial surface oxidant–antioxidant balance in cystic fibrosis and support the rationale for further clinical evaluation. An interesting case report suggests that treatment with nebulized GSH led to rapid resolution of an acute respiratory failure secondary to emphysema and apparent bronchial infection in a 95-year-old man [24•]. Most recently, children with middle ear epithelial cell damage from antioxidants released in chronic otitis media with effusion, have been treated with nebulized GSH. Patients in the treatment group received 600 mg GSH in 4 ml saline per day, administered in five 2-min nasal aerosol doses every 3–4 h for 2 weeks. Clinical improvements were observed in 66.6% of GSH patients compared to 8% in the saline controls ($P<0.01$) leading the authors to advocate GSH for the non-surgical management of chronic otitis media with effusion [25••].

Arterial

GSH could be used to restore intracellular redox imbalance and prevent inactivation of endothelial-derived nitric oxide, on the abnormal vasomotor reactivity in spastic coronary arteries by either intracoronary infusion or by incorporation into cardioplegia solutions

Kugiyama *et al.* [26] in a controlled research study demonstrated that GSH infusion (50 mg/min for 6 min) suppressed constrictor response of epicardial diameter to acetylcholine (50 μg/min) in patients with coronary spastic angina, whereas it had no significant effect in control subjects. GSH may therefore have an important role in the regulation of coronary vasomotor function in patients with coronary spastic angina.

On the other hand, Nakamura *et al.* [27•] hypothesized that GSH detoxifies the $ONOO^-$ radical and reverses defects in endothelial function and systolic function when present in crystalloid cardioplegia solution (Plegisol, Abbott Laboratories, Health Santé, Canada). The cardioplegia solution contained 5 μmol/l authentic $ONOO^-$; catalase was included to attenuate the potential antioxidant effects of GSH and to unmask the effect on $ONOO^-$. In one group (five patients) the cardioplegia solution contained 500 μmol/l GSH, whereas a second group (six patients) received crystalloid cardioplegia solution without GSH. Myocardial neutrophil accumulation (myeloperoxidase activity) was attenuated with cardioplegia solution plus GSH versus cardioplegia solution alone (2.2 ± 0.7 versus 5.4 ± 1.2, $P<0.05$). The adherence of neutrophils to post-experimental coronary arteries as a measure of endothelial function was less with cardioplegia solution plus GSH than with cardioplegia solution alone (98 ± 18 versus 234 ± 36 neutrophils/mm^2). Therefore, GSH in crystalloid cardioplegia solution does appear to detoxify $ONOO^-$ by forming cardioprotective nitrosoglutathione, resulting in attenuated neutrophil adherence and selective endothelial protection through the inhibition of neutrophil-mediated damage.

Intraperitoneal

Although the intraperitoneal route is not the best way to increase GSH concentrations in the body, experimental animal studies by Chen *et al.* [28] demonstrated that intraperitoneal injection of 10 mmol/kg of GSH monoethyl ester to mice could correct GSH and cysteine deficiency after acetaminophen (paracetamol) administration. Despite this, intraperitoneal GSH deserves further evaluation in order to find any clinical applicability.

Intramuscular

Intramuscular GSH administration appears to be a good therapeutic option for patients with chronic diseases. Italian researchers have injected 600 mg/day intramuscularly for 10 days to a group of 15 patients with type 2 diabetes mellitus. They hypothesized that treatment with GSH may improve platelet constitutive nitric oxide synthase activity. With respect to the basal values on the tenth day of treatment, the red blood cell GSH concentration and platelets constitutive nitric oxide synthase increased (1.4 ± 0.1 versus 1.9 ± 0.1 μmol/10^{10} red blood cells, $P<0.01$ and 0.7 ± 0.1 versus

2.9 ± 0.2 fmol/min^{-1} 10^{-9} platelets, $P < 0.001$, respectively) and the plasma plasminogen activator inhibitor type 1 levels diminished (81.4 ± 3.7 versus 68.7 ± 4 ng/ml, $P < 0.002$). These data suggest that intramuscular administration of GSH to patients with type 2 diabetes mellitus is able to improve platelet constitutive nitric oxide synthase activity together with a reduction of plasminogen activator inhibitor type 1 [29•].

Pharmacokinetics of intravenous glutathione

Bianchi *et al.* in 1997 [30], evaluated the GSH kinetics in normal subjects compared with cirrhotic patients. Following GSH infusion, plasma GSH rapidly increased, reaching a first steady state value approximately twice as high in controls compared to cirrhotic patients. (11.80 μM versus 24.63 μM for control group, $P < 0.05$) During the second hour, when the infusion rate was doubled, plasma GSH further increased to a second steady state approximately 50 μM in controls and 25 μM in cirrhosis. In summary, high dose intravenous GSH is safe. It distributes in the extracellular compartment and is eliminated from the circulation (80 ml/min/m^2) with a half-life of between 5 and 7 min [31].

Pharmaceutical considerations of glutathione as a drug

A competent clinician is expected to be familiar not only with all the drugs he or she prescribes and uses in the course of care, but also those drugs prescribed by other specialists with whom he or she must interact. Antioxidant therapy as a therapeutic alternative thus requires extensive medical knowledge, particularly in pharmacology. The practicalities of GSH supplementation require such an approach with knowledge of the important sources, indications, methods and routes of administration, including therapeutic drug monitoring parameters, stability and compatibility (pH, effect of light, solution, and drug compatibility), which are summarized below.

(1) Sources of products: includes all known preparations used for the referenced clinical or experimental research studies:
   (a) Intravenous;
      (i) GSH sodium salt 0.646 g/4 ml and 2.5 g/25 ml (TAD®, Biomedica Foscama, Italy) [15•] or Tationil, Roche Products, Italy;
      (ii) GSH monoethyl ester 800 mmol/l (not commercially available) [32];
   (b) Oral;
      (i) Antioxidant mixture includes L-GSH (Nutrition House™ Canada Inc);
      (ii) GSH 25, 50 mg; GSH reduced 50, 100, 200 mg; GSH power powder 50 mg; GSH reduced powder 50 mg (Gaines Nutrition, USA);
      (iii) Amino acid, vitamins and antioxidant mixture with GSH 50 mg (Prolete power, ProActive Nutrition, Auckland, New Zealand);
   (c) Nebulization;
      (i) $97 \pm 3\%$ of reduced GSH/4 ml of sterile 0.9% saline (not commercially available) [23];
      (ii) Reduced GSH 150 mg/ml (4 ml of 0.9% NaCl) [33];
   (d) Arterial: no products commercially available;
   (e) Intraperitoneal: GSH monoethyl ester (not commercially available);
   (f) Intramuscular: the GSH sodium salt (TAD®) is also approved for intramuscular use.

(2) Dosing and administration: typical dosing regimens that have been used include:
   (a) Intravenous: there have been clinical reports with different schedules;
      (i) 70 mg/kg infused every day over 6 h to critically ill patients for 5 days [15•];
      (ii) 2.4 g/day given to cirrhotic patients over 30 days [17];
      (iii) Reduced GSH given intravenously at a dose of 1200 mg at the end of each dialysis session for at least nine months to patients with chronic renal failure [33];
   (b) Oral: there have been only a few studies with oral GSH;
      (i) 3000 mg (0.15 mmol/kg) as a single dose in seven healthy volunteers [21];
      (ii) Clinical report of GSH as component of an antioxidant mixture, 1 g GSH daily during 7 days prior to an exercise protocol [34];
   (c) Nebulization;
      (i) 600 mg of reduced GSH, administered in six doses by aerosol every 12 h [23];
      (ii) 600 mg nasal aerosol every 3–4 h for 2 weeks [25];
      (iii) 600 mg of reduced GSH as a single dose [35];
   (d) Arterial;
      (i) 300 mg of reduced GSH in a 6-min infusion (50 mg/min) [26];
      (ii) Reduced GSH incorporated into cardioplegia solution at concentration of 500 μmol/l [27•];
   (e) Intraperitoneal: GSH monoethyl ester administered as a single dose at 10 mmol/kg [28];
   (f) Intramuscular: 600 mg/day intramuscular for 10 days [29•].

(3) Monitoring: therapeutic drug monitoring of GSH levels should be routinely considered. The best ways to evaluate the GSH effect are by knowing the antioxidant balance between oxidants and antioxidants [36], by measuring biological lipid peroxida-

tion [37] or protein oxidation [14], and finally assessment of the reduced and oxidised GSH levels.

(4) Stability:
   (a) Product stability: the sodium salt of GSH is stable in lyophilised form for 3 years and has been safely administered to animals at doses up to 7500 mg/kg intravenous or intraperitoneal.
   (b) Solution pH: the pH of GSH products in aqueous solution are close to neutral;
      (i) GSH monoethyl ester 800 mmol/l: pH 7.2–7.4 [32];
      (ii) GSH sodium salt: pH 6.8–7.4 [38•];
      At present there are no published data on the effect of pH on GSH stability but care should be taken (see below) to monitor the pH of GSH-containing admixtures
   (c) Solution stability: the most recent report of GSH stability in nutritional solutions was published last year. Valencia *et al.* [38•] demonstrated that GSH as the lyophilised sodium salt was stable in a standard lipid-free total parenteral nutrition (TPN) mixture for 72 h at 4–8°C. However, it appeared less stable in a glutamine enriched mixture and in the glucose control. Both TPN mixtures contained the same total amount of different amino acids but the more stable mixture (pH 6.3), contained NAC and taurine. These two amino acids were not present in the glutamine enriched mixture (pH 7.2) or the glucose control (pH 7.1). Although the presence of glutamine in the less stable TPN mixture could have affected GSH activity this cannot account for the loss of GSH in dextrose. The lower pH of the stable mixture may also have been a factor requiring further investigation, but it is more likely that NAC and/or taurine preferentially reacted with dissolved oxygen in their mixture and provided protection against GSH oxidation. This is borne out by similar results that we have recently observed; i.e. GSH is equally stable when added to vitamin C-containing admixtures with or without glutamine (Valencia E, Marin A and Hardy G, unpublished data).
   (d) Solution compatibility: since there have been few research studies with intravenous GSH in clinical practice, to date there have been no reports of any incompatibilities that could have clinical repercussion for patients. However the absence of information implies neither compatibility nor incompatibility.
   (e) Light protection: to date there have been no reports of any adverse effects of light on GSH, but a study by Bhatia *et al.* [39] in 1992 demonstrated that light exposure of parenteral nutrients adversely affected hepatobiliary function and amino acid homeostasis in rats. Biliary total glutathione (GSH+GSSG) levels were significantly greater, whereas inorganic phosphate and glucose were lowered, with light protection. As it is now standard procedure to routinely protect TPN mixtures from light, it is advisable to follow this practice for GSH enriched mixtures.

(5) Drug compatibility (co-administration with other medications): GSH is considered compatible if published information supports either physical or chemical compatibility with the other medication tested, either by y-site administration, syringe, or by mixing the two medications. To date there have been no reports of any co-administration problems from clinical treatments with reduced GSH.

Conclusion

Treatment strategies that maintain GSH levels or replenish depleted stores may minimize the susceptibility of patients to free radical mediated tissue injury and help improve outcome of the critically ill. Stable GSH products appear to be reasonably compatible with other nutrients and can be administered safely by most of the common routes, including as a supplement to certain TPN admixtures.

Much research is currently in progress and our knowledge is rapidly improving and/or changing. While every effort has been made to ensure that the information contained within this review is accurate and in accordance with the data available at the time of publication, it is possible, as new research and experience broaden our knowledge, that changes in clinical practice, therapeutic intervention, drug interactions, or compatibility information may occur. The reader is therefore advised to check, prior to the clinical administration of GSH, the specific product information included in the package insert (or SPC, Summary of Product Characteristics) to be certain that changes have not been made that could modify the validity of earlier information, in order to ensure the best possible clinical practice.

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