Anti-oxidant strategies to treat stroke

Bernhard H.J. Juurlink

Department of Anatomy and Cell Biology, College of Medicine, University of Saskatchewan,
107 Wiggins Road, Saskatoon, SK, S7N 5E5, Canada

Introduction

There is an abundance of evidence that there is significant oxidative stress following stroke [1]. Human trials with therapies aimed at reducing oxidative stress have, however, been disappointing. The trials with the 21-aminosteroid tirilazad mesylate suggests that this anti-oxidant therapy does not result in better outcome following stroke [2] and may even result in worse outcome [3]. The reasons for these failures are understandable when pathways of oxidative stress are examined. A consideration of the consequences of oxidative stress at the cellular level and mechanisms used by the cell to minimize oxidative stress ought to provide the theoretical basis for rationally pursuing therapies directed towards minimizing oxidative stress.

Oxidative stress and cell dysfunction

Strong oxidants such as the hydroxyl radical [4] or peroxynitrous acid [5] can cause polyunsaturated fatty acid peroxidation that, in the presence of oxygen, initiates a self-perpetuating chain reaction of lipid peroxidation causing alterations in membrane fluidity [6], increased permeability of membranes [7] and decreased membrane ATPase activity [8]. This results in increased Na^+ and Ca^{2+} influx resulting in depletion of cellular ATP stores and all the problems associated with cellular Ca^{2+} overload [9, 10]. Lipid radicals and peroxides also break down (Fig. 1) forming pro-inflammatory isoprostanes [11] and isoleukotrienes [12] or strong oxidants including dicarbonyls such as malondialdehyde [13] and α,β -unsaturated aldehydes [14, 15]. The latter are strong oxidants that can interfere with critical cellular functions such as glutamate uptake [14, 16], alter membrane protein configuration [7], interfere with maintenance of ion homeostasis [17], as well as mitochondrial respiration [18]. In addition, other oxidants such as peroxynitrous acid inhibit the activity of the mitochondrial respiratory chain proteins [19].

Oxidative stress can promote activation of pro-inflammatory gene cascades activating nuclear factor κB (NF- κB), reviewed in [20]. NF- κB is a transcriptional

factor complex whose activation is required for maximal transcription of a wide array of pro-inflammatory proteins important in the generation of acute inflammation [21].

Mechanisms of strong oxidant production and scavenging

Glutathione (GSH)-dependent scavenging mechanisms

This is illustrated in Figure 1. References for these pathways can be found in [20, 22]. Note that GSH plays important roles in: (1) scavenging peroxides, both hydrogen peroxide and lipid peroxides, catalysed by the selenoproteins glutathione per-

Figure 1

Some pathways involved in generation and scavenging of strong oxidants. To reduce clutter, the reactions are not necessarily balanced and sometimes leave out intermediate steps. Superoxide can interact with another molecule of superoxide (#1) to form the strong oxidant, singlet oxygen. Superoxide can also interact with nitric oxide to give rise to peroxynitrous acid (#2) that in turn can give rise to strong oxidants such as the nitrogen dioxide and hydroxyl radicals (#3). Hence, superoxide is scavenged by superoxide dismutase (SOD) to form hydrogen peroxide and molecular oxygen (#4). In the presence of transition metal ions, hydrogen peroxide can give rise to the hydroxyl radical (#5). Hydrogen peroxide is scavenged by glutathione peroxidase (GPx) that requires GSH as the electron donor (#6). The oxidized-glutathione is reduced by glutathione reductase (GRed) that uses NADPH as the electron donor (#7). The hydroxyl radical can abstract an electron from a polyunsaturated fatty acid (LH) to form a carbon-centred lipid radical (#8). The lipid radical can interact with molecular oxygen to form a peroxyl radical that in turn can abstract an electron from a polyunsaturated fatty acid initiating a lipid peroxidation chain reaction (#9). The oxidative stress that ensues can activate NF- κ B. The lipid peroxides that are formed are scavenged by either thioredoxin reductase using thioredoxin (Tr[SH]_2) as electron donor (#10) or glutathione peroxidase using GSH as the electron donor (not presented). It is necessary to scavenge the lipid peroxides since they can interact with transition metal ions to give rise to new lipid peroxyl radicals (#11) that can initiate new rounds of lipid peroxidation. Lipid peroxyl radicals are quenched by vitamin E (TOH) giving rise to an innocuous vitamin E radical (#12). The vitamin E radical is reduced by ascorbic acid (#13) and the oxidized ascorbate is reduced by GSH (#14). Lipid peroxides can also break down (#15) into: strong oxidants such as malondialdehyde (#16); 4-hydroxynonenal (#17) which is scavenged by glutathione S-transferase (GST) by formation of an adduct with GSH; isoleukotrienes (#18); and isoprostanes (#19). In the presence of transition metal ions, superoxide anion can also convert sugars to strong oxidants such as the dicarbonyl glyoxal (#20). Dicarbonyls are scavenged by the glyoxalase pathway that uses GSH as a cofactor (#21).

oxidases [23]; (2) the reduction of oxidized ascorbate [24], which is essential for the ultimate regeneration of vitamin E from the vitamin E radical; (3) in neutralizing strong oxidants including α,β -unsaturated aldehydes such as 4-hydroxynonenal by forming glutathione adducts catalyzed by various glutathione S-transferases (GSTs) [25]; (4) as an essential cofactor in the scavenging of α -oxo-aldehydes such as glyoxal and methylglyoxal [26]. Cellular levels of GSH typically vary from 2–10 mM [27].

Vitamin E, along with ascorbic acid, plays an important, albeit limited, role in peroxide scavenging by inactivating lipid peroxyl radicals, giving rise to innocuous vitamin E radicals plus lipid hydroperoxides. Inactivation of lipid peroxyl radicals does not eliminate the problems encountered with the lipid peroxidation cascade since lipid hydroperoxides can interact with transition metal ions giving rise to alkoxyl radicals, peroxyl radicals, α,β -unsaturated aldehydes or reactive aldehydes such as malondialdehyde and 4-hydroxyalkenals, as well as isoprostanes and isoleukotrienes: this is likely why anti-oxidant approaches such as administering tirilazad mesylate have not been proven to be efficacious treatments for oxidative stress.

Thioredoxin-dependent scavenging mechanisms

Thioredoxin(Trx)-dependent peroxidases (peroxiredoxins) [28] also scavenge peroxides; the significance of the peroxiredoxins in peroxide scavenging is still not clear. Oxidized-Trx is reduced by the selenoprotein Trx reductase (TrxRed). The typical concentration of Trx within cells is approximately 10 μ M [29, 30]; hence, the efficiency by which Trx-dependent scavenging occurs is dependent upon the ability of the cell to reduce oxidized-Trx. TrxRed has been shown to have a number of other important anti-oxidant functions that include: (1) reduction of the ascorbyl radical [31] as well as oxidized-ascorbate [32] and lipoic acid [33]; and (2) with selenocyst(e)ine as a cofactor, TrxRed can also directly scavenge lipid peroxides [34] as well as peroxynitrite [35].

Central anti-oxidant players

GSH

During oxidative stress the ability to synthesize GSH is critical. Regulation of GSH synthesis is complex and involves the following two reactions [36]:

- (1) $\text{l-glutamic acid} + \text{l-cysteine} + \text{ATP} \leftrightarrow \text{l-}\gamma\text{-glutamyl-l-cysteine} + \text{ADP} + \text{P}_i$
- (2) $\text{l-}\gamma\text{-glutamyl-l-cysteine} + \text{ATP} + \text{glycine} \leftrightarrow \text{glutathione} + \text{ADP} + \text{P}_i$

The first reaction, catalyzed by 1- γ -glutamyl-1-cysteine synthase (GCS), is the rate-limiting reaction that is inhibited by GSH with the GSH-dependent inhibition alleviated by glutamate [37]. This first reaction is also rate-limiting in that cysteine is the rate-limiting amino acid. Cellular cysteine content is regulated principally by the uptake of cystine since cysteine is readily auto-oxidized. Cystine is taken up (or released) by the X_c^- antiporter in exchange for glutamate [38]; intracellularly, one molecule of cystine is reduced to two molecules of cysteine. The intracellular concentration of glutamate, thus, has a two-fold regulatory effect on intracellular GSH concentration: (1) alleviating the GSH inhibition of GCS, and (2) regulating uptake of cystine.

Phase 2 proteins

Another approach to increase intracellular GSH is to increase GCS activity. GCS is a heterodimeric enzyme whose 2 subunits are under the control of the anti-oxidant response element (ARE) [39]. The transcription of genes with an ARE in the promoter region can be induced by phase 2 protein inducers. There are a number of compounds (mainly Michael reaction acceptors, quinones and certain isothiocyanates) at low micromolar concentrations that selectively activate the anti-oxidant response; such phase 2 protein-inducing compounds can form components of our diet, see [22]. In addition to GCS, the following proteins belong to the phase 2 protein family: quinone reductase, GSTs, epoxide hydrolase, UDP-glucuronosyl-transferase [40], TrxReds, both cytosolic and mitochondrial isoforms [41], inducible heme oxygenase, peroxiredoxin I, X_c^- cystine/glutamate antiporter [42] and ferritin H [43].

There has been considerable study of phase 2 enzymes and their induction in the context of cancer prevention, e.g., [44, 45], but little attention has been paid in the context of other diseases. In principle, induction of phase 2 proteins ought to decrease oxidative stress.

Decreasing oxidative stress in the context of stroke

Stroke prevention

There is a link between diet and atherosclerosis, with diets high in fruits and vegetables being correlated with lower incidence of heart attacks and stroke [46, 47]. Factors that predispose one to atherogenesis all cause oxidative stress at the level of the endothelium, e.g., hypercholesterolemia, hypertension [48–50], homocysteine [51–55], hyperglycemia associated with diabetes increases the formation of advanced glycation endproducts [56, 57] which also causes increases in oxidative

stress, while bacterial endotoxins activate pro-inflammatory gene expression in endothelial cells [58]. Therefore, decreasing oxidative stress ought to inhibit development of atherosclerosis. I have argued elsewhere [22] that a diet rich in phase 2 protein inducers will possibly inhibit atherogenesis; hence, I shall not dwell at length on this topic.

Decreasing oxidative stress once a stroke has occurred

One of the problems with nutritional studies in relationship to disease is that such studies tend to focus on one nutritional component without considering the interactions amongst nutritional components. For example, there are many studies on vitamin E which do not take into consideration the interaction between vitamin E radical and vitamin C, nor the importance of GSH and selenoproteins in the reduction of oxidized ascorbate and scavenging of the lipid hydroperoxides that are an endproduct of lipid peroxidation.

Nutritional intake of stroke patients is often suboptimal and malnutrition can occur secondary to stroke [59–61]. Following a stroke there is a rapid reduction of plasma anti-oxidants [62]. To date, nutritional support following trauma to the CNS been mainly targeted toward achieving caloric and nitrogen balance to prevent protein-energy malnutrition [63]. It is suggested that following a stroke there be aggressive nutritional intervention that not only targets caloric and nitrogen balance, but also promotes anti-oxidant activities of tissues.

Selenium and vitamins C and E

GPx's and TrxRed's are selenoproteins and the synthesis of these proteins is dependent upon the availability of selenium. Low serum selenium levels are associated with increased risk of stroke [64] and selenium supplementation has been shown to reduce the risk of stroke mortality in one trial [65]. The synthesis of selenoproteins is regulated by availability of dietary selenium [66]. Recommended daily allowance for selenium is 55 µg/day (National Academy of Sciences Institute of Medicine's Dietary Reference Intake value) with maximal safe intake being around 400 µg/day [67].

Vitamin E plays an important role in inactivating lipid peroxy radicals while vitamin C plays an important role in reducing the vitamin E radical to vitamin E. The blood levels of these anti-oxidant vitamins decrease following a stroke [62]. The recommended daily allowance of vitamin is 15 mg/day of the RRR- α -tocopherol/day, while for vitamin C it is 75 and 100 mg/day for females and males respectively with an additional 35 mg/day for smokers.

It is recommended that stroke patients be administered selenium and vitamins C and E at a dose that is at least the daily recommended allowance.

GSH

Factors regulating GSH synthesis are (1) availability cysteine, (2) activity of GCS, and (3) cellular concentration of glutamate since glutamate can (a) promote cystine uptake and (b) alleviate the inhibition of GSH on GCS activity.

Cysteine delivery vehicles

Since cysteine auto-oxidizes and cystine has low solubility, an efficient way to increase intracellular cysteine is to administer cysteine-containing dipeptides that do not auto-oxidize readily. Thus, 1- γ -glutamyl-1-cysteine and especially 1-cysteinyl-glycine [68] have been shown to be efficient ways of increasing neuronal GSH by increasing intracellular cysteine. Dipeptides are increasingly used in clinical nutrition [69] and such dipeptides can be administered enterally or parenterally. A traditional nutritional approach might also be used. For example, an increase in GSH levels in tissues is seen following the consumption of whey [70–73]. The likely explanation for this is that bovine serum albumin has a high content of glutamyl-cysteine residues that are released from the undenatured, but not denatured, protein as 1- γ -glutamyl-1-cysteine dipeptides in the gut. Pharmacological approaches might also be used by administering compounds that can be converted to cysteine intracellularly. Two such compounds are N-acetylcysteine (NAC) and 1-2-oxothiazolidine-4-carboxylate (OTC). We have shown that administration of OTC following spinal cord crush injury promotes GSH synthesis, decreases oxidative stress thereby minimizing tissue damage, enabling otherwise paraplegic rats to locomote [74]. OTC has been used in a number of small human clinical trials showing no untoward effects [75–78]. Although studies suggest that the procysteine compound NAC, unlike OTC [80], does not readily cross the blood-brain barrier [79], it has been shown to be neuroprotective following ischemia and reperfusion [81,82] in animal models. NAC is used clinically to treat acetaminophen-induced liver damage [83].

Increasing intracellular glutamate

Elevating intracellular glutamate promotes (1) cystine uptake and alleviates the glutamate-induced inhibition of GCS activity, and (2) ATP production since it protects against cataplerotic losses of Krebs cycle intermediates. An effective way to do this is to use glutamine as the delivery system since glutamine is readily taken up by cells and converted to glutamate by glutaminase. Since glutamine is somewhat unstable, it can be administered as the dipeptides 1-alanyl-1-glutamine or glycyl-1-glutamine [69]. Rats fed parenterally with glutamine or glutamine-containing dipeptides better maintained liver GSH than non-supplemented animals [84] and also had improved cardiac function following ischemia and reperfusion [85]. 1-Alanyl-1-glutamine supplementation parenterally has been demonstrated to be clinically safe and led to a better nitrogen balance in post-operative human patients with a significant-

ly better clinical outcome in the supplemented patients as opposed to placebo controls [86]. Although increasing intracellular glutamate may be counter-intuitive, once a stroke has occurred it is not likely to exacerbate damage.

Induction of phase 2 protein

Increasing phase 2 enzyme proteins decreases oxidative stress *via* a large number of mechanisms. Inducers of phase 2 proteins are encountered in our diet and have been shown to increase phase 2 enzymes in rats, e.g., ellagic acid [87]; the isothiocyanate sulforaphane [88]; green tea polyphenolics [89]; soy meal [90], likely mediated by genistein [91]; certain polyphenolics in blueberries/cranberries [92]; the flavonol kaempferol [93]; curcumin, a component of turmeric [94]; and the lignan phytoestrogen enterolactone [91], the metabolite of the major flax seed lignan secoisolariciresinol diglucoside (SDG). Administration of dietary phase 2 protein inducers inhibits chemically-induced tumour formation (reviewed in [22]), suggesting that such dietary compounds might also decrease oxidative stress and accompanying inflammation.

Concluding remarks

There has been an enormous effort to develop drugs that block various glutamate receptors and calcium channels with little therapeutic effects seen in human clinical trials. There has been some effort directed toward minimizing oxidative stress following stroke and trauma; however, most of these efforts have been directed toward stopping the lipid peroxidation chain by scavenging lipid peroxyl radicals and, again, these approaches have not been promising. As can be seen from this review, scavenging lipid radicals does not eliminate the problems associated with lipid peroxides. Promoting scavenging of peroxides is an efficient way to decrease oxidative stress and this may be accomplished, in part, through a nutritional approach. It is suggested that there be an aggressive nutritional intervention following stroke to ensure that there is adequate intake of: (1) sulfur amino acids for glutathione synthesis, (2) vitamins E and C enabling efficient quenching of lipid peroxyl radicals, (3) selenium to ensure that there is optimal selenoproteins being synthesized, thus increasing peroxide scavenging efficiency.

It is also recommended that research be carried out on experimental animal models of stroke to determine whether administration of glutamine promotes glutathione synthesis and better maintains energy stores. It is also recommended that the therapeutic potential of phase 2 enzyme inducers be examined experimentally.

It seems most probable that a nutritional approach could be designed to better enable the vascular and the nervous tissues to cope with oxidative stress and decrease damage following a stroke.

Acknowledgements

I thank the Medical Research Council of Canada (now the CIHR), the Heart & Stroke Foundation of Saskatchewan, the Neurotrauma Initiative of Saskatchewan and the Saskatchewan Agriculture Development Fund for supporting my research.

References

- 1 Love S (1999) Oxidative stress in brain ischemia. *Brain Pathol* 9: 119–131
- 2 Scott P, Barsan W, Frederiksen S, Kronick S, Zink BJ, Domeier RM, Mitchiner JC, Judge FP, Levy RJ, Alexiou A et al (1996) A randomized trial of tirilazad mesylate in patients with acute stroke (RANTTAS). *Stroke* 27: 1453–1458
- 3 The Tirilazad Steering Committee (2000) Tirilazad mesylate in acute ischemic stroke: A systematic review. *Stroke* 31: 2257–2265
- 4 Braughler JM, Hall ED (1989) Central nervous system trauma and stroke. I. Biochemical considerations for oxygen radical formation and lipid peroxidation. *Free Rad Biol Med* 6: 289–301
- 5 Rubbo H, Radi R, Trujillo M, Telleri R, Kalyanaraman B, Barnes S, Kirk M, Freeman BA (1994) Nitric oxide regulation of superoxide and peroxynitrite-dependent lipid peroxidation. Formation of novel nitrogen-containing oxidized lipid derivatives. *J Biol Chem* 269: 26066–26075
- 6 McGrath LT, Douglas AF, McClean E, Brown JH, Doherty CC, Johnston GD, Archbold GP (1995) Oxidative stress and erythrocyte membrane fluidity in patients undergoing regular dialysis. *Clin Chim Acta* 235: 179–188
- 7 Subramaniam R, Roediger F, Jordan B, Mattson MP, Keller JN, Waeg G, Butterfield DA (1997) The lipid peroxidation product, 4-hydroxy-2-trans-nonenal, alters the conformation of cortical synaptosomal membrane proteins. *J Neurochem* 69: 1161–1169
- 8 Rauchova H, Ledvinkova J, Kalous M, Drahotka Z (1995) The effect of lipid peroxidation on the activity of various membrane-bound ATPases in rat kidney. *Int J Biochem Cell Biol* 27: 251–255
- 9 Hossmann KA (1994) Glutamate-mediated injury in focal cerebral ischemia - the excitotoxin hypothesis revised. *Brain Pathol* 4: 23–36
- 10 Siesjö BK, Zhao Q, Pahlmark K, Siesjö P, Katsura K, Folbergrova J (1995) Glutamate, calcium, and free radicals as mediators of ischemic brain damage. *Ann Thorac Surg* 59: 1316–1320
- 11 Liu T-Z, Stern A, Morrow JD (1998) The isoprostanes: Unique bioactive products of lipid peroxidation. *J Biomed Sc* 5: 415–420
- 12 Harrison KA, Murphy RC (1995) Isoleukotrienes are biologically active free radical products of lipid peroxidation. *J Biol Chem* 270: 17273–17278
- 13 Esterbauer H, Zollner H, Schauer RJ (1990) Aldehydes formed by lipid peroxidation: mechanisms of formation, occurrence, and determination. In: C Vigo-Pelfrey (ed): *Membrane lipid peroxidation*. CRC Press, Boca Raton, FL, 239–268

- 14 Springer JE, Azbill RD, Mark RJ, Begley JG, Waeg G, Mattson MP (1997) 4-hydroxynonenal, a lipid peroxidation product, rapidly accumulates following traumatic spinal cord injury and inhibits glutamate uptake. *J Neurochem* 68: 2469–2476
- 15 Comporti M (1998) Lipid peroxidation and biogenic aldehydes: from the identification of 4- hydroxynonenal to further achievements in biopathology. *Free Radic Res* 28: 623–635
- 16 Blanc EM, Keller JN, Fernandez S, Mattson MP (1998) 4-hydroxynonenal, a lipid peroxidation product, impairs glutamate transport in cortical astrocytes. *Glia* 22: 149–160
- 17 Mark RJ, Lovell MA, Markesbery WR, Uchida K, Mattson MP (1997) A role for 4-hydroxynonenal, an aldehydic product of lipid peroxidation, in disruption of ion homeostasis and neuronal death induced by amyloid β -peptide. *J Neurochem* 68: 255–264
- 18 Picklo MJ, Amarnath V, McIntyre JO, Graham DG, Montine TJ (1999) 4-Hydroxy-2(E)-nonenal inhibits CNS mitochondrial respiration at multiple sites. *J Neurochem* 72: 1617–1624
- 19 Brown GC, Bolanos JP, Heales SJR, Clark JB (1995) Nitric oxide produced by activated astrocytes rapidly and reversibly inhibits cellular respiration. *Neurosci Lett* 193: 201–204
- 20 Christman JW, Blackwell TS, Juurlink BHJ (2000) Redox regulation of nuclear factor kappa B: Therapeutic potential for attenuating inflammatory responses. *Brain Pathol* 10: 153–162
- 21 Blackwell TS, Christman JW (1997) The role of nuclear factor-kappa B in cytokine gene regulation. *Am J Respir Cell Mol Biol* 17: 3–9
- 22 Juurlink BHJ (2001) Therapeutic potential of dietary phase 2 enzyme inducers in ameliorating diseases that have an underlying inflammatory component. *Can J Physiol Pharmacol* 79: 266–282
- 23 Flohé L (1989) The selenoprotein glutathione peroxidase. In: D Dolphin, O Avramović, R Poulson (eds): *Glutathione chemical, biochemical, and medical aspects. Part A*. Krieger Publishing, Malabar, FL, 643–731
- 24 Fornai F, Saviozzi M, Piaggi S, Gesi M, Corsini GU, Malvaldi G, Casini AF (1999) Localization of a glutathione-dependent dehydroascorbate reductase within the central nervous system of the rat. *Neuroscience* 94: 937–948
- 25 Goon D, Saxena M, Awasthi YC, Ross D (1993) Activity of mouse liver glutathione S-transferases toward trans,trans-muconaldehyde and trans-4-hydroxy-2-nonenal. *Toxicol Appl Pharmacol* 119: 175–180
- 26 Thornalley PJ (1998) Glutathione-dependent detoxification of alpha-oxoaldehydes by the glyoxalase system: involvement in disease mechanisms and antiproliferative activity of glyoxalase I inhibitors. *Chem Biol Interact* 111–112: 137–151
- 27 Uhlig S, Wendel A (1992) The physiological consequences of glutathione variations. *Life Sci* 51: 1083–1094
- 28 Chae HZ, Kang SW, Rhee SG (1999) Isoforms of mammalian peroxiredoxin that reduce peroxides in presence of thioredoxin. *Methods Enzymol* 300: 219–226

- 29 Björnstedt M, Kumar S, Bjorkhem L, Spyrou G, Holmgren A (1997) Selenium and the thioredoxin and glutaredoxin systems. *Biomed Environ Sci* 10: 271–279
- 30 Das KC, White CW (1998) Detection of thioredoxin in human serum and biological samples using a sensitive sandwich ELISA with digoxigenin-labeled antibody. *J Immunol Methods* 211: 9–20
- 31 May JM, Cobb CE, Mendiratta S, Hill KE, Burk RF (1998) Reduction of the ascorbyl free radical to ascorbate by thioredoxin reductase. *J Biol Chem* 273: 23039–23045
- 32 May JM, Mendiratta S, Hill KE, Burk RF (1997) Reduction of dehydroascorbate to ascorbate by the selenoenzyme thioredoxin reductase. *J Biol Chem* 272: 22607–22610
- 33 Arner ES, Nordberg J, Holmgren A (1996) Efficient reduction of lipoamide and lipoic acid by mammalian thioredoxin reductase. *Biochem Biophys Res Commun* 225: 268–274
- 34 Björnstedt M, Hamberg M, Kumar S, Xue J, Holmgren A (1995) Human thioredoxin reductase directly reduces lipid hydroperoxides by NADPH and selenocystine strongly stimulates the reaction via catalytically generated selenols. *J Biol Chem* 270: 11761–11764
- 35 Arteel GE, Briviba K, Sies H (1999) Function of thioredoxin reductase as a peroxynitrite reductase using selenocystine or ebselen. *Chem Res Toxicol* 12: 264–269
- 36 Meister A (1983) Glutathione. *Ann Rev Biochem* 52: 711–760
- 37 Deneke SM, Fanburg BL (1989) Regulation of cellular glutathione. *Am J Physiol* 257: L163–L173
- 38 Bannai S (1984) Transport of cystine and cysteine in mammalian cells. *Biochim Biophys Acta* 779: 289–306
- 39 Galloway DC, Blake DG, Shepherd AG, McLellan LI (1997) Regulation of human gamma-glutamylcysteine synthetase: co-ordinate induction of the catalytic and regulatory subunits in HepG2 cells. *Biochem J* 328: 99–104
- 40 Presterla T, Holtzclaw WD, Zhang Y, Talalay P (1993) Chemical and molecular regulation of enzymes that detoxify carcinogens. *Proc Natl Acad Sci USA* 90: 2965–2969
- 41 Eftekharpour E, Holmgren A, Juurlink BHJ (2000) Thioredoxin reductase and glutathione synthesis is upregulated by t-butylhydroquinone in cortical astrocytes but not in cortical neurons. *Glia* 31: 241–248
- 42 Ishii T, Itoh K, Sato H, Bannai S (1999) Oxidative stress-inducible proteins in macrophages. *Free Radic Res* 31: 351–355
- 43 Tsuji Y, Ayaki H, Whitman SP, Morrow CS, Torti SV, Torti FM (2000) Coordinate transcriptional and translational regulation of ferritin in response to oxidative stress. *Mol Cell Biol* 20: 5818–5827
- 44 Benson AM, Hunkeler MJ, Talalay P (1980) Increase of NAD(P):quinone reductase by dietary antioxidants: possible role in protection against carcinogenesis. *Proc Natl Acad Sci USA* 77: 5216–5220
- 45 Presterla T, Zhang Y, Spencer SR, Wilczak CA, Talalay P (1993) The electrophile counterattack response: protection against neoplasia and toxicity. *Advan Enzym Reg* 33: 281–296

- 46 Keli SO, Hertog MG, Feskens EJ, Kromhout D (1996) Dietary flavonoids, antioxidant vitamins, and incidence of stroke: the Zutphen study. *Arch Intern Med* 156: 637–642
- 47 Yochum L, Kushi LH, Meyer K, Folsom AR (1999) Dietary flavonoid intake and risk of cardiovascular disease in postmenopausal women. *Am J Epidemiol* 149: 943–949
- 48 Nakazono K, Watanabe N, Matsuno K, Sasaki J, Sato T, Inoue M (1991) Does superoxide underlie the pathogenesis of hypertension? *Proc Natl Acad Sci USA* 88: 10045–10048
- 49 Ohara Y, Peterson TE, Harrison DG (1993) Hypercholesterolemia increases endothelial superoxide anion production. *J Clin Invest* 91: 2546–2551
- 50 Quyyumi AA (1998) Endothelial function in health and disease: new insights into the genesis of cardiovascular disease. *Am J Med* 105: 32S–39S
- 51 Stamier JS, Osborne JA, Jaraki O, Rabbani LE, Mullins M, Singel D, Loscalzo LE (1993) Adverse vascular effects of homocysteine are modulated by endothelium-derived relaxing factor and related oxides of nitrogen. *J Clin Invest* 91: 308–318
- 52 Loscalzo J (1996) The oxidant stress of hyperhomocyst(e)inemia. *J Clin Invest* 98: 5–7
- 53 Welch GN, Upchurch GR, Jr., Loscalzo J (1997) Homocysteine, oxidative stress, and vascular disease. *Hosp Pract (Off Ed)* 32: 81–82, 85, 88–92
- 54 de Jong SC, van den Berg M, Rauwerda JA, Stehouwer CD (1998) Hyperhomocysteinemia and atherothrombotic disease. *Semin Thromb Hemost* 24: 381–385
- 55 Hempel SL, Moses BL, O'Malley YQ (1998) Mixed disulfides of homocysteine and cysteine inhibit cystine uptake, decreasing intracellular glutathione. *Free Rad Biol Med* 25: S33
- 56 Brownlee M (1995) Advanced protein glycosylation in diabetes and aging. *Ann Rev Med* 46: 223–234
- 57 Cooper ME, Gilbert RE, Jerums G (1997) Diabetic vascular complications. *Clin Exp Pharmacol Physiol* 24: 770–775
- 58 Seitz CS, Kleindienst R, Xu QB, Wick G (1996) Coexpression of heat-shock protein 60 and intercellular-adhesion molecule-1 is related to increased adhesion of monocytes and T cells to aortic endothelium of rats in response to endotoxin. *Lab Invest* 74: 241–252
- 59 Paterson PG, Juurlink BHJ (1999) Nutritional regulation of glutathione in stroke. *Neurotoxicity Res* 1: 99–112
- 60 Gariballa SE (2000) Nutritional factors in stroke. *Br J Nutr* 84: 5–17
- 61 Finestone HM (2000) Safe feeding methods in stroke patients. *Lancet* 355: 1662–3
- 62 Cherubini A, Polidori MC, Bregnocchi M, Pezzuto S, Cecchetti R, Ingegni T, di Iorio A, Senin U, Mecocci P (2000) Antioxidant profile and early outcome in stroke patients. *Stroke* 31: 2295–2300
- 63 Roberts PM (1995) Nutrition in the head-injured patient. *New Horiz* 3: 506–517
- 64 Virtamo J, Valkeila E, Alfthan G, Punsar S, Huttunen JK, Karvonen MJ (1985) Serum selenium and the risk of coronary heart disease and stroke. *Am J Epidemiol* 122: 276–282
- 65 Mark SD, Wang W, Fraumeni JF, Jr., Li JY, Taylor PR, Wang GQ, Dawsey SM, Li B, Blot

- WJ (1998) Do nutritional supplements lower the risk of stroke or hypertension? *Epidemiology* 9: 9–15
- 66 Burk RF, Hill KE (1993) Regulation of selenoproteins. *Ann Rev Nutr* 13: 65–81
- 67 Yang GQ, Xia YM (1995) Studies on human dietary requirements and safe range of dietary intakes of selenium in China and their application in the prevention of related endemic diseases. *Biomed Environ Sci* 8: 187–201
- 68 Dringen R, Pfeiffer B, Hamprecht B (1999) Synthesis of the antioxidant glutathione in neurons: supply by astrocytes of CysGly as precursor for neuronal glutathione. *J Neurosci* 19: 562–569
- 69 Fürst P, Kuhn KS (2000) Amino-acid substrates in new bottles: implications for clinical nutrition in the 21st century. *Nutrition* 16: 603–606
- 70 Bounous G, Gervais F, Amer V, Batist G, Gold P (1989) The influence of dietary whey protein on tissue glutathione and the diseases of aging. *Clin Invest Med* 12: 343–349
- 71 Zommara M, Toubou H, Sakono M, Imaizumi K (1998) Prevention of peroxidative stress in rats fed on a low vitamin E-containing diet by supplementing with a fermented bovine milk whey preparation: effect of lactic acid and beta-lactoglobulin on the antiperoxidative action. *Biosci Biotechnol Biochem* 62: 710–717
- 72 Lands LC, Grey VL, Smountas AA (1999) Effect of supplementation with a cysteine donor on muscular performance [published erratum appears in *J Appl Physiol* 2000 Jan; 88 (1): following table of contents]. *J Appl Physiol* 87: 1381–1385
- 73 Lothian B, Grey V, Kimoff RJ, Lands LC (2000) Treatment of obstructive airway disease with a cysteine donor protein supplement: a case report. *Chest* 117: 914–916
- 74 Kamencic H, Griebel RW, Lyon A, Paterson PG, Juurlink. BHJ (2001) Promoting glutathione synthesis following spinal cord trauma decreases secondary damage and promotes retention of function. *FASEB J* 15: 243–250
- 75 Kalayjian RC, Skowron G, Emgushov R-T, Chance M, Spell SA, Borum PR, Webb LS, Mayer KH, Jackson LB, Yen-Lieberman B et al (1994) A phase I/II trial of intravenous L-2-oxothiazolidine-4-carboxylic acid (Procysteine) in asymptomatic HIV-infected subjects. *J Acquir Immune Defic Syndr* 7: 369–374
- 76 Barditch-Crovo P, Noe D, Skowron G, Lederman M, Kalayjian RC, Borum P, Buier R, Rowe WB, Goldberg D, Lietman P (1998) A phase I/II evaluation of oral L-2-oxothiazolidine-4-carboxylic acid in asymptomatic patients infected with human immunodeficiency virus. *J Clin Pharmacol* 38: 357–363
- 77 Bernard GR, Wheeler AP, Arons MM, Morris PE, Paz HL, Russell JA, Wright PE (1997) A trial of antioxidants N-acetylcysteine and procysteine in ARDS. The Antioxidant in ARDS Study Group. *Chest* 112: 164–172
- 78 Vita JA, Frei B, Holbrook M, Gokce N, Leaf C, Keaney JF (1998) L-2-oxothiazolidine-4-carboxylic acid reverses endothelial dysfunction in patients with coronary artery disease. *J Clin Invest* 101: 1408–1414
- 79 McLellan LI, Lewis AD, Hall DJ, Ansell JD, Wolf CR (1995) Uptake and distribution of N-acetylcysteine in mice: tissue-specific effects on glutathione concentrations. *Carcinogenesis* 16: 2099–2106

- 80 Anderson ME, Meister A (1989) Marked increase of cysteine levels in many regions of the brain after administration of 2-oxothiazolidine-4-carboxylate. *FASEB J* 3: 1632–1636
- 81 Carroll JE, Howard EF, Hess DC, Wakade CG, Chen Q, Cheng C (1998) Nuclear factor-kappa B activation during cerebral reperfusion: effect of attenuation with N-acetylcysteine treatment. *Brain Res Mol Brain Res* 56: 186–191
- 82 Cuzzocrea S, Mazzon E, Costantino G, Serraino I, Dugo L, Calabro G, Cucinotta G, De Sarro A, Caputi AP (2000) Beneficial effects of N-acetylcysteine on ischaemic brain injury. *Br J Pharmacol* 130: 1219–1226
- 83 Speeg KV, Bay MK (1995) Prevention and treatment of drug-induced liver disease. *Gastroenterol Clin North Am* 24: 1047–1064
- 84 Matilla B, Ortiz J, Gonzalez P, Garcia-Diez F, Jorquera F, Culebras JM, Gonzalez-Gallego J, Tunon MJ (2000) Effects of parenteral nutrition supplemented with glutamine or glutamine dipeptides on liver antioxidant and detoxication systems in rats. *Nutrition* 16: 125–128
- 85 Khogali SE, Harper AA, Lyall JA, Rennie MJ (1998) Effects of L-glutamine on post-ischaemic cardiac function: protection and rescue. *J Mol Cell Cardiol* 30: 819–827
- 86 Jian ZM, Cao JD, Zhu XG, Zhao WX, Yu JC, Ma EL, Wang XR, Zhu MW, Shu H, Liu YW (1999) The impact of alanyl-glutamine on clinical safety, nitrogen balance, intestinal permeability, and clinical outcome in postoperative patients: a randomized, double-blind, controlled study of 120 patients. *JPEN J Parenter Enteral Nutr* 23: S62–S66
- 87 Barch DH, Rundhaugen LM, Pillay NS (1995) Ellagic acid induces transcription of the rat glutathione S-transferase-Ya gene. *Carcinogenesis* 16: 665–668
- 88 Fahey JW, Zhang Y, Talalay P (1997) Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. *Proc Natl Acad Sci USA* 94: 10367–10372
- 89 Khan SG, Katiyar SK, Agarwal R, Mukhtar H (1992) Enhancement of antioxidant and phase II enzymes by oral feeding of green tea polyphenols in drinking water to SKH-1 hairless mice: possible role in cancer chemoprevention. *Cancer Res* 52: 4050–4052
- 90 Appelt LC, Reicks MM (1997) Soy feeding induces phase II enzymes in rat tissues. *Nutr Cancer* 28: 270–275
- 91 Wang W, Liu LQ, Higuchi CM, Chen H (1998) Induction of NADPH:quinone reductase by dietary phytoestrogens in colonic Colo205 cells. *Biochem Pharmacol* 56: 189–195
- 92 Bomser J, Madhavi DL, Singletary K, Smith MA (1996) *In vitro* anticancer activity of fruit extracts from *Vaccinium* species. *Planta Med* 62: 212–216
- 93 Uda Y, Price KR, Williamson G, Rhodes MJ (1997) Induction of the anticarcinogenic marker enzyme, quinone reductase, in murine hepatoma cells *in vitro* by flavonoids. *Cancer Lett* 120: 213–216
- 94 Dinkova-Kostova AT, Talalay P (1999) Relation of structure of curcumin analogs to their potencies as inducers of Phase 2 detoxification enzymes. *Carcinogenesis* 20: 911–914