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Nutritional Regulation of Glutathione in Stroke

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In contrast to cardiovascular disease, the impact of nutritional status on the prevention and outcome of stroke has received limited investigation. We present a mechanism based on animal studies, clinical data, and epidemiological data by which protein-energy status in the acute stroke and immediate postinjury periods may affect outcome by regulating glutathione (GSH), a key component of antioxidant defense. As cysteine is the limiting amino acid for GSH synthesis, the GSH concentration of a number of nonneural tissues has been shown to be decreased by fasting, low-protein diets, or diets limiting in sulfur amino acids. The mechanism may also be relevant in brain since GSH in some brain regions is responsive to dietary sulfur amino acid supply and to the pro-cysteine drug, L-2-oxothiazolidine-4-carboxylate. The latter is an intracellular cysteine delivery system used to overcome the toxicity associated with cysteine supplementation. These findings may provide the mechanism to explain both the inverse correlation between dietary protein and stroke mortality and the documented association between suboptimal protein-energy status and diminished functional status following a stroke. Future investigations should examine the role of nutritional intervention in neuroprotective strategies aimed at improving stroke outcome. Pharmacological interventions such as L-2-oxothiazolidine-4-carboxylate should be investigated in animal models of stroke, as well as the impact of nutritional status on the response to these agents. Finally, micronutrient deficiencies that may accompany protein-energy malnutrition, such as selenium, should also be investigated for their role in antioxidant defense in cerebral ischemia.

Keywords: Glutathione, Glutathione peroxidase, L-2-oxothiazolidine-4-carboxylate, Stroke, Cerebral ischemia, Protein-calorie malnutrition, Elderly

INTRODUCTION

Stroke, a reduction in blood flow to a region of the brain (Juurlink and Sweeney, 1997), remains the third most common cause of death and a major cause of chronic disability in North America (Petrasovits and Nair, 1994; Shuaib and Kanthan, 1997). With an aging population, stroke represents a significant burden when measured on the basis of death, disability, and health care costs (Petrasovits and Nair, 1994). While stroke mortality has declined over the last 40 years, due to factors such as improved detection and treatment of high blood pressure (Petrasovits and Nair, 1994), fewer advances have been made in treating patients once a stroke has occurred. A future increase in the incidence of stroke is also anticipated as populations in industrialized countries age; these older patients are also likely to be more disabled by stroke (Pohjasvaara *et al.*, 1997). Given the huge psychological and economic costs

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associated with disability, there is a demand for new forms of treatment which will minimize brain damage following stroke (Juurlink and Sweeney, 1997). Nutritional factors may play a role. In contrast to cardiovascular disease, the impact of dietary factors and nutritional status on the prevention and outcome of stroke has received little attention. Most previous work has focused on nutrition as it relates to treating risk factors for stroke such as hypertension and diabetes mellitus.

Increased generation of reactive oxygen species is an important neurodestructive mechanism during and subsequent to a stroke, and current strategies aim to enhance the ability of the brain to scavenge these reactive compounds as a means of reducing disability (Hall, 1993; Juurlink and Sweeney, 1997). The intracellular glutathione (GSH) concentration is a critical component of antioxidant function that may be influenced by the stroke patient's nutritional status (Taylor *et al.*, 1996). Our laboratories have become interested in identifying nutritional factors that can regulate the GSH levels in brain, and in turn, to use this information to develop nutritional therapies to replete or enhance brain GSH for optimal antioxidant function. We review here the evidence that the protein-energy status of patients at the time of a stroke will affect stroke outcome. Micronutrients that may also impact on these processes are also briefly discussed.

PEROXIDE SCAVENGING IN CEREBRAL ISCHEMIA

While the precise mechanisms by which neural cells die during ischemia are still being defined, key interacting components include depletion of ATP, glutamate excitotoxicity, calcium overload, and production of strong oxidants that can overwhelm the antioxidant defense system. These events have been extensively reviewed in this symposium (Juurlink, 1999). While the increased superoxide anion produced under ischemia and reperfusion conditions can adversely affect cell function, it can also interact to produce powerful oxidizing agents, including the hydroxyl radical. The superoxide dismutase family constitutes the mechanism developed by cells to dismutate the increased superoxide anion produced during ischemia into hydrogen peroxide. It is essential that this hydrogen peroxide be removed as it can be reduced to the hydroxyl radical as mediated by reduced transition metals such as ferrous iron. The hydroxyl radical can damage cells by causing DNA strand breaks, protein oxidation, and lipid peroxidation. The latter is a particularly damaging effect since once initiated, this starts a chain of peroxidations, ultimately resulting in the formation of lipid peroxyl radicals and lipid hydroperoxides that disturb cell membrane function. The lipid hydroperoxides in the presence of iron can be converted to alkoxy and peroxy radicals resulting in new chains of lipid peroxidation (Chan, 1994; Grace, 1994; Gutteridge and Halliwell, 1990; Hall, 1993; Halliwell, 1994; 1996; Juurlink, 1996; 1997). An upregulation of proinflammatory genes also occurs as a result of the oxidative stress with upregulation of cell adhesion molecules on the endothelium and leucocyte infiltration, resulting in an inflammatory state that also contributes to the tissue damage (reviewed in Juurlink and Paterson, 1998).

The key importance of GSH and glutathione peroxidase (GPX) to efficient peroxide scavenging in neural cells has been described in some detail (Juurlink, 1996; Juurlink and Paterson, 1998; Juurlink and Sweeney, 1997). In most tissues, about 90% of cellular GPX activity is GPX1, the classical isoform localized to the cytosol and mitochondria that can scavenge hydrogen peroxide as well as organic peroxides such as free fatty acid hydroperoxides. Other major cellular activity is accounted for by a phospholipid hydroperoxide glutathione peroxidase (GPX4) that can scavenge membrane-bound phospholipid hydroperoxides and other organic peroxides (Gutteridge and Halliwell, 1990; Hall, 1993; Halliwell, 1994; 1996; Halliwell and Cross, 1994;

Halliwell and Gutteridge, 1990; Juurlink, 1996; 1997). GPX activity is dependent upon the presence of GSH which is oxidized in the process. As the efficiency of GPX for scavenging peroxides increases as a function of GSH concentration, small changes in GSH can have a large influence on the ability of the cell to scavenge peroxides (Juurlink, 1996). GSH also has a role in regenerating vitamin E that is important for scavenging lipid peroxyl radicals in membranes; GSH reduces oxidized ascorbate which directly reduces the α-tocopherol radical. GSH also exerts direct antioxidant effects (Chan, 1994; Grace, 1994; Gutteridge and Halliwell, 1990; Hall, 1993; Halliwell, 1994; 1996; Halliwell and Gutteridge, 1990; Juurlink, 1996; 1997). Evidence is also reviewed by Juurlink (1999) in this symposium that GSH influences other cell processes that are important in determining the extent of cell injury. These include prevention of the formation of advanced glycation products and inhibition of the transcription factor, NFκB, that is required for the expression of pro-inflammatory genes.

That brain GSH and GPX activity are important determinants of tissue damage associated with stroke has been illustrated in a number of ways. Ebselen (2-phenyl-1,2-benzisoselenazol-3[2H]-one), a seleno-organic compound that is believed to inhibit lipid peroxidation by mimicking GPX activity, protects against both permanent and transient ischemic brain damage in animal models (Dawson *et al.*, 1995; Johshita *et al.*, 1990). Based on work in cultured myocytes, ebselen has also been shown to increase the intracellular concentration of both reduced and oxidized glutathione and to elevate glutathione reductase (Hoshida *et al.*, 1997). Most recently, ebselen has been shown in a placebo-controlled, double-blind clinical trial in acute ischemic stroke to be protective if administered within 24 h of stroke onset (Yamaguchi *et al.*, 1998). Some protection against brain damage in patients with delayed neurological deficits after subarachnoid hemorrhage has also been demonstrated with this agent (Saito *et al.*, 1998). GSH depletion by buthionine sulfoximine enhances ischemic injury in rat cerebrum (Mizui *et al.*, 1992), and increasing brain GSH by administering a GSH ester immediately after an ischemic insult also offers neuroprotection (Gotoh *et al.*, 1994). The administration of N-acetylcysteine (NAC), a compound that promotes GSH synthesis, is also protective in some transient brain ischemia models (Knuckey *et al.*, 1995). The overproduction of GPX using transgenic mice overexpressing GPX1 reduced the volume of infarction, brain edema, and behavioral deficits in a model of focal cerebral ischemia followed by reperfusion (Weisbrot-Lefkowitz *et al.*, 1998). A moderate overexpression of GPX1 in hippocampus quite dramatically enhances the recovery of synaptic transmission in the CA1 area of hippocampal slices after transient exposure to hypoxia (Ghribi *et al.*, 1998).

GSH is decreased during ischemia and reperfusion in rat brain (Cooper *et al.*, 1980; Gotoh *et al.*, 1994; Noguchi *et al.*, 1989; Rehncrona *et al.*, 1980; Shivakumar *et al.*, 1992; 1995), suggesting an inability to maintain GSH homeostasis when generation of reactive species is increased. Shivakumar *et al.* (1995) have showed in a model using bilateral carotid artery occlusion and reperfusion that although an increase in oxidized glutathione (GSSG) levels accounts for a small portion of depleted GSH ($< 1\%$), the rest can be essentially recovered as protein–GSH mixed disulfide with accompanying loss of protein thiols. Brain GSH concentrations have also been shown to decrease with aging in animal models (Chen *et al.*, 1989; Ravindranath *et al.*, 1989), providing an important mechanism by which the aged brain will be more susceptible to injury by oxidative stress.

NUTRITIONAL REGULATION OF GSH

Current approaches in stroke management are attempting to enhance the ability of the brain to scavenge reactive oxygen and nitrogen species as a means of reducing the extent of brain damage

associated with stroke. Given the multiple and critical functions by which GSH can protect against cell injury, we suggest that nutritional intervention targeted to replete or enhance brain GSH may be an effective means of reducing this damage and associated disability. As cysteine is the limiting amino acid for GSH synthesis, the sulfur amino acid content of the diet is a major determinant of intracellular GSH levels. Conversely, specific nutrient deficiencies, acting through the same mechanism, may worsen the degree of functional recovery. The elderly are known to be vulnerable to a variety of nutritional problems resulting from altered physiological processes, medical, and social factors (Abbasi and Rudman, 1994; Lipschitz, 1991; Marcus and Berry, 1998).

It is during the 4–6 h window of opportunity following the stroke that therapies may limit the extent of brain injury (Shuaib and Kanthan, 1997). These rescue attempts are directed towards the penumbral region, that is, that area surrounding the core region in which blood flow is also reduced, but enough blood flow is present to allow the tissue to be viable (Juurlink and Sweeney, 1997). In addition to other medical therapies under study (Shuaib and Kanthan, 1997), it may be that nutritional strategies could also play a role in neuroprotection during this period. The increase in the permeability of the blood–brain barrier during reperfusion of ischemic tissue (Yang and Betz, 1994) may allow greater delivery of nutritional substrates such as sulfur amino acids or L-2-oxothiazolidine-4-carboxylate (discussed below) that is not possible under normal physiological circumstances.

GSH utilization, the reduction of GSSG by glutathione reductase, and the *de novo* synthesis of GSH will determine the GSH status of tissues under conditions of oxidative stress. Cooper and Meister (1993) have reviewed the regional GSH concentrations in the brain; whole brain levels from a number of species vary from 1 to 3 mM, with a maximum cerebral GSSG concentration of 1% of total GSH. In the synthesis of GSH (L-γ-glutamyl-L-cysteinylglycine), γ-glutamylcysteine is formed in the initial rate-limiting step that is catalyzed by γ-glutamylcysteine synthetase. This initial step is considered to be an important mechanism for regulating the maximum tissue concentration of GSH *in vivo*. In the second step, GSH synthetase catalyzes the reaction between glycine and γ-glutamylcysteine to form GSH (Bray and Taylor, 1993; Meister *et al.*, 1986; Taylor *et al.*, 1996). As plasma cysteine concentrations are low, additional sources of cysteine for this reaction are supplied from reduction of cystine and by synthesis from methionine via the cystathionine pathway. In many extrahepatic tissues, the amino acid substrates for GSH synthesis are also provided by efflux of hepatic GSH into plasma and the uptake of plasma GSH via the γ-glutamyltranspeptidase reaction into these tissues. However, while intraorgan and interorgan cycles of GSH are well described for tissues such as liver and kidney, much less is known about the involvement of brain in these cycles (Cooper and Meister, 1993).

While the enzymes of the GSH cycle and their regional specificity provide the basis for intracellular maintenance of tissue GSH concentrations, diet and nutritional status also play a role in determining GSH concentrations. A number of comprehensive reviews have been published on this topic (Bray and Taylor, 1993; 1994; Taylor *et al.*, 1996). Cysteine is the limiting amino acid for GSH synthesis, and the sulfur amino acid content of the diet is a major determinant of GSH concentration in tissues such as liver (Taylor *et al.*, 1996). The GSH concentration of tissues such as lung and liver is decreased by fasting, low-protein diets, or diets limiting in sulfur amino acids (Bauman *et al.*, 1988a; Benuck *et al.*, 1995; Taylor *et al.*, 1992). The response of liver GSH to dietary protein is found only in the physiological range, with no further increase with excessive dietary protein (Bauman *et al.*, 1988a; Hum *et al.*, 1992). Figure 1 demonstrates the response of liver cysteine and GSH to dietary protein; 4% and 7.5% dietary protein are below the requirement of

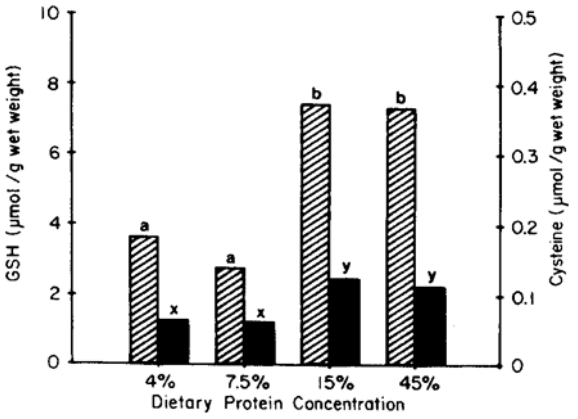

FIGURE 1 Effect of dietary protein on rat hepatic GSH (hatched bar) and cysteine (solid bar) concentrations. Values are a mean for five animals per group. Mean values not sharing a common letter are significantly different ($P < 0.05$) by Tukey's test. SEM are 0.336 for GSH and 0.0085 for cysteine. Data are reprinted with permission from *Can. J. Physiol. Pharmacol.* **66**, 1048–1052, 1988. Copyright (1988) National Research Council of Canada.

the growing rat, and 45% represents excess dietary protein. Sulfur amino acid deficiency also dampens the ability to restore GSH levels in liver and lung in response to an inflammatory challenge (Hunter and Grimble, 1997).

We have begun to investigate whether brain GSH homeostasis is also responsive to these nutritional factors. In our studies, adult female Long–Evans rats confronted with a short-term dietary deficiency of sulfur amino acids show decreases of 10–14% in the GSH concentration of neocortex, hippocampus, thalamus, and striatum (Paterson *et al.*, 1998). Although regional differences in brain GSH concentration were apparent, as previously suggested (Cooper and Meister, 1993), all areas studied appear to be susceptible to the deficiency. We propose that this modest depletion of brain GSH by the reduced supply of dietary precursors will be important at the time of a stroke when the rate of GSH utilization and the need for synthesis are increased. Thorburne and Juurlink, (1996) have previously shown that relatively small changes in intracellular GSH markedly increase the ability of oligodendroglial cells to handle oxidative stress. In contrast to our work, Benuck *et al.* (1995) reported no change in total GSH concentration of brain cortex, cerebellum, or pons medulla in response to a 48 h fast in either young or aged rats. It will be of interest to determine whether the effects on brain GSH metabolism induced by an acute, severe sulfur amino acid deficiency or a chronic moderate protein deficiency exacerbates tissue damage in an animal model of hypoxia–ischemia.

PROTEIN-ENERGY STATUS IN STROKE

While the work reviewed above suggests that nutritional status may be critical to the maintenance of brain GSH, evidence indicates that a subset of the most elderly, the group at highest risk for stroke (Mayo, 1993), are compromised with respect to protein-energy status. The elderly, both in their own homes and in institutions, are at high risk for nutritional deficiencies because of anorexia, poor dental status that impairs eating, drug therapy, decreased basal metabolic rate and activity levels, and decreased access to familiar foods (Abbasi and Rudman, 1994; Lipschitz, 1991; Marcus and Berry, 1998). The problem of refusal to eat in elderly in both institutional and community settings is complex and causes include aging-associated physiological changes, dementia, and depression as well as a variety of other medical and social factors (Marcus and Berry, 1998). These factors may be combined with a chronic disease that increases nutrient requirements. Protein-energy malnutrition is a common outcome (Constans *et al.*, 1992; Lesourd, 1995; Mowé and Bøhmer, 1991; Potter *et al.*, 1995) that has been documented in 30–50% of American nursing home residents (Abbasi and Rudman, 1994). In some cases, the onset is associated with a relatively minor stress of short duration (Lipschitz, 1991). Protein-energy malnutrition has been identified as an independent risk factor for morbidity and mortality in the elderly (Mühlethaler *et al.*,

1995; Sullivan and Walls, 1994; Sullivan *et al.*, 1995), yet it is known to be underdiagnosed and undertreated (Lipschitz, 1991; Mowé and Bøhmer, 1991).

Evidence has emerged from Spain (Dávalos *et al.*, 1996), Sweden (Axelsson *et al.*, 1988), and the United Kingdom (Gariballa *et al.*, 1998) that the problem of suboptimal protein-energy status exists in a significant proportion of patients at the time of admission for an acute stroke. These assessments, using both biochemical and anthropometric markers of protein-energy status, were done at timepoints varying from less than 24 h to 4 days following the stroke. Axelsson *et al.* (1988) and Dávalos *et al.* (1996) reported poor protein-energy status in approximately 16% of acute stroke admissions. A study from Korea (Choi-Kwan *et al.*, 1998) documented undernutrition to be as high as 25% in patients with cerebral infarction and 62% in patients with intracerebral hemorrhage as compared to 13% in age-matched controls. Since their anthropometric assessments were done up to one week following admission, nutritional problems occurring secondary to the stroke may partially account for the higher proportion of malnourished patients. There are two other important findings from these studies. Both Dávalos *et al.* (1996) and Gariballa *et al.* (1998) have shown a correlation between the clinical markers of protein-energy malnutrition at admission for acute stroke and increased risk of morbidity and mortality. Secondly, there is good evidence that nutritional status deteriorates during the stroke patient's hospital stay (Axelsson *et al.*, 1988; Gariballa *et al.*, 1998). Canadian data suggest that rates of malnutrition can be as high as 49% among stroke patients at the time of transfer from acute treatment to rehabilitation services (Finestone *et al.*, 1995). While nutritional problems occurring as a result of the stroke, such as dysphagia and physical disability, will be major contributors to this high rate, these data also suggest that nutritional intervention is not likely sufficient in the immediate postinjury period to optimize antioxidant defense mechanisms.

EPIDEMIOLOGICAL STUDIES: DIETARY PROTEIN AND STROKE INCIDENCE AND MORTALITY

The epidemiological evidence supporting an inverse correlation between dietary protein and stroke incidence and mortality has been reviewed by Klag and Whelton (1993). Much of the information comes from studies that either correlate within-population trends in stroke mortality with trends in food consumption or compare levels of mortality with differences in food consumption between countries or regions. Accompanying the decline in cerebrovascular disease mortality in Japan since 1970 (Kodama, 1993) has been an increase in per capita intake of protein, particularly from animal sources (Omura *et al.*, 1987). In an ecologic study of 600 geographic areas within Japan, intake of animal protein appeared to have a protective effect (Omura *et al.*, 1987). An inverse association between protein intake and total stroke incidence was also found in the Honolulu Heart Program, a longitudinal cohort study (Kagan *et al.*, 1985). In 16-year follow-up data from the Honolulu study, animal protein was inversely associated with the incidence of both fatal and nonfatal thromboembolic stroke, independent of other stroke risk factors (Lee *et al.*, 1988). In fact, a diet low in food from animal sources is one of the key dietary factors that has been associated with the paradox of high risk of stroke in populations with low risk of coronary heart disease (Reed, 1990). In contrast, the percentage of energy obtained from protein was not associated with 12-year stroke mortality in a prospective population-based study of Swedish women (Lapidus *et al.*, 1986), nor was total dietary protein, adjusted for energy intake, related to 12-year stroke mortality in a southern Californian cohort (Khaw and Barrett-Connor, 1987). It has been suggested that the discrepancy in these results may be due to the higher level and narrower range of protein intake in the Swedish and California populations (Klag and Whelton, 1993). However, data from the First National Health and

Nutrition Examination Survey (NHANES I) Epidemiologic Follow-up Study in the United States shows low serum albumin level to be a risk factor for stroke (Gillum *et al.*, 1994). White men aged 65–74 years with serum albumin concentrations $>4.4\,g/dL$ had a risk of stroke incidence over a follow-up period of 9–16 years of approximately two-thirds that of men with serum albumin concentrations of $<4.2\,g/dL$, an effect that remained after controlling for multiple stroke risk variables. In blacks aged 45–74 years, serum albumin concentrations $>4.4\,g/dL$ were associated with a risk of stroke incidence of one-half and a risk of stroke death of one-fourth that seen at serum albumin levels $<4.2\,g/dL$, after controlling for other risk factors. The mechanism for the effect of serum albumin on stroke risk is unknown, but it is important to note that low serum albumin concentration, although responsive to many nonnutritional factors, is also an indicator of depleted visceral protein status (Gibson, 1990). Thus, these data contribute further support to the idea that suboptimal protein status may be an important risk factor for stroke outcome. While it is unknown whether these trends are causally related, the relationship between protein-energy status and tissue GSH described above could provide a possible explanation for the epidemiological studies.

Data from the stroke-prone spontaneously hypertensive rat support the epidemiological evidence for a link between protein status and stroke susceptibility. The incidence of stroke in this model was dramatically reduced by the diet fed in rat colonies in the United States when compared to the diet originally used with this strain in Japan (Yamori *et al.*, 1984). This finding was independent of a change in blood pressure, and the most dramatic difference between the diets was the lower protein composition of the Japanese diet. These findings are intriguing and illustrate the potential for nutritional status to exert either a permissive or preventative effect on the expression of risk factors for stroke.

INTERACTION BETWEEN NUTRITIONAL AND PHARMACOLOGIC STRATEGIES TO ENHANCE BRAIN GSH

It has been reported that the administration of a GSH ester, although effective in enhancing the GSH concentration in a number of tissues, does not increase brain GSH (Anderson *et al.*, 1985). However, the findings by Gotoh *et al.* (1994) and Noguchi *et al.* (1989) that administering a GSH ester immediately after an ischemic insult increases brain GSH and offers neuroprotection illustrates the need to test the efficacy of such compounds under situations in which the requirement for GSH is increased by oxidative stress. An alternative approach that has been effective in a number of tissues is to enhance GSH synthesis by administering one of the pro-cysteine drugs, L-2-oxothiazolidine-4-carboxylate (OTC) or NAC, the antidote for acetaminophen overdose (Hazelton *et al.*, 1986). Anderson and Meister (1987) and Meister *et al.* (1986) have discussed this strategy for intracellular delivery of GSH, and Juurlink and Paterson (1998) have reviewed some of the current applications of NAC and OTC being investigated in clinical medicine. Among the latter are the adult respiratory distress syndrome (Bernard *et al.*, 1997), human immunodeficiency viral infection (Barditch-Crovo *et al.*, 1998; Kalayjian *et al.*, 1994), and diabetes (De Mattia *et al.*, 1998). These compounds have been shown to be protective in a variety of tissue ischemia and oxidative stress-induced tissue damage models (DiMari *et al.*, 1997; Lüthen *et al.*, 1997; Nakano *et al.*, 1995; Radice *et al.*, 1997; Tsan *et al.*, 1985).

OTC, which readily promotes GSH synthesis (Meister *et al.*, 1986; Williamson *et al.*, 1982; Williamson and Meister, 1981), is converted intracellularly to cysteine by the enzyme 5-oxoprolinase, and has been used as an intracellular cysteine delivery system to overcome the toxicity associated with cysteine supplementation (Anderson and Meister, 1987). OTC is also active as a cysteine precursor when administered orally

(Chung *et al.*, 1990). Meister *et al.* (1986) showed that OTC can be transported into brain and converted to cysteine and to glutathione. Increases in rat brain GSH with a peak rise of 41% have been reported following subcutaneous administration of OTC (Mesina *et al.*, 1989), although others have been unable to reproduce these data (Pileblad and Magnusson, 1992). Anderson and Meister (1989) reported a 25% increase in rat brain cortex GSH at 6 h following intraperitoneal administration of OTC with smaller changes in other brain regions.

There is evidence that nutritional status interacts with such pharmacological agents to enhance or replete tissue glutathione. The work of Bauman *et al.* (1988b) suggests that the previous protein status of a patient may influence the GSH response to sulfur amino acid supplementation whether delivered by pharmacological or nutritional means. Their study showed that rats previously exposed to protein deficiency had a more rapid increase in liver GSH concentration in response to a diet adequate in protein or a dietary supplement of the cysteine prodrug, OTC, than animals fed a diet adequate in protein throughout the study (Bauman *et al.*, 1988b). This may have direct relevance for the elderly stroke patient who is protein-energy-compromised. The OTC also increased hepatic GSH concentration above the physiological maximum when liver GSH had been previously depleted; the mechanism responsible for this overshoot phenomenon is unknown (Bauman *et al.*, 1988b). The responsiveness of tissue GSH to nutritional intervention would also be expected to vary with the extent of oxidative stress given that the rate of utilization of GSH will also be involved in controlling GSH turnover. This is supported by the work of Taylor *et al.* (1992) who showed that OTC administered to rats exposed to hyperoxia effectively increased the GSH concentration of lung and protected against pulmonary damage (Fig. 2) in both protein-malnourished and control rats. The assessment of lung damage shown in Fig. 2 was also confirmed by *in vivo* proton magnetic resonance imaging. A study by

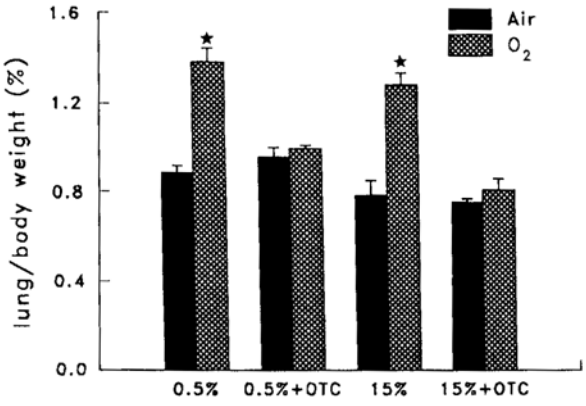

FIGURE 2 Effect of oral supplementation of OTC and hyperoxia exposure on lung-to-body weight ratios in rats fed 0.5% or 15% protein diet. Values are expressed as mean ± SEM, $n = 3$ or 4. Significant ($P < 0.05$) main effects are diet, hyperoxia exposure, and diet and hyperoxia exposure interaction. Values marked with a star are significantly different from both the value in the respective air control group and the value in the respective OTC-supplemental group. Data are reprinted with permission from *FASEB J.* **6**, 3101–3107, 1992. Copyright (1992) Federation of American Societies for Experimental Biology.

Levy *et al.* (1998) has highlighted the usefulness of combining OTC as a short-term strategy for elevating GSH in a target organ in protein-calorie malnutrition with long-term nutritional intervention. They showed in severely protein-calorie malnourished rats that OTC was more effective than protein repletion in restoring GSH levels (Fig. 3) and protecting the lung against hyperoxia-induced damage. The control group in Fig. 3 refers to the PEM (protein-energy malnourished) rats which did not receive either a protein repletion diet or OTC. OTC has also been shown to maintain liver and lung GSH concentrations under conditions of sulfur amino acid deficiency (Jain *et al.*, 1995). The efficacy of a dietary supplement of OTC in maximizing brain GSH is yet to be studied under varying protein-energy or sulfur amino acid status. A recent report has emphasized the difference in tissue specificity for different GSH precursors (Li *et al.*, 1998), which will be important for the development of strategies to protect the CNS against free radical damage.

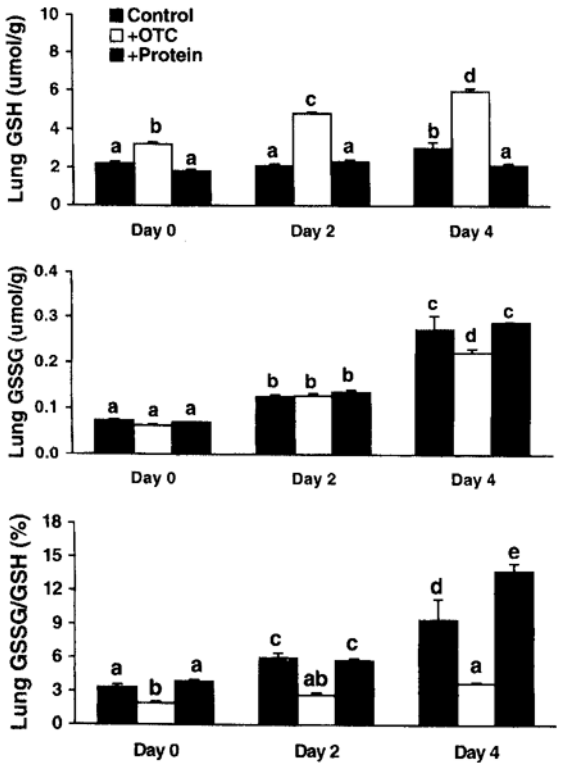

FIGURE 3 Effect of L-2-oxothiazolidine-4-carboxylate (OTC) supplementation or protein repletion on lung reduced glutathione (GSH), oxidized glutathione (GSSG) and the ratio of GSSG/GSH in PEM rats during 4 days of hyperoxia exposure. Values are expressed as means ± SEM, $n = 6$. Values not sharing a common letter are significantly different ($P < 0.05$). Data are reprinted with permission from *J. Nutr.* **128**, 671–676, 1998. Copyright (1998) American Society for Nutritional Sciences.

OTHER NUTRITIONAL FACTORS

While we have emphasized the importance of the supply of dietary sulfur amino acids and energy for maintenance of GSH, impaired protein-energy status is often accompanied by micronutrient deficiencies which may also influence tissue GSH and GPX activity. For example, glutathione reductase, which plays a key role in regulating intracellular GSH levels by regenerating GSH from its oxidized form, is a flavin enzyme, and thus its activity is influenced by riboflavin intake (Beutler, 1989). The trace element, selenium, may be of particular importance. Low plasma selenium levels have been reported in critically ill patients, suggesting that selenium metabolism is disturbed (Forceville *et al.*, 1998). If this proves to be the case in patients with stroke, Se deficiency may contribute to neural damage through a number of interrelated mechanisms, resulting in increased peroxidative damage. As a structural component of the active center of GPX (Burk and Hill, 1993), Se has a clear role in ensuring efficient peroxide scavenging. Thus, Se deficiency may depress GPX activity, with the resultant oxidative stress inactivating thiol enzymes such as glutathione reductase (Tabatabaie and Floyd, 1994), providing a mechanism for further depletion of GSH. Se deficiency also appears to have a direct influence on GSH metabolism. Se deficiency in the rat increases liver GSH synthesis and release into the blood, resulting in a 2–3-fold elevation of plasma GSH concentration (Hill and Burk, 1982; 1985). The mechanism responsible for this altered interorgan metabolism of GSH is unknown. However, Burk *et al.* (1995) examined the relationship between GSH and Se by administering phorone, a glutathione-depleting agent to Se-deficient rats. Depletion of GSH in Se deficiency resulted in marked liver and kidney necrosis associated with increased lipid peroxidation. The influence of dietary selenium on GSH metabolism in tissues other than liver has rarely been studied. Brain GSH concentration in response to either inadequate or supplemental selenium supply has not been reported.

We have begun to address the first question by examining whether dietary Se regulates the expression of GPX in brain. The family of GPX selenoproteins incorporate Se into their active sites in the form of selenocysteine. In the majority of mammalian tissues, there is a relationship between GPX activity, dietary Se level, and susceptibility to certain types of oxidative stress (Buckman *et al.*, 1993). In rat liver, Se deficiency can result in a loss of GPX1 activity to less than 1% of control values and this is accompanied by loss of immunoreactive GPX1 protein (Knight and Sunde, 1987). Selenoproteins are also regulated

individually through changes in their mRNA levels. This is thought to allow maintenance of certain selenoproteins at the expense of others when selenium supply is inadequate for optimal expression of all selenoproteins (Burk and Hill, 1993). Lei *et al.* (1995) have demonstrated in liver, heart, kidney, and lung that GPX1 activity and mRNA levels are more susceptible to selenium depletion than GPX4. The data on dietary Se and isoforms of GPX in brain are much less clear although it has been shown that brain is better able to retain Se at the expense of other tissues when Se supply is limited (Buckman *et al.*, 1993). Also, few data have been available on the response of brain GPX to supplementing dietary Se above requirement. Some have reported dietary Se restriction to decrease total GPX activity in rat brain by 25–39% (Beckett *et al.*, 1989; Lawrence *et al.*, 1974; Watanabe and Satoh, 1994; Whanger and Butler, 1988). Castaño *et al.* (1993) have even reported decreases of 21–22% in substantia nigra and striatum after only 15 days on a low Se diet. In contrast, Prohaska and Ganther (1976) and Buckman *et al.* (1993) found no influence of dietary selenium on total GPX activity in brain. The discrepancy in results may be due to varying degrees of Se deficiency among experiments or to contamination of the tissue with red blood cells. Methodological differences in measurement of GPX activity likely also account for some of the differences as GPX activity is extremely low in brain. Studies of the distribution of selenium content and GPX activity show brain to be among the lowest of tissues studied in the rat (Behne and Wolters, 1983). The low activity of GPX in neurons has been shown to correlate with low GPX protein levels (Eftekharpour *et al.*, 1998).

Our preliminary studies suggest that total GPX1 activity in at least some brain regions is not sensitive to either deficient levels of dietary Se or to excess Se provided above the dietary requirement (Paterson, 1998). Other brain regions are currently being examined for GPX activity and GPX1 protein and mRNA to fully answer this question. It may be that the tissue hierarchy for Se partitioning protects the brain when Se is in short supply. It has been shown that with decreased selenium intake, brain has very high retention of selenium relative to other tissues (Behne *et al.*, 1988). Previous work of Lei *et al.* (1995) has also illustrated important differences among tissues in how dietary Se regulates the expression of selenoproteins. In contrast to liver, heart, kidney, and lung, dietary Se deficiency causes a much smaller decrease in testis GPX1 activity and has no influence on GPX1 mRNA; activity and mRNA level for GPX4 are unaltered. In similar studies in liver, heart, and thyroid, Bermano *et al.* (1995) have confirmed these differences in regulation of expression of these two enzymes by dietary Se both within and among tissues. The important question, however, still remains unanswered, and this is whether Se deficiency dampens the ability to upregulate these selenoproteins in brain under conditions of increased oxidative stress generated during acute stroke and the reperfusion period.

CONCLUSIONS

In contrast to cardiovascular disease, the impact of nutritional status on the prevention and outcome of stroke has received little attention. Nutritional intervention has previously been appreciated in prevention strategies for treating risk factors for stroke such as hypertension and diabetes mellitus. In this review, a mechanism has been proposed by which nutritional status in the acute stroke and immediate postinjury periods may affect outcome by regulating a key component of antioxidant defense. We have predicted that an important relationship exists between protein-energy status and the enhancement of brain GSH for optimal antioxidant defense on the basis of animal studies, data from stroke patients, and epidemiological studies. As nutritional status is compromised for a significant proportion of patients during acute stroke, nutritional intervention should be examined as a component of neuroprotective strategies aimed at improving

stroke outcome. The efficacy of OTC should be examined in cerebral ischemia models via its role in enhancing GSH synthesis, and the existing data suggest that nutritional status should be examined for its impact on the response to this agent. Finally, deficiencies of micronutrients such as selenium that may accompany protein-energy malnutrition should also be investigated for their role in optimizing GSH and GPX for optimal antioxidant defense in cerebral ischemia.

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