Glutathione Deficiency: Therapeutic Target in Human Immunodeficiency Virus Infection

Sean K. Waterson, B.Sc.[1]

It has been demonstrated that asymptomatic persons infected with the human immunodeficiency virus (HIV) have significantly reduced levels of extracellular glutathione.[1] Serum glutathione was only one-third of the level of healthy controls. The level of glutathione (GSH) in lung epithelial lining fluid was only 60% of that in controls. All subjects were non-smokers.

It was initially thought that the immune dysfunction seen in acquired immune deficiency syndrome (AIDS) was solely due to increased mortality of infected helper T-cells. However, it is becoming increasingly clear that other factors must contribute to a complex dysregulation of normal immune function.[2,3,4]

Glutathione plays an important role in protecting cells from oxidative stress and in maintaining normal immune response. The documentation of severe GSH deficiency in HIV infection raises urgent therapeutic questions.

Background

Glutathione is a tripeptide formed from cysteine, glutamate and glycine. Glutathione is the most abundant intracellular low-molecular weight sulfur-containing compound in almost every aerobic organism ever studied.[5]

GSH is synthesized intracellularly in two steps:[6]

1) L-glutamate + L-cysteine + ATP =+== gamma - L-glutamyl - L-cysteine + ADP + Pi
2) gamma-L-glutamyl-L-cysteine + glycine + ATP === gamma - L-glutamyl - L-cysteinyl glycine + ATP + Pi

The first reaction is catalyzed by gamma-glutamyl-cysteine synthetase and the second by glutathione synthetase. The first reaction is considered to be the rate-limiting step and it may depend on intracellular levels of cysteine which are generally an order of magnitude lower than glutamate levels.[7]

Degradation of GSH occurs through the transfer of the gamma-glutamyl portion of the peptide to an acceptor.[6] This reaction is catalyzed by gamma-glutamyltransferase. Certain amino acids can function as the acceptor. If a water molecule is the acceptor then hydrolysis of GSH results.

Glutathione performs a number of functions in the cell. Perhaps its best known function is as an anti-oxidant in the reaction catalyzed by the enzyme glutathione peroxidase. Toxic hydroperoxides formed as a byproduct of oxygen metabolism are inactivated by this selenium-dependent enzyme as follows:[5] R-O-O-H + 2GSH—>R-OH + GSSG + H_20

GSSG is readily reduced to glutathione by GSSG reductase.[8]

Glutathione transferases are a group of versatile enzymes. They are responsible for the first step in a pathway that eliminates potentially cytotoxic electrophilic compounds by conjugating them with GSH. They are so important that up to 4% of hepatic cytosolic proteins are glutathione transferases.[9] Glutathione transferases can also detoxify fatty acid hydroperoxides and protect lipid membranes from peroxidation. These enzymes constitute 5% of rat liver mitochondrial membranes.[10]

GSH and Immunity

The importance of GSH in maintaining competent immune function has been well documented. In human peripheral blood mononuclear cells, in which the intracellular GSH level had been decreased to 30-40% of normal, the spontaneous cell-mediated cytotoxicity was inhibited by 50%.[11] Antibody-dependent cellular cytotoxicity was also significantly reduced by low GSH levels. In human peripheral blood lymphocytes, in which GSH levels had been reduced to 20% of controls, blast transformation and [^{3}H]-thymidine uptake were 80-90% suppressed.[13] Depletion

1. Canadian College of Naturopathic Medicine, 60 Berl Avenue, Toronto, ON, Canada M8Y 3C7.

of rat liver GSH resulted in corresponding decreases in the in vivo phagocytic activity of Kupffer cells as measured by clearance of colloidal carbon.[14] Repletion of GSH levels resulted in a return of normal function. In mice injected with GSH, the activity of cytotoxic T-lymphocytes was five times greater than in controls.[12]

The cause of glutathione deficiency in HIV infection is unknown. It is thought that the enzymes of GSH synthesis are present in cells throughout the body. Plasma levels are dependent on tissue synthesis. Efflux of GSH has been demonstrated in hepatocytes, human fibroblasts and lymphoid cells.[6]

Intracellular levels of GSH have been shown to be heavily dependent on protein intake in general and intake of sulfur-containing amino acids in particular. Glutathione-dependent enzyme activity was markedly decreased in rats fed a low-protein diet.[8] When animals on deficient protein diets were fed proportionately more sulfur-containing amino acids, GSH levels in liver approached those of normal. When laboratory rats were starved for 48 hours, the hepatic GSH levels were reduced by 50-60%.[7] When refed, their GSH levels returned to normal. The increase in GSH was dependent on cysteine intake. Cysteine was incorporated into GSH when supplied in excess of what was required for protein synthesis.

Cysteine

Decreased plasma cysteine has been documented in HIV-infected persons.[15] In persons with AIDS or lymphadenopathy syndrome, plasma cysteine levels were approximately one-third of the levels of healthy controls. In HIV-seropositive persons without symptoms, plasma cysteine levels were slightly more than half the level of controls.

The reason for the decreased cysteine levels in HIV infection is unknown. However, since cysteine is the rate-limiting substrate for GSH synthesis, it seems likely that low GSH levels are due to low cysteine levels.

Cysteine is not an essential amino acid. It can be synthesized from methionine. Except for the liver, however, most body cells lack this ability. Moreover, methionine is preferentially incorporated into cell proteins and in case of low methionine levels the conversion to cysteine would be minimized.[7] One of the enzymes in this conversion pathway, cystathionine synthase, requires Pyridoxine (Vitamin B_6) as part of the apoenzyme.[16] In a University of Florida Medical School study, Pyridoxine deficiency was found in 52% of HIV seropositive patients.[17] In severe Pyridoxine deficiency cysteine may become an essential amino acid.

HIV Enteropathy

Malabsorption, weight-loss and diarrhea are common clinical features associated with HIV infection. Opportunistic infections of the bowel with bacteria, fungi and protozoa are frequent findings. An HIV-associated enteropathy characterized by villous atrophy, crypt hyperplasia and decreased or absent enzyme activity has been found even in persons in the early stages of HIV infection with no identifiable enteric pathogens.[18,19]

Gastric acid secretion in patients with AIDS has also been studied and found to be markedly reduced.[20] [21] This not only contributes to malabsorption, but inhibits destruction of acid-sensitive pathogens. The combination of impaired gastric acid secretion and enteropathy could easily result in protein malabsorption.

Researchers at the University of Medicine and Dentistry of New Jersey studied protein and energy metabolism in patients with AIDS.[22] Nine subjects with CDC-defined AIDS were assessed for whole-body protein synthesis and glucose cycling using labeled substrates. They found that protein turnover, fibrinogen fractional synthesis and glucose cycling were significantly decreased compared to controls. All of the AIDS patients were free from acute infections, diarrhea, anorexia and had stable body weights. It was also found that total plasma amino acids and the ratio of essential to non-essential amino acids were significantly depressed. The researchers stated that their findings were consistent with a starvation-type response and adaptation to chronic energy shortage.

The similarities between the immune dysfunction in persons with AIDS and in those with protein-calorie malnutrition (PCM) have been noted by a number of researchers. Cutaneous energy, decreased numbers of T-lymphocytes, decreased helper/suppressor T-cell ratios and infection with *Pneumocystis carinii* pneumonia are common in both AIDS and PCM.[23] [24] [25] [26] Glutathione depletion may

be a common pathway contributing to the immune deficiency in both groups. The immune deficiency seen in PCM is reversible. The therapeutic possibilities of GSH augmentation in AIDS requires prompt and thorough investigation.

GSH Supplementation

Several approaches to GSH repletion are possible. Dietary supplementation with oral glutathione may be an effective approach. GSH has been shown to be absorbed intact by pinocytotic vesicles in pig jejunum.[27] This mechanism was strongly dependent on GSH concentrations and in other than ideal conditions significant quantities of GSH are likely to be hydrolyzed to constituent compounds. In rats, when GSH was used to replace cysteine in the diet, GSH was incorporated into plasma and hepatic proteins only 80% as efficiently as cysteine.[7]

Other researchers found that oral glutathione protected mouse intestinal epithelium from the degeneration induced by GSH deficiency.[28] In a series of experiments these researchers showed that mice which were made GSH-deficient by buthionine sulfoximine (a gamma-glutamylcysteine synthetase inhibitor) exhibited marked degeneration of the intestinal mucosa, characterized by 50% loss of height of the epithelial layer and microvillus desquamation. This degeneration was prevented by oral GSH administration but not by intra-peritoneal GSH injection. The authors therefore surmised that orally-administered GSH was absorbed by gut epithelium as di-peptides such as gamma-glutamyl cysteine which could then be used to re-synthesize GSH intracellularly, bypassing the inhibited gamma-glutamylcysteine synthetase.

The above authors note that bile contains significant amounts of GSH and they suggest that GSH plays a protective role in the intestinal lumen. They conclude that oral GSH and GSH monoesters can be an important source of cysteinyl residues with protective effects on intestinal mucosa. Their findings are particularly relevant for persons with AIDS who demonstrate GSH deficiency and intestinal degeneration.

The therapeutic potential of GSH in persons with AIDS is beginning to be investigated. A team of researchers from Cornell University Medical School and the National Institute of Health have found that GSH can inhibit HIV replication by up to 90% in vitro.[29] More studies are needed to assess the effect of GSH in people with HIV/AIDS.

Cysteine Supplementation

Cysteine supplementation is another possible approach to GSH restoration. Dietary cysteine supplementation has been viewed with caution by some authors due to the possibility that significant quantities of ingested cysteine may be oxidized to cystine in the gut lumen. However, cystine is readily absorbed by intestinal mucosa except in patients with cystinuria.[30] The absorption system can be saturated and competitively inhibited by the di-basic amino acids arginine, lysine and ornithine. In plasma, cystine levels are ordinarily higher than those of cysteine and cystine is readily taken up by cells of the kidney, fibroblasts and hepatocytes except in cystinurics.[30] Intracellular cystine is quickly reduced to cysteine. Within two minutes 62%, of cystine is converted to cysteine and 10% has been incorporated into GSH in cultured human fibroblasts.[31]

Lymphocytes, on the other hand, very poorly take up cystine, but readily take up cysteine. They seem to be dependent on plasma cysteine which depends on dietary intake and efflux from hepatocytes and other cells.[30] It has been shown that in stimulated lymphocytes, DNA synthesis, protein synthesis and cell viability were positively correlated with extracellular cysteine concentration and that this effect was not achieved by substituting with methionine or cystine.[15] Since macrophages take up cystine and release cysteine, it has been postulated that part of the mechanism by which macrophages activate lymphocytes is by exposing them to higher than normal cysteine concentrations when these cells come into close contact.[32]

While cystine is well tolerated by the healthy body, other products of cysteine oxidation may be of concern. The production of toxic oxygen species, including superoxide and hydrogen peroxide, during cysteine auto-oxidation has been documented.[33] Other potentially toxic sulphur residues such as cysteic acid may also be of concern. In well fed rats who were not GSH deficient, large amounts of cysteine (0.5-1.0 g/kg bodyweight) given intra-peritoneally or orally decreased GSH

levels in liver and brain.[34 35]

N-acetylcysteine has been shown to have a protective effect in animals subjected to oxidative stress such as acetaminophen overdose.[36] This effect is due to enhanced GSH synthesis. Persons with HIV infection have been shown to have evidence of oxidative stress.[37] Malondialdehyde (MDA), a marker of lipid peroxidation, was elevated in persons with HIV infection and was higher in persons with more progressed disease.

In addition to protection from oxidative stress, NAC has also been shown to inhibit HIV multiplication in vitro.[38] NAC may also have immune stimulating abilities. The growth of T-cells from people with AIDS/ARC was increased more than 200% when treated with NAC in vitro.[39]

The therapeutic possibilities of NAC in people with HIV is being investigated at Stanford University in the United States.

Dietary Approaches

In addition to amino acid supplementation, dietary modification may be a way of optimizing cysteine intake. Eggs are particularly rich in cysteine. However, the balanced amino acid profile of eggs may not be optimal for promoting cysteine incorporation into GSH.

Bounous argues that whey protein is an excellent source of dietary cysteine.[40] He points out that whey contains substantially more cysteine per gram than does casein and he has demonstrated that in mice, whey protein ingestion enhances splenic GSH levels and results in significant augmentation of the immune response as measured by plaque-forming cell assay.[41] The immune-enhancing effect of whey protein was greater than the effect of giving equivalent amounts of protein plus free L-Cysteine.

Treatment of infectious diseases with whey has historical antecedents as far back as Hippocrates.[40] He reportedly advised one to two litres of whey per day on a short term basis. Since whey protein contains a large proportion of bovine immunoglobulins, its usefulness in HIV infection may be limited. British researchers found that 25% of patients with HIV infection had abnormally high small intestine permeability.[42] Those persons with low ($< 0.1 \times 10^9$) T-cell counts and weight loss were more likely to have abnormal permeability. In these people, the bovine immunoglobulins may represent an additional antigenic load on an already compromised system.

The antigenicity of whey proteins could be reduced by hydrolysis. A group of American researchers reported that when they supplemented the diets of HIV-seropositive persons with whey peptides and medium chain triglycerides, positive results were achieved within eight weeks.[43] Ten out of seventeen subjects experienced weight gains and thirteen of seventeen reported decreases in diarrhea. Many of these people had been unable to tolerate whole protein supplements. Further investigations seem appropriate.

Other Approaches

It is possible that the effects of cysteine/ GSH deficiency could be partially compensated by vitamin and mineral supplementation. Adequate levels of Pyridoxine, for instance, would ensure efficient conversion of methionine to cysteine.

The anti-oxidant potential of the available GSH may be dependent upon adequate selenium intake. In normal rats exposed to hyperbaric pure oxygen, glutathione delays the onset of seizures and prolongs survival. However, the protective effect of glutathione was absent in selenium deficient rats, suggesting that glutathione peroxidase activity is necessary for protection from hyperoxia.[44] Selenium and glutathione peroxidase deficiencies have been documented in persons with AIDS.[45 46] In mice with selenium deficiency the ability of neutrophils to kill *Candida albicans* was impaired.[47] When healthy human volunteers were given 400 micrograms of sodium Selenite daily, the activity of their natural killer cells was significantly augmented after two weeks.[48] Selenium supplementation in persons with reduced GSH levels should proceed cautiously. In mice who were 80% GSH depleted, selenium was toxic at half the dosage that was toxic to mice with normal GSH levels.[49] Researchers from the University of Miami Medical School found that in persons with HIV, high levels of plasma selenium were associated with lower levels of IgG and IgM compared to those with normal or low selenium levels.[50] Low plasma selenium was associated with reduced natural killer cell cytotoxicity. Both excessive and low sele nium levels appear to be undesirable.

Another nutrient which may help to offset GSH deficiency is vitamin E. In patients with low GSH levels due to inborn glutathione synthetase deficiency, vitamin E supplementation resulted in increased erythrocyte survival and improved polymorphonuclear leukocyte function.[51] [52] This improvement was thought to be due to the anti-oxidant abilities of vitamin E. Vitamin E has also been shown to enhance the immune function of calves,[53] rats,[54] sheep,[55] mice[56] and humans.[57] It has been reported that persons with HIV infection have significantly lower vitamin E levels than controls.[46]

In experiments with newborn rats it was shown that vitamin C has a protective effect against the organ damage produced by GSH deficiency. In rats that were made GSH deficient by the injection of BSO, ascorbate reduced mortality in a dose-dependent fashion.[58] Vitamin C has also been shown to suppress HIV replication in vitro.[59]

Summary

It has been found that persons with HIV infection are deficient in glutathione and cysteine as well as folic acid, Pyridoxine, cyanocobalamin, zinc, selenium and vitamin E. These deficiencies are common and begin to appear early in the course of infection. They may be a result of a malabsorption syndrome which is accompanied by demonstrable changes in the intestinal epithelium. The nutrients in question have been shown to play an important role in normal immune response. The impact of these deficiencies is likely to be significant and could contribute to the progressive deterioration so often seen in this disease.

Replenishment of GSH levels is known to be possible using precursors such as cysteine or N-acetylcysteine and also by giving GSH and GSH monoesters orally. The beneficial effects of this approach has been demonstrated in a number of conditions. The low cost, ease of administration and lack of major toxicity make these possibilities attractive.

New therapeutic approaches to HIV infection are urgently needed. Current anti-viral pharmaceuticals possess significant toxicity and are palliative at best. The frequent incidence of severe toxicity is accepted because of the assumption that a debilitating disease must require 'strong' medicine. This largely unexamined, intuitive notion combines with political and economic considerations to result in intensive research on the development of new chemotherapeutic agents. In this context, the suggestion that natural substances, derived from food sources, can have a significant effect on the outcome of a life-threatening disease is all too easily dismissed. Whether aggressive nutritional therapy can forestall disease progression and improve the quality of life is a question that requires prompt investigation.

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