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Antioxidant Therapy in Acute Central Nervous System Injury: Current State

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***Abstract*——Free radicals are highly reactive molecules generated predominantly during cellular respiration and normal metabolism. Imbalance between cellular production of free radicals and the ability of cells to defend against them is referred to as oxidative stress (OS). OS has been implicated as a potential contributor to the pathogenesis of acute central nervous system (CNS) injury. After brain injury by ischemic or hemorrhagic stroke or trauma, the production of reactive oxygen species (ROS) may increase, sometimes drastically, leading to tissue damage via several different cellular molecular pathways. Radicals can cause damage to cardinal cellular components such as lipids, proteins, and nucleic acids (e.g., DNA), leading to subsequent cell death by modes of necrosis or apoptosis. The damage can become more widespread due to weakened cellular antioxidant defense systems.**

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Moreover, acute brain injury increases the levels of excitotoxic amino acids (such as glutamate), which also produce ROS, thereby promoting parenchymatous destruction. Therefore, treatment with antioxidants may theoretically act to prevent propagation of tissue damage and improve both the survival and neurological outcome. Several such agents of widely varying chemical structures have been investigated as therapeutic agents for acute CNS injury. Although a few of the antioxidants showed some efficacy in animal models or in small clinical studies, these findings have not been supported in comprehensive, controlled trials in patients. Reasons for these equivocal results may include, in part, inappropriate timing of administration or suboptimal drug levels at the target site in CNS. Better understanding of the pathological mechanisms of acute CNS injury would characterize the exact primary targets for drug intervention. Improved antioxidant design should take into consideration the relevant and specific harmful free radical, blood brain barrier (BBB) permeability, dose, and time administration. Novel combinations of drugs providing protection against various types injuries will probably exploit the potential synergistic effects of antioxidants in stroke.

I. Introduction

A. Acute Central Nervous System Injury Mechanisms

Stroke is a sudden loss of brain function resulting from interference with the blood supply to the central nervous system (CNS[1]). Acute stroke can be classified either as ischemic (80% of stroke cases), which can be further classified to extra-cranial embolism and intracranial thrombosis, or a hemorrhagic stroke (20% of stroke cases), which can be further classified to intracerebral hemorrhage and subarachnoid hemorrhage (SAH; Fig. 1).

Stroke is the third most common cause of death in Europe and North America, and is a major cause of morbidity particularly in the middle-aged and elderly population (Bronner et al., 1995; De Freitas and Bogousslavsky, 2001). CNS damage occurs in stroke as a result of hypoxia. In cerebral ischemia there is an ischemic gradient that can be divided into the core, which is the central ischemic zone, and the penumbra, which is located in more peripheral zones. In the penumbra, functional alterations occur in the neurons and glial cells. Neurons are most vulnerable to hypoxia due to their dependence on the oxidative metabolism of glucose for energy. The principal pathophysiological processes in acute CNS injury, such as stroke, mechanical trauma, or subarachnoid hemorrhage, are extremely complex and involve pathological permeability of blood brain barrier (BBB, in part of the CNS injuries), energy failure, loss of cell ion homeostasis, acidosis, increased intracellular calcium, excitotoxicity, and free radical-mediated toxicity. This can lead to ischemic necrosis, which occurs in the severely ischemic regions and is associated with loss of calcium and glutamate homeostasis. It can also lead to apoptosis, which is more likely to occur in the moderately ischemic regions, evolves more slowly, and depends on the activation of a sequence of genes (Pulsinelli, 1992; Gennarelli, 1997; Dirnagl et al., 1999; Fig. 2)

[1] Abbreviations: EAA, excitatory amino acids; NMDA, *N*-methyl-D-aspartate; CNS, central nervous system; BBB, blood brain barrier; ROS, reactive oxygen species; OS, oxidative stress; SAH, subarachnoid hemorrhage; PBN, phenyl-α-*tert*-butyl nitrone; SOD, superoxide dismutase; NF-κB, nuclear factor-κB; CHI, closed head injury; LMWA, low molecular weight antioxidants; MCA-O, middle cerebral artery occlusion; GSH, reduced glutathione.

B. Reactive Free Radicals and Oxidative Stress in Acute Central Nervous System Injury

A free radical is any chemical compound that contains one or more unpaired electrons in its outer orbits. Unpaired electrons alter the chemical reactivity of an atom or molecule, usually making it more reactive than the corresponding nonradical, because they act as an electron acceptor and essentially "steal" electrons from other molecules. This electron loss is called oxidation and free radicals are referred to as oxidizing agents (Halliwell and Gutteridge, 1989). Humans are constantly exposed to free radicals created by external sources from the environment (e.g., radon and cosmic radiation) or man-made and by internal cellular metabolisms. The most commonly occurring cellular free radical is superoxide radical ($O_2^{\cdot-}$), which is produced when an oxygen molecule gains one electron from another substance. Excess amount of $O_2^{\cdot-}$ leads to tissue damage by promoting hydroxyl radical ($OH^{\cdot}$) formation through hydrogen peroxide (H_2O_2) (the iron-mediated Haber-Weiss reaction; Jenner and Olnaw, 1996; Simonian and Coyle, 1996; Fig. 3A). Alternatively, $O_2^{\cdot-}$ may lead to $OH^{\cdot}$ formation through an interaction with endogenously formed nitric oxide ($NO^{\cdot}$) by nitric oxide synthetase, an enzyme that is concentrated in certain neurons and activated by Ca^{2+}. The interaction of $NO^{\cdot}$ with $O_2^{\cdot-}$ leads to the formation of peroxynitrite ($ONOO^-$), which can generate nitrosyl radical (ONOOH), which decomposes to form $OH^{\cdot}$ (Fig. 3B). Free radicals and related molecules are often classified together as reactive oxygen species (ROS) to signify their ability to lead to oxidative changes within the cell (Simonian and Coyle, 1996). These radicals can cause cellular damage to cardinal cellular components

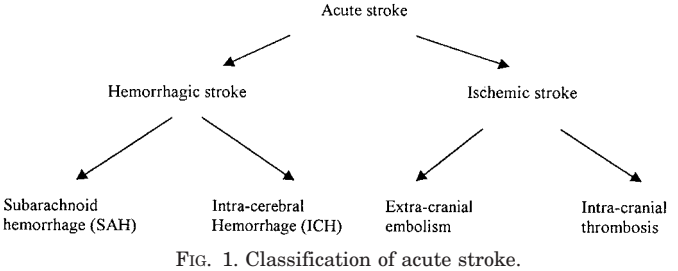

FIG. 1. Classification of acute stroke.

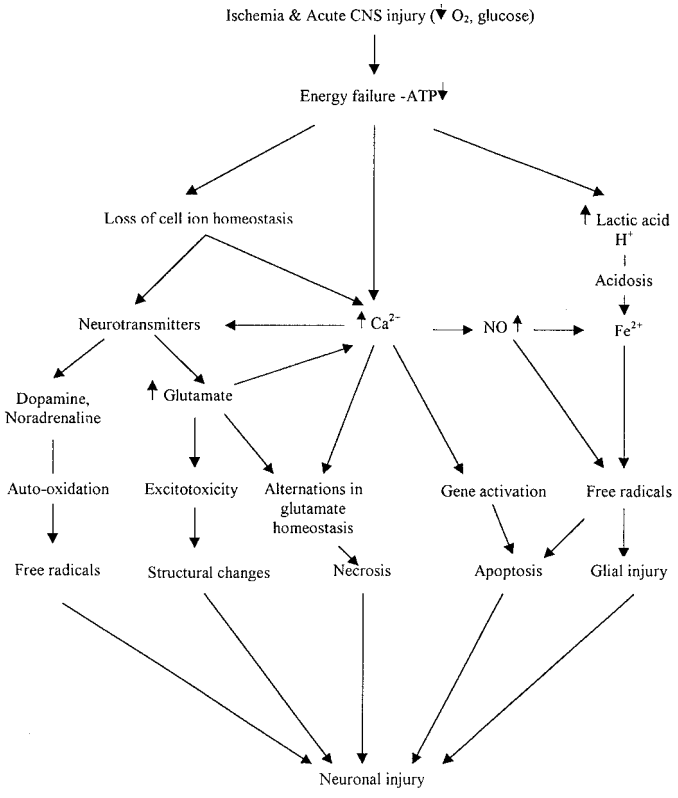

FIG. 2. Cellular mechanisms that may be involved in acute ischemia and CNS injury.

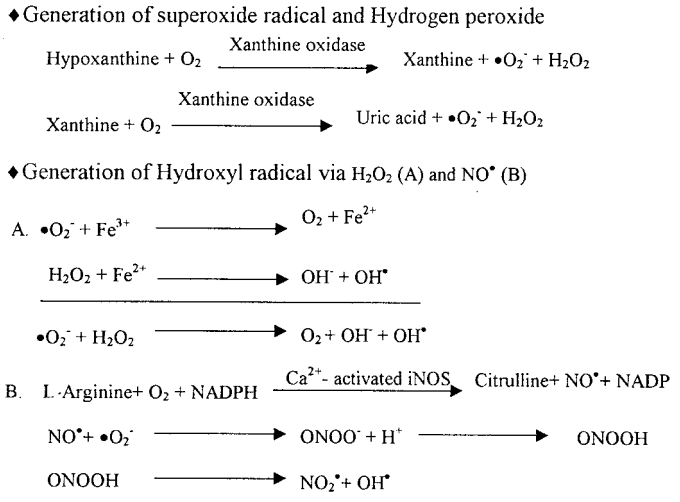

FIG. 3. Reactive oxygen species reactions that may lead to OH• production. NADP, nicotinamide adenine dinucleotide phosphate; NADPH, reduced form of NADP; NOS, nitric oxide synthetase.

such as lipids. Polyunsaturated fatty acids are particularly vulnerable to free radical attack, because the double bonds within membranes allow easy removal of hydrogen ions by ROS such as OH• (Halliwell and Gutteridge, 1989). Free radicals can also damage proteins and nucleic acids (e.g., DNA), leading to subsequent cell death by mode of necrosis or apoptosis. Cells normally have a number of mechanisms acting to defend against damage induced by free radicals (Evans, 1993; Simonian and Coyle, 1996). Problems occur when production of ROS exceeds their elimination by the antioxidant protective systems or when the latter are damaged. This imbalance between cellular production of ROS and the inability of cells to defend against them is called oxidative stress (OS) (Ebadi et al., 1996; Jenner and Olnaw, 1996; Simonian and Coyle, 1996). OS is involved in acute and chronic CNS injury and is a major factor in the pathogenesis of neuronal damage (Facchinetti et al., 1998).

1. Oxidative Stress-Mediated Brain Damage. Some of the pathological processes in acute CNS injury involve the generation of oxygen free radicals either as a cause or a result of disease progression (Love, 1999; Lewen et al., 2000). Free radicals are generated by the constant use of oxygen in the mitochondria to supply the energy needs of the brain. Some enzymes expressed in the brain including monoamine oxidase, tyrosine hydroxylase, and L-amino acid oxidase produce H_2O_2 as a normal byproduct of their activity. The activity of other neuronal enzymes yields oxidants such as the Ca^{2+}-dependent activation of phospholipase A_2. That may lead to arachidonic acid release, producing O_2^- through its subsequent metabolism by lipoxygenases and cyclo-oxygenases to form eiconasoids. Auto-oxidation of endogenous substances, e.g., ascorbic acid and catecholamines, may yield high levels of H_2O_2 (Coyle and Puttfarcken, 1993). Therefore, ROS have been the focus of interest as possible candidates for the elicitation of various pathological responses in the pathogenesis of acute CNS injury and as a therapeutic target (Bromont et al., 1989; Hall, 1989; Oliver et al., 1990). It is well known that glial cells are more resistant to OS than neurons, probably due to transcriptional up-regulation of glutathione synthesis (Rice and Russo-Menna, 1998; Iwata-Ichikawa et al., 1999).

Studies have demonstrated that free radicals play an important role in the pathogenesis of ischemia, especially superoxide, which was shown to produce during the reperfusion phase and interact with NO•, leading to peroxynitrite formation (Cazevielle et al., 1993). Love et al. (1999) showed that free radicals and related ROS mediate much of the damage that occurs after transient brain ischemia and in the penumbral region of infarcts caused by permanent ischemia. Demirkaya et al. (2000) found that patients with acute ischemic hemispheric stroke had significantly higher levels of malondialdehyde in their red blood cells on the first and seventh days after stroke onset, compared with controls. Moreover, superoxide dismutase (SOD) and glutathione peroxidase activities were significantly decreased compared with control subjects. These results showed a significant correlation with infarct size, initial stroke severity, and poor short-term prognosis. Other acute CNS injuries like SAH and trauma also involve ROS production. Gaetani et al. (1998) have shown an imbalance of the antioxidant enzymatic activities in the human brain after SAH. This antioxidant imbalance precedes the oc-

currence of symptomatic vasospasm in patients undergoing an operation in the early stage of SAH. The presence of free hemoglobin in nerve tissue may also exacerbate the potential for ROS-effected damage caused by the appearance of free iron salts (Sandrzadeh et al., 1987). Taking all that together, treatment with free radical scavengers and antioxidants is a rational therapeutic strategy for stroke or CNS trauma.

2. Excitotoxicity Insults. One of the first pathophysiological events leading to neuronal damage in acute CNS injury involves glutamate accumulation in the extracellular space. Glutamate is the major excitatory amino acid among the excitatory amino acids (EAA) in the brain, acting mainly through activation of its ionotropic receptors. These receptors can be distinguished by their pharmacological and electrophysiological properties: the α-amino-3-hydroxy-5-methyl-4-isoxasole-propionic acid, kainic acid, and the *N*-methyl-D-aspartate (NMDA) receptors. Activation of these receptors leads to depolarization and neuronal excitation. However, if for any reason receptor activation becomes excessive or prolonged, the target neurons become damaged and eventually die. In the ischemic brain, extracellular glutamate is elevated rapidly after the onset of ischemia and declines after reperfusion. The mechanisms that are responsible for the elevation of extracellular glutamate include enhanced efflux of glutamate and the reduction of glutamate uptake. This process seems to involve sustained elevations of intracellular calcium levels through glutamate transporters operating in the reverse mode, and owing to imbalance of sodium ions across plasma membranes. Moreover, the fact that the brain can neither synthesize nor store energy reserves, means that any interruption in cerebral blood flow may lead to rapidly and irrevocably energy failure and dramatic fall in intracellular levels of ATP. The consequences will be an increase in the concentrations of extracellular glutamate and neuronal sensitization to excitotoxic cell death. It is well established that high levels of glutamate in the extracellular space appear rapidly after the onset of ischemia. Nevertheless, a direct linkage between the enhanced release of glutamate and the neuronal injury has not been fully established (Coyle and Puttfarcken, 1993; Bondy, 1995; Doble, 1999). Pharmacological studies in rodents and recent clinical studies in humans have shown that the extra-neuronal concentration of glutamate rose to toxic levels under ischemic (Benveniste et al., 1984; Hagberg et al., 1985; Siesjo, 1992a,b; Bullock et al., 1995; Davalos et al., 1997) and traumatic (Faden et al., 1989) conditions. In addition, NMDA antagonists that were added to neuronal cultures, rescued cells treated with glutamate receptor agonists. Neuroprotection can be achieved by blocking the presynaptic release of glutamate and/or by blocking the excitation of postsynaptic neurons occurring after an ischemic episode. In this regard, the voltage-sensitive calcium channels and glutamate receptors may be suitable targets for therapy (Coyle and Puttfarcken, 1993; Nishizawa, 2001).

3. Oxidative Stress and Excitotoxicity. It is well known that EAA and neurotransmitters, whose metabolism produces ROS, are unique in the brain as sources of OS (Coyle and Puttfarcken, 1993). It has been proposed that during CNS, ROS (mainly $O_2^{\cdot-}$ and $NO^{\cdot}$) and EAA (mainly glutamate) may cooperate in the pathogenesis of neuronal damage, involving loss of cellular calcium homeostasis (White et al., 1984; Bose et al., 1992; Siesjo, 1993).

The chain of occurrences has not been well established. Excitatory events may stimulate ROS, and there is evidence that ROS can lead to release of EAA, suggesting a bi-directional relationship (Pellegrini-Giampeitro et al., 1990). Several studies provide evidence that the two phenomena are interrelated. Transient ischemia elevates cerebral levels of both excitatory amino acids and rates of hydroxyl radical formation (Delbarre et al., 1991). The resultant low oxygen supply in the brain tissue after CNS injury can lead to reduced energy supply. This anabolic deficit may then result in diminution of the ionic gradients across the plasma membrane. The influx of extracellular calcium may then stimulate release of neurotransmitters including glutamate. In addition, the capacity of the energy-requiring high-affinity re-uptake systems is diminished. Thus, extracellular levels of glutamate may rise. A hyperexcitable state will ensue, resulting from both the reduced axonal membrane potential and increased calcium-stimulated neurotransmitter release. Subsequent reperfusion will lead to an abrupt return of glucose and oxygen to neurons, which disrupts mitochondrial function. Such uncoupling will then increase the generation rate of ROS. Elevated intracellular calcium will also exacerbate ROS levels by phospholipase activation. This will initiate a cycle leading to increasingly severe neuronal damage. The above mechanism may explain why both ROS scavengers and calcium channel antagonists afford protection against ischemic states. The role of NO in hypoxic neuronal damage is ambiguous. Influx of calcium into the cell can activate NO synthetase, thus increasing NO levels. This radical can interact with the superoxide radical to form the intensely reactive nitroperoxyl radical, which can be a cytotoxic mediator in neuronal injury during stroke and NMDA activation (Cazevielle et al., 1993; Fagni et al., 1994). However, nitric oxide has also been found to be neuroprotective, perhaps by reducing the toxicity of hydrogen peroxide (Wink et al., 1993). Thus, under some circumstances, inhibition of NO synthesis can potentiate excitotoxicity (Hayberry et al., 1992). The dual nature of nitric oxide may best be accounted in terms of its ability either to exacerbate the harmfulness of some oxidant species such as superoxide or to form less reactive compounds such as hydrogen peroxide. The effect of NO may then depend on the precise ROS species that are coexisting in the tissue. Taken together, it is now clear that

tissue damage associated with excitotoxicity may be blocked, not only by antagonists of EAA receptors, but also by agents that inhibit generation of ROS (e.g., O_2^- and NO$^\bullet$). Such approaches include agents that inhibit ROS producing enzymes (e.g., nitric oxide synthetase or xanthine oxidase) and ROS scavenging agents.

4. Oxidative Stress-Induced Gene Expression. A large number of gene products appear after an ischemic insult, making it difficult to decipher which genes are really involved in the mechanism of tissue injury. ROS were shown to influence gene expression and to play a role in the events that lead to neuronal death. In global cerebral ischemia, the oxidative responsive transcription factor, nuclear factor-κB (NF-κB), is persistently activated. Overall, persistent NF-κB activation enhances ischemic neuronal death (Schneider et al., 1999), but its effects differ between cell types. Activation of NF-κB in neurons induces production of anti-apoptotic gene products and proteins involved in modulating synaptic plasticity and increases their survival after stroke. Activation of NF-κB in glial cells (astrocytes and microglia) results in the production of proinflammatory cytokines and potentially neurotoxic ROS and excitotoxins, thus, promoting ischemic neuronal degeneration (Mattson and Camandola, 2001). The NF-κB translocation into the nucleus and binding to the NF-κB site can activate many inducible genes, including but not only cyclooxygenase-2, inducible nitric oxide, metalloproteinases, intercellular adhesion molecules, and cytokines. The expression of these genes may lead to formation of ROS and BBB breakdown, which may lead to apoptosis or necrosis or both (Chan, 2001). In addition to NF-κB, many transcription factors such as AP-1, HIF-1, SP-1, and EIK-1 are known to be redox-sensitive proteins (Sen, 1998), and their regulation of gene expression by OS in cerebral ischemia has yet to be determined (Sharp et al., 2000).

C. Blood-Brain Barrier Integrity

The BBB is a major barricade that separates the brain microenvironment from the blood within the cerebrovascular tree to allow complex neural signaling without external interference. According to ultrastructural studies, endothelial cells in the brain differ fundamentally in two ways from those in peripheral tissues. First, they have very few endocytotic vesicles, limiting the amount of *trans*-cellular flux. Second, they are coupled by tight junctions or a zipper-like structure that seal the intercellular cleft and restrict par-cellular flux (Reese and Karnovsky, 1967). Normally, the tight junctions of the BBB permit the diffusion of only very small amounts of water-soluble compounds (par-cellular aqueous pathway), whereas the large surface area of the lipid membranes of the endothelium offers an effective diffusive route for lipid-soluble agents (*trans*-cellular lipophilic pathway; Rowland et al., 1992). Pathological permeability of BBB may occur after closed head injury (CHI) and SAH, enabling easier drug penetration to the brain. In other acute CNS injuries such as cerebral ischemia, the BBB is intact, at least in part, leading to reduction in drug penetration into the brain. This problem has prompted researchers to develop methods to induce transient opening of the tight junctions of the brain endothelial cells, such as osmotic opening with mannitol or arabinose (Gumerlock and Neuwelt, 1992).

II. Antioxidants in the Treatment of Acute Central Nervous System Injury

A. Antioxidants

Antioxidants are exogenous (natural or synthetic) or endogenous compounds acting in several ways including removal of O_2, scavenging reactive oxygen species or their precursors, inhibiting ROS formation and binding metal ions needed for catalysis of ROS generation. The natural antioxidant system can be classified into two major groups: enzymes and low molecular weight antioxidants (LMWA). The enzymes include SOD, catalase, peroxidase, and some supporting enzymes. The LMWA group of molecules can be further classified into directly acting antioxidants (e.g., scavengers and chain breaking antioxidants) and indirectly acting antioxidants (e.g., chelating agents). The former are extremely important in defense against OS. This subgroup currently contains several hundred compounds. Most of them, including ascorbic and lipoic acids, polyphenols, and carotenoids, are derived from dietary sources (Shohami et al., 1997). The cell itself synthesizes a minority of these molecules, such as glutathione and NADPH. The distribution of protective antioxidants in the body has some interesting features. For instance, there is a relatively high concentration of the water-soluble antioxidant vitamin C in the brain. However, vitamin E concentrations in CNS are not remarkably different from those in other organs. The concentrations of antioxidants also vary within the different regions of the brain itself. For instance, the lowest concentration of vitamin E is found in the cerebellum (Vatassery, 1992). It was also shown that enzymatic antioxidants, such as catalase, are in lower concentrations in the brain than in other tissues.

B. Experimental and Clinical Treatments of Acute Central Nervous System Injury

As with other neuroprotectants, to achieve high efficacy, antioxidants must penetrate through the BBB, and be given as early as possible and within the "neuroprotective window" (the time interval where they significantly reduce or prevent cerebral damage). The therapeutic window for successful attenuation of an infarct volume was shown to be 3 to 4 h in rats (Kaplan et al., 1991; Memezawa et al., 1992) and cats (Heiss and Rosner, 1983) and 6 to 8 h in nonhumans primates (Jones et al., 1981). Current early-phase trials of neuroprotectants in stroke (e.g., NMDA antagonists) adhered to

the 4- to 6-h time frame within which tissue rescue may be possible (Ginsberg, 1994; Pulsinelli, 1995). This is supported by statistical analysis of relevant animal studies suggesting that irreversible focal injury begins within a few minutes and is complete within 6 h (Zivin, 1998).

1. Vitamins.

a. In Prevention. An important finding of epidemiological studies on stroke is the lower risk of cerebral ischemic events among individuals with frequent consumption of fruit and vegetables (Acheson and Williams, 1983; Vollset and Bjelke, 1983; Joshipura et al., 1999). The specific nutrients responsible for this effect remain elusive, but antioxidant vitamins, such as vitamin E, β-carotene, and vitamin C, which are free radical scavengers, may be major contributing factors to this phenomenon.

Vitamin E is a fat-soluble vitamin known to be one of the most potent antioxidants. It breaks the propagation of the free radical chain reaction in the lipids of biological membranes. Vitamin E deficiency in humans is caused by either fat malabsorption or genetic abnormalities, leading to peripheral neuropathy and ataxia (Traber and Sies, 1996). Low levels of antioxidants such as vitamin E, ascorbic acid, and reduced glutathione (GSH) could lead to tissue peroxidation disability in rats. Vitamin E deficiency also influences the activities of SOD, catalase, and glutathione peroxidase (De Kumar and Rukmini, 1988). Carotenoids are also natural lipid-soluble antioxidants (Bendich, 1993). β-Carotene is the best-known carotenoid due to its importance as a vitamin A (retinol) precursor. β-Carotene possesses antioxidant activity somewhat analogous to that of vitamin E. Studies showed that within 24 h after the clinical event, acute ischemic stroke patients had lowered levels of carotenoids and vitamin E as compared with matched controls (Chang et al., 1998).

Vitamin C (ascorbic acid) is a water-soluble antioxidant that is found throughout the body as the ascorbate anion. It inhibits peroxidation of membrane phospholipids and acts as a scavenger of free radicals (Padh, 1990; Rice, 2000). Another important role of vitamin C is the regeneration of vitamin E (Chan, 1993). Brain concentration of vitamin C is 10-fold higher than its plasma levels (Frei and England, 1989; Schreiber and Trojan, 1991; Rose and Bote, 1993). This may indicate its potential role as a cerebro-protective agent.

i. Clinical Studies. Epidemiological studies examining the correlation between antioxidant vitamin consumption and stroke incidence and mortality produced conflicting results. On the one hand, Gey et al. (1993), Keli et al. (1996), and Daviglus et al. (1997) found that increased antioxidant vitamin intake resulted in a decreased risk of stroke. On the other, two randomized trials showed that β-carotene supplements (Hennekens et al., 1996) and intake of other antioxidant vitamins (Blot et al., 1993) were not associated with a reduced stroke risk. Yochum et al. (2000) found an inverse association between stroke mortality and dietary vitamin E in postmenopausal women. These conflicting results may indicate that only people with extraordinarily low OS and BBB disruption will benefit from vitamin E supplementation.

b. In Treatment. OPC-14117, a vitamin E analog, was found to attenuate edema formation and behavioral deficits after cortical contusion in rats (Kawamata et al., 1997). MDL 74,722**,** another vitamin E analog, was found to reduce infarct volume by 49% after transient middle cerebral artery occlusion (MCA-O) in rats (Van der Worp et al., 1999).

To summarize, epidemiological studies preformed using vitamins to prevent stroke demonstrated conflicting results. The inconsistent results can be explained by the fact that vitamin C and vitamin E may be oxidized to form ascorbyl radical and α-tocopherol radical, which may act as toxic pro-oxidants in some ischemic circumstances (Dyatlov et al., 1998; Rice, 2000). No controlled clinical study was performed to verify whether vitamins may be beneficial as a treatment for acute stroke patients. Thus, vitamins may reduce stroke complications only if they are given at a specific dose, and within specific time window. Moreover, it may be that vitamins are beneficial only when the severity of stroke is mild with a low OS level in the ischemic zone.

2. Coenzyme Q_{10}. Coenzyme Q_{10} (ubiquinone) is a mobile and lipid-soluble compound within the hydrophobic core of the phospholipid bilayer of the inner membrane of the mitochondria. It is an essential cofactor in the electron transport chain, where it accepts electrons from complexes 1 and 2 (Beyer, 1992; Ernster and Dallner, 1995; Do et al., 1996). Coenzyme Q_{10} also serves as an important antioxidant in lipid membranes (Noack et al., 1994; Forsmark et al., 1997) either directly or by regenerating vitamin E. Its levels are known to decrease with age in both human and rat tissues (Beyer et al., 1985; Kalen et al., 1989; Battino et al., 1995). This decrease may be caused by reduced synthesis or age-dependent increases in lipid peroxidation (Forsmark et al., 1997).

The effect of coenzyme Q_{10} on the survival of Mongolian gerbils with unilateral carotid ligation-induced stroke was examined. The control stroke gerbils died within 24 h. However, with a subcutaneous implantation of a 250-mg pellet of coenzyme Q_{10}, an improvement was observed, with 45% survival at 4 weeks (Ogawa et al., 1986). Another study showed that oral administration of coenzyme Q_{10} (10 mg/kg per 6 days) prevented the development of ischemic brain lesions in a rabbit model of SAH-induced symptomatic vasospasm (Grieb et al., 1997). More studies should be performed to evaluate coenzyme Q_{10} efficacy in acute CNS injury.

3. Melatonin. Melatonin (*N*-acetyl-5-methoxytryptamine) is an indoleamide secreted by the pineal gland, which has structural similarities to serotonin.

Melatonin is known as a biological modulator of many physiological mechanisms (e.g., circadian rhythms and sleep). It is highly lipophilic and, when administered exogenously, can readily cross the BBB and gain access to neurons and glial cells. There is experimental evidence that melatonin influences aging and age-related processes and disease states (Beyer et al., 1998). These roles are apparently related to its potency as a free radical scavenger (Beyer et al., 1998).

Several studies have examined the neuroprotective effects of melatonin as an antioxidant in cerebral ischemia. Borlongan et al. (2000) showed that oral administration of melatonin, 1 h before MCA occlusion in rats, significantly enhanced glial cell survival. Moreover, another study showed that pinealectomy in rats increases the infarct volume after MCA occlusion. Injection of melatonin (4 mg/kg) to pinealectomized rats before both ischemia and reperfusion reduced infarct volume by 40%. Melatonin also significantly improved neurological deficit scores in pinealectomized and in the sham-operated group subjected to MCA occlusion (Manev et al., 1996; Kilic et al., 1999). It was found to decrease the infarct area, prevent neuronal death after MCA-O for 1 h in rats and increase the expression of neuronal bcl-2 in the penumbral area of the ischemic brain (Ling et al., 1999). This may suggest that melatonin has a potential role in inhibiting apoptosis after cerebral ischemia. However, melatonin treatment may be problematic due to its various physiological roles and multiple undesirable side effects.

4. α-Lipoic Acid. α-Lipoate is a LMWA absorbed from the diet, which crosses the BBB (Packer, 1992; Packer et al., 1997). It is intracellularly reduced to dihydrolipoate, which is exported to the extracellular medium. Both α-lipoate and especially dihydrolipoate are potent antioxidants and reduce lipid peroxidation. Hence, protection is potentially afforded to both intracellular and extracellular environments.

α-Lipoate was shown to scavenge hydroxyl radicals, singlet oxygen, and nitric oxide. In addition, α-lipoate chelates a number of transition metals, recycles other antioxidants (such as vitamin C and vitamin E), raises intracellular levels of glutathione, and modulates transcription factors activities, especially that of NF-κB (Packer et al., 1997; Packer, 1998).

Prehn et al. (1992) and Backhaus et al. (1992) found that treatment with dihydrolipoate, but not with α-lipoate, reduced infarct size after MCA occlusion in mice. Others reported a protective effect of α-lipoate, only when given subcutaneously, but not intraperitoneally or intracisternally. It was found that the *S*-enantiomer was more effective than the *R*-enantiomer when administered only 1 h before ischemia (Woltz and Krieglstein, 1996). Cao and Phillis (1995) observed a protective effect of α-lipoate against ischemia-reperfusion injury in the Mongolian gerbil model. Gerbils treated with α-lipoate for 7 days before ischemia-reperfusion exhibited less change in locomotor activity and less damage to the CA1 hypocampal pyramidal cell layer, than the saline-treated controls. Controlled clinical studies should be performed to evaluate its advantages in acute CNS injury.

5. Ebselen. Glutathione peroxidase, in both selenium-dependent and -independent forms, is one of the major enzymes responsible for the degredation of hydrogen peroxide and organic peroxides in the brain. The seleno-organic compound ebselen has antioxidant activity through a glutathione peroxidase-like action. This data has led to extended research of this molecule (Muller et al., 1984; Wendel et al., 1984).

i. Animal Models. Johshita et al. (1990) showed that ebselen significantly ameliorated the postischemic hypoperfusion after MCA occlusion. Another study showed that oral ebselen administration (30 mg/kg) 40 min before MCA occlusion in rats reduced the volume of ischemic damage in the cerebral hemisphere and cerebral cortex by 48 and 53%, respectively (Dawson et al., 1995). Takasago et al. (1997) showed that 10 mg/kg ebselen administration reduced the volume of ischemia in the cerebral hemisphere and cerebral cortex by 31.8 and 36.7%, respectively, in a rodent model of focal cerebral ischemia (permanent MCA occlusion). Ebselen (50 mg/kg) ameliorated delayed cerebral vasospasm as detected angiographically in a canine model of SAH (Watanabe et al., 1997).

ii. Clinical Studies. Based on encouraging evidence of the neuroprotective role of ebselen in animal models, Saito et al. (1998) conducted a randomized, placebo-controlled clinical study in 286 SAH patients. Ebselen, given orally at a dose of 300 mg per person per day for 2 weeks, did not affect the incidence of symptomatic vasospasm but significantly ameliorated delayed ischemic neurological deficits and subsequent cerebral infarction, leading to an improvement in the overall outcome. In humans, this compound has come to be regarded as a neuroprotective agent rather than an antivasospastic agent.

In 300 patients with acute ischemic stroke, treatment with ebselen (150 mg twice a day for 2 weeks) within 48 h of stroke onset showed, at 1 month, a significantly improved outcome, as measured by the Glasgow outcome scale (Yamaguchi et al., 1998). The improvement was maintained at 3 months, although this failed to reach statistical significance. Ogawa et al. (1999) conducted a randomized, double-blind, placebo-controlled trial of ebselen in 99 patients with complete MCA-O. Ebselen (150 mg) was given orally within 12 h of onset and continued for 2 weeks. There was a corresponding significant reduction in the volume of cerebral infarct and an improvement in the outcome of patients who had started treatment within 6 h of onset. These findings suggest that ebselen may protect the human brain from ischemic damage in the acute stage. Ebselen was given in optimal administration route (i.v.), providing rapid

and controlled delivery of the drug into the brain and is now in phase 3 clinical stroke trials. These preliminary clinical results are promising, but further studies are needed to establish its beneficial effects in stroke.

6. Human Superoxide Dismutase/Superoxide Dismutase-Like Molecules. SOD converts superoxide to hydrogen peroxide (H_2O_2) and represents the first line of defense against oxygen toxicity. Three forms have been described in man: The first isoform, containing copper and zinc at its active site (Cu/Zn SOD-1), is found in the cytoplasm of cells. Another isoform, containing manganese at its active site, is located in the mitochondria (Mn SOD-2). The third isoform is present in extracellular fluids such as plasma (Cu/Zn SOD-3). It was found that the traces of copper, zinc, and manganese metals are essential for maintaining the antioxidant activity of SOD (Halliwell, 1994).

i. Animal Studies. Preischemic administration of recombinant human SOD has been reported to attenuate the ischemic damage induced in gerbils by 5-min bilateral carotid artery occlusion (Tagaya et al., 1992). Further studies have shown that transgenic mice expressing the human Cu-Zn isoform (SOD-1) have a reduced infarct volume after MCA occlusion. Nitroxides, which are cell-permeable, nontoxic stable radicals, display SOD-like activity. Beit-Yannai et al. (1996) and Zhang (1998) showed that nitroxides (50 mg/kg i.v.) reduced edema, ameliorated BBB disruption, and markedly improved outcome when administered within a therapeutic window of 4 h after CHI in rats.

ii. Clinical Studies. In a multicenter, randomized controlled trial, polyethylene glycol-conjugated SOD therapy (pegorgotein) failed to improve outcome of patients with severe head injury, when given within 8 h after injury (Young et al., 1996). This relatively long therapeutic time window may explain the failure of SOD to exhibit a protective effect in this clinical trial.

7. Spin-Trap Scavenging Agents. Spin-trap scavenging agents are molecules (usually with a nitrone moiety) that have been used in electron paramagnetic studies for trapping highly reactive, unstable radicals. These compounds have been shown to protect experimental animals from pathology, mainly associated with ischemia-reperfusion injury, physical trauma, and aging (Carney et al., 1991; Hensley et al., 1997). Phenyl-α-*tert*-butyl nitrone (PBN) is a synthetic antioxidant capable of scavenging oxygen- and carbon-based free radicals (Kotake, 1999).

In gerbils given PBN before brain ischemia-reperfusion injury, survival significantly improved as compared with controls (Carney and Floyd, 1991). Preischemic systemic administration of 100 mg/kg PBN, combined with 100 mM topical PBN, produced a significant attenuation of hydroxyl radical adduct during ischemia-reperfusion injury of rat brain (Sen and Phillis, 1993). PBN was shown to reduce the infarct size after transient MCA occlusion in rats (Zhao et al., 1994) and to improve the recovery of brain energy state when given 1 h after reperfusion (Folbergrova et al., 1995). It was shown to reduce neuronal necrosis in the neocortex when given 30 min postischemia but not when given before or 6 h after the ischemic event (Pahlmark and Siesjo, 1996). PBN also attenuated the secondary mitochondrial dysfunction after transient focal cerebral ischemia in rats when given 1 h after the start of recirculation (Kuroda et al., 1996). Schultz et al. (1997) reported that PBN administration (25 mg/kg i.v.) 5 min before and 30 min after MCA occlusion in rats provided protection of the vascular endothelium, leading to enhanced postischemic reperfusion. To promote these molecules for clinical applications, intensive toxicity studies should be preformed. These agents should be design for short therapeutic time windows to combat the earlier ischemic events.

8. N-Acetylcysteine. NAC is a thiol-containing compound used in clinical practice since the mid-1950s. NAC has been demonstrated to effectively reduce free radical species and other oxidants, especially OH and H_2O_2 (Moldeus et al., 1986). NAC is not synthesized endogenously and cannot cross the BBB after exogenous administration. This fact limits the efficacy of NAC in vivo.

Animal studies (mostly rodent models) have indicated that NAC may be beneficial in the treatment of ischemia-reperfusion-induced oxidant injury in a variety of tissues. NAC has been shown to have a neuroprotective capacity during periods of transient forebrain or hippocampal ischemia in rats. However, the efficacy of the drug decreased as the duration of the ischemic period increased (Knuckey et al., 1995).

Another recent study showed that preischemic administration of *S*-allylcysteine, another cysteine-containing compound, inhibits free radical production, lipid peroxidation, and neuronal damage in MCA occlusion model in rats (Numagami and Ohnishi, 2001). Because NAC was used and demonstrated to be helpful in various nonneurological diseases, its brain-penetrated derivates should be developed and employed in stroke.

9. Glutathione. GSH is a ubiquitous tri-peptide formed from three amino acids—glutamate, glycine, and cysteine—and synthesized by two ATP-dependent enzymatic reactions (Richman and Meister, 1975; Meister and Anderson, 1983). It can also be generated from metabolism of NAC. GSH has major intracellular antioxidant activity, mainly due to the thiol group within the molecule. It plays a critical role in detoxification of peroxides and electrophilic toxins as a substrate for GSH peroxidase and GSH transferase (Larsson et al., 1983; Meister and Anderson, 1983).

It was shown that GSH depletion (e.g., by buthionine sulfoximine, which inhibits γ-glutamylcysteine synthetase, the producing enzyme of GSH) enhances cerebral ischemic injury in rats (Mizui et al., 1992). Shivakumar et al. (1995) showed that GSH levels were decreased in brain regions during reperfusion for 1 h

after moderate or severe ischemia for 0.5 h. However, Zaidan and Sims (1996) showed a 150% increase in GSH levels in the mitochondria after 30 min of forebrain ischemia in rats. Thus, rapid restoration of thiol homeostasis in the brain during reperfusion may help the brain recover from ischemia-reperfusion injury.

The glutathione analog YM737 was shown to reduce lethality, increase brain-water levels, and decrease malondialdehyde levels in cerebral ischemic rats when given immediately after ischemia, suggesting that its antiischemic effects are due, in part, to inhibition of lipid peroxidative responses (Yamamoto et al., 1993). These short therapeutic time windows for intervention in GSH system make the task of repeating these results in clinical trials even more difficult.

10. Metal Ion Chelators. Free metal ions are associated with the pathology of various neurodegenerative diseases (e.g., copper in Wilson's disease and iron in the substantia nigra in Parkinson's disease). Therefore, proteins that are involved in the binding of metal ions were suggested to act as antioxidants. These may include transferrin (binds iron), ceruloplasmin (binds copper), and hemopexin (binds heme, a catalyst in oxidative reactions). Desferral, a potent chelator of redox-active metals, was shown to facilitate the clinical recovery of traumatized rats in a model of CHI (Zhang et al., 1998). Deferoxamine, an iron catalyst in the generation of free radicals and lipid peroxides, given prior or soon after the ischemic episode improved survival and physiological functions in rats (Palmer et al., 1994), dogs (Hurn et al., 1995), and mice (Sarco et al., 2000). However, Fleischer et al. (1987) did not find any benefit of deferoxamine in complete cerebral ischemia in dogs.

In an experimental model of SAH (Vollmer et al., 1991), the administration of deferoxamine 16 h before the induction of SAH, showed a consistent attenuation of vascular contraction of cerebral vessels of 77%. The significance of these results to clinical practice in stroke and other OS-involved diseases is quite limited, due to the short therapeutic time windows. Because metal ions have an important role in many enzyme activities, such intervention may be toxic and limit the clinical applications.

11. Uric Acid. Uric acid is a waste product of the living cell, which is produced by xanthine oxidase. It is widely distributed in relatively high concentrations throughout the body. Urate contributes up to 60% of the total plasma antioxidant activity in healthy subjects (Wayner et al., 1987, Benzie, 1996). It acts as an antioxidant by interacting with 10 to 15% of the hydroxyl radicals produced daily and by efficiently scavenging both peroxyl radicals and singlet oxygen (Ames et al., 1981). It also binds iron (Davies et al., 1986) and acts indirectly by stabilizing plasma ascorbate (Sevanian et al., 1991). In contrast, Benzie and Strain (1996) hypothesized that urate at high concentrations acts as a prooxidant and suggested that hyperuricemia is a risk factor for oxidative stress-associated disorders.

It was shown that the cerebral uric acid level increases 10-fold after experimental CHI in rats 24 and 48 h after injury (Tayag et al., 1996). Administration of uric acid to rats either 24 h before MCA occlusion (62.5 mg/kg, i.p.) or 1 h after reperfusion (16 mg/kg, i.v.) resulted in a highly significant reduction of ischemic damage to cerebral cortex and striatum. Uric acid also improved the behavioral outcome in these rats (Yu et al., 1998). Implementation of these results to clinical practice is quite limited, due to the above short therapeutic time windows.

12. Creatine. Creatine (*N*-[aminoiminomethyl]-*N*-methyl glycine) is a tri-peptide endogenously produced from glycine, methionine, and arginine in the liver, kidney, and pancreas (McArdle et al., 1999). Creatine can be found in the muscle, but also in brain tissue (Mujika and Padilla, 1997). Recent experimental findings have demonstrated that creatine provides significant neuroprotection against ischemic and oxidative insults (Holtzman et al., 1998; Balestrino et al., 1999). Sullivan et al. (2000) showed that chronic administration of creatine ameliorated the extent of cortical damage by as much as 36% in mice and 50% in rats after experimental traumatic brain injury. The protection seemed to be related to creatine-induced maintenance of mitochondrial bioenergetics. Mitochondrial membrane potential was significantly increased, intramitochondrial levels of ROS and calcium were significantly decreased, and ATP levels were maintained. This new agent should be intensively investigated before clinical studies for acute CNS injury are performed.

13. Lazaroids. Lazaroids are 21-aminosteroids derived from glucocorticosteroids, but they lack glucocorticoid and mineralocorticoid activities. They scavenge lipid peroxyl radicals and inhibit iron-dependent lipid peroxidation (Hall, 1995). Tirilazad mesylate (U-74006F), one of the lazaroid series, is a lipophilic compound with a high affinity for vascular endothelium (Hall et al., 1994). It was shown to protect the BBB against traumatic or SAH-induced permeability. The penetration of tirilazad to brain parenchyma is enhanced after acute CNS injury and disruption of the BBB (Hall et al., 1994).

i. Animal Model Studies.

Ischemia. Lazaroids were shown to protect against ischemic damage in several species (Hall and Braughler, 1989; Hall, 1995; Clark et al., 1995). Tirilazad provided a neuroprotective effect in animal model systems of focal cerebral ischemia-reperfusion injury to the brain or spinal cord (Braughler and Hall, 1989; Xue et al., 1992; Park, 1994; Hall, 1995). It has been demonstrated to decrease infarct size secondary to permanent MCA occlusion in rats (Beck and Bielenberg, 1991; Park and Hall et al., 1994) and cats (Silvia et al., 1987). It improved both 24- and 48-h survival in gerbils subjected

to 3 h of unilateral carotid occlusion (Hall et al., 1988). Other studies have shown that the novel lazaroid LY231617 protects against ischemia-induced neuronal damage in rat models of global cerebral ischemia (Clemens et al., 1993; O'Neill et al., 1997).

Subarachnoid hemorrhage. Tirilazad mesylate prevented SAH-induced chronic vasospasm in a rabbit model (Zuccarello et al., 1989). Intravenous administration of the 21-aminosteroid U74389G, another potent inhibitor of lipid peroxidation and a free radical scavenger, in a dog model of SAH has significantly decreased vasospasm (Macdonald et al., 1998).

Closed head injury. Administration of a single i.v. dose (0.003–30 mg/kg) of tirilazad mesylate produced a significant improvement in the neurological status 1 h postinjury in head-injured mice (Hall, 1988a). Similarly, it reduced BBB disruption in a controlled cortical impact injury rat model (Smith et al., 1994) and attenuated post-traumatic mortality and brain edema (Mcintosh et al., 1992; Sanada et al., 1993).

Spinal cord injury. Tirilazad has been reported to improve the neurological recovery of cats after a moderately severe compression injury to the lumbar spinal cord (Anderson et al., 1988, 1991; Hall, 1988b). It also improved the subacute neurological recovery of rats subjected to a compression spinal injury (Holtz and Gerdin, 1991).

ii. Clinical Studies. After demonstrating its cerebroprotective efficacy in animal models, tirilazad has been clinically evaluated in acute human neurological disorders.

Subarachnoid hemorrhage. In two very similar multicenter trials of tirilazad in SAH, one in Europe, Australia, and New Zealand and the other in North America, conflicting results have been reported. Kassel et al. (1996) showed reduced mortality in patients treated with tirilazad (6 mg/kg/d for 10 days) and a better 3-month neurological outcome compared with those given placebo. Gender differences were observed, probably due to the pharmacokinetics of tirilazads, which is metabolized by the P450 enzyme system in the liver (Fleishaker et al., 1995). In contrast, Haley et al. (1997) found no differences between the tirilazad and placebo groups. These conflicting results can be explained by differences in patient admission characteristics, standard of care, or the use of anti-convulsive drugs, which decreases the bioavailability of tirilazad.

Closed head injury. Two large multicenter trials of tirilazad in moderate and severe closed head injury failed to show any clear differences in outcome between the treated and the placebo groups (Marshall et al., 1998; Maas et al., 1999).

Spinal cord injury. Treatment with tirilazad (2.5 mg/kg every 6 h for 2 days) seemed to have equal efficacy compared with 24-h infusion of methylprednisolone in acute spinal injury patients (Bracken et al., 1997).

Huang et al. (2001) suggested that although the lazaroid compounds inhibit lipid peroxidation, they do not reduce the frequency of deoxyribonucleic acid (DNA) damage, adenosine tri-phosphate depletion, or loss of cell replication, which occurs later. One of the major problems concerning the use of 21-aminosteroids as neuroprotective agents is that they have low oral bioavailability and brain uptake (Hall, 1991; Raub et al., 1993). Due to the conflicting results, more experimental and clinical data is needed to elucidate whether lazaroids can be used in acute CNS injury.

TABLE 1
Antioxidants and free radical scavengers currently available for the treatment of acute CNS injury

- Endogenous enzymes, e.g., SOD and glutathione peroxidase-like molecules (e.g., ebselen)
- Endogenous antioxidant compounds (also found in diet), e.g., tocopherols (vitamin E), ascorbic acid (vitamin C), carotenoids (β-carotene)
- Other endogenous antioxidant substances, e.g., uric acid, glutathione, and glutathione precursors e.g., cysteine (given as NAC), melatonin, and creatine
- Endogenous antioxidant cofactors, e.g., coenzyme Q_{10}
- Metal chelators, e.g., deferoxamine, desferal, and nitrone-based free radical traps e.g., PBN and lipoic acid
- Naturally occurring plant substances, e.g., flavonoids
- Synthetic free radical compounds, e.g., 21-aminosteroids (lazaroids) and pyrrolopyrimidines
- Other compounds, e.g., polyamines, MCI-186

14. Nicaraven. Two recent studies using nicaraven (also called AVS (±)-*N*,*N*′-propylenedinicotinamide), a hydroxyl radical scavenger, confirmed its antivasospastic and brain-protective activities, accompanied by improved cerebral blood flow and glucose use in a rat model of SAH (Germano et al., 1998; Yamamoto et al., 2000).

Nicaraven has been tried in a prospective, placebo-controlled, double-blind, multicenter trial (Asano et al., 1996) for evaluation as an antivasospastic agent in SAH. Nicaraven seemed to reduce symptomatic vasospasm significantly (34.5%) and improved Glasgow outcome scale at 1 month. At 3 months, the differences in the Glasgow outcome scale between the groups became marginal, but the percentage of good outcome tended to increase, and the cumulative incidence of death was significantly reduced.

15. Other Antioxidants.

i. 2,4-Diamino-Pyrrolo[2,3-D] Pyrimidines. In vivo models of oxidative injury in mice (Hall et al., 1997) and ischemia models in rats (Schmid-Elasesser et al., 1997) have recently shown some efficacy of the novel 2,4-diamino-pyrrolo [2,3-D] pyrimidines. These molecules, administered orally, were identified as having a much greater BBB penetration capacity (Hall et al., 1997) and high-lipophilic antioxidant activity with protective effects (Bundy et al., 1995).

ii. Polyamines. It has been well established that alterations in polyamines (e.g., spermidine and spermine), which are potent antioxidants and anti-inflammatory agents, occur in animal models of focal and global ischemia and traumatic brain injury (Johnson, 1998). Gilad and Gilad (1991, 1992) found that polyamines could

TABLE 2
Efficacy of antioxidants in the treatment of cerebral ischemia and intracranial hemorrhage in clinical studies

Antioxidant	Disease	Efficacy	References
Vitamins (mainly E, C, carotenoids (β-carotene), and flavonoids	Ischemic stroke	+	Gey et al., 1993; Keli et al., 1996; Daviglus et al., 1997
		−	Blot et al., 1993; Hennekens et al., 1996
		+/−	Yochum et al., 2000 (just from food)
Ebselen		+	Yamaguchi et al., 1998
		+	Ogawa et al., 1999
	SAH	−	Saito et al., 1998
Tirilazad mesylate	SAH	+	Kassel et al., 1996
		−	Haley et al., 1997
	Head injury	−	Marshall et al., 1998; Maas et al., 1999
	Spinal cord injury	−	Bracken et al., 1997
Superoxide dismutase-like	Head injury	−	Young et al., 1996
Nicaraven	SAH	+	Asano et al., 1996

protect against ischemia-induced nerve cell death in the gerbil forebrain.

iii. MCI-186. This is a free radical scavenger that was shown to inhibit in vitro both nonenzymatic lipid peroxidation and lipoxygenase activity. MCI-186 (3 mg/kg, i.v.) markedly attenuated the ischemic and postischemic brain swelling after MCA-O (3 and 6 h of ischemia) and reperfusion in rats (Abe et al., 1988).

III. Conclusion and Future Strategies

An increasing amount of evidence suggests that OS is important in either the primary or the secondary pathophysiological mechanisms underlying acute CNS injury. In addition, reduction in the endogenous antioxidant defense system due to environmental and genetic factors may contribute to OS evolution. Therefore, the discovery and development of potent antioxidant agents has been one of the most interesting and promising approaches in the search for treatment of CNS injury. Antioxidants of varying chemical structures have been investigated as therapeutic agents in the treatment of acute CNS injury (Table 1). Although some of the antioxidants showed efficiency in animal models, most of them did not show beneficial effect in clinical trials performed to date (Table 2). To achieve efficacy, the antioxidant must be given during the "time window" available between the vascular event and irreversible neuronal loss. They also should fit to the precise OS physiology, e.g., the type of ROS involved, the place of generation, and the severity of the damage. Moreover, antioxidants must penetrate the BBB to attain a critical therapeutic level within the CNS. Thus, pharmacotherapy for closed head injury and SAH is less problematic than for other acute CNS injuries, because there is obvious disruption of the BBB, enabling easier drug penetration to the brain. Potential reasons for antioxidant failure to achieve neuroprotection in clinical trials include narrow "time window", suboptimal drug dose, inappropriate drug levels at the target CNS site, and discrepancy in drug mechanism and pathophysiological processes (Table 3). Antioxidants may have differential effects in protecting nucleic acids, proteins, and lipids from free radical damage and some compounds may be preferentially localized within specific subcellular organelles. Thus, antioxidant cocktails or antioxidants combined with other drugs such as calcium antagonists, glutamate antagonists, or anti-apoptotic agents, may have more successful synergistic effects. Better understanding of the underlying pathologic al mechanisms of acute CNS injury and improvement of the molecular design of antioxidants will open a

TABLE 3
Possible reasons for lesser efficacy of antioxidants in clinical studies

- Current theories about mode of drug action in arresting propagation of neuronal damage in the ischemic territory are inappropriate.
- Current experimental models of ischemia are inappropriate to human stroke.
- Effective neuroprotection by drug in the models cannot predict success in the human illness.
- Drug that inhibits one parameter of injury might be insufficient to inactivate other parallel pathways of the ischemic destructive cascade.
- Stroke is associated with several injuries induced by various mechanisms. Single treatment might miss the "time window" opportunity, e.g., glutamate toxicity may occur early and last only a short time.
- Antioxidants should be given in very narrow range of therapeutic dosages. As with vitamins, antioxidants given in high doses in a certain redox situation might become pro-oxidants and toxic.
- Inadequate circulation in collateral vessels preventing adequate delivery of the drug to a major portion of the ischemic tissue.
- Inadequate penetration into salvageable portion of the ischemic zone that maintains BBB integrity.
- Genetic, environmental, age, and dietary background differences between the study trials.
- Source of supplementation: natural vs. synthetic agents might influence the study outcome.
- The high heterogeneity of the brain damage size and neurological outcome in human stroke patients, making it difficult to obtain statistically significant effects of therapeutic agents.
- Brain structure, function, and vascular anatomy of humans and animals differ.

full spectrum of possibilities for treatment of various types of injuries.

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