

## Review

# Oxidants, antioxidants and the ischemic brain

David S. Warner<sup>1,3,4,\*</sup>, Huaxin Sheng<sup>1</sup> and Ines Batinić-Haberle<sup>2</sup>

*Departments of <sup>1</sup>Anesthesiology, <sup>2</sup>Radiation Oncology, <sup>3</sup>Neurobiology and <sup>4</sup>Surgery (Neurosurgery), The Multidisciplinary Neuroprotection Laboratories, Duke University Medical Center, Durham, NC 27710, USA*

\*Author for correspondence (e-mail: warne002@mc.duke.edu)

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### Summary

**Despite numerous defenses, the brain is vulnerable to oxidative stress resulting from ischemia/reperfusion. Excitotoxic stimulation of superoxide and nitric oxide production leads to formation of highly reactive products, including peroxynitrite and hydroxyl radical, which are capable of damaging lipids, proteins and DNA. Use of transgenic mutants and selective pharmacological antioxidants has greatly increased understanding of the complex interplay between substrate deprivation and ischemic outcome. Recent evidence that reactive oxygen/nitrogen species play a critical role in initiation of**

**apoptosis, mitochondrial permeability transition and poly(ADP-ribose) polymerase activation provides additional mechanisms for oxidative damage and new targets for post-ischemic therapeutic intervention. Because oxidative stress involves multiple post-ischemic cascades leading to cell death, effective prevention/treatment of ischemic brain injury is likely to require intervention at multiple effect sites.**

Key words: brain, ischemia, oxidative stress, antioxidant.

### Introduction

Oxidative stress has been defined as 'a disturbance in the pro-oxidant-antioxidant balance in favour of the former, leading to potential damage' (Sies, 1991). The brain consumes a large quantity of oxygen, making it particularly susceptible to oxidative stress. Natural formation of oxidants during mitochondrial electron transport, auto-oxidation of some neurotransmitters (e.g. norepinephrine, dopamine) and initiation of events during hypoxia or ischemia, can result in oxidant formation and subsequent tissue damage. Oxidative stress can be traced primarily to formation of superoxide and nitric oxide. Both molecules have important roles in health, serving as regulators of blood flow and neurotransmission. Perturbation in the production and/or metabolism of either molecule can have pathologic consequences.

Principal sources of superoxide include electron leak during mitochondrial electron transport, perturbed mitochondrial metabolism and inflammatory responses to injury (Halliwell and Gutteridge, 1999). The brain has potent defenses against superoxide including dietary free-radical scavengers (ascorbate,  $\alpha$ -tocopherol), the endogenous tripeptide glutathione, and enzymatic antioxidants. Enzymatic antioxidants regulate superoxide concentration by dismutation of superoxide to hydrogen peroxide (superoxide dismutase or SOD; Fridovich, 1995), which is then converted to water (peroxidases such as glutathione peroxidase and peroxiredoxin) or dismutated to water and oxygen (catalase).

Although increased expression of these enzymes can occur in response to ischemia (Fukui et al., 2002), endogenous antioxidant capacity can be overwhelmed, leading to increased superoxide and hydrogen peroxide concentrations.

Nitric oxide formation is both constitutive and inducible. Ischemia-induced nitric oxide overproduction is in part caused by glutamatergic-mediated increases in intracellular calcium concentration, resulting in a calmodulin-dependent upregulation of nitric oxide synthase (NOS; Dawson et al., 1991; Garthwaite et al., 1988, 1989). Nitric oxide can be consumed by reacting with hemoglobin (Ignarro et al., 1987; Joshi et al., 2002). Flavohemoglobin-based enzymes (nitric oxide reductase, nitric oxide dioxygenase) capable of specifically metabolizing nitric oxide have been identified in bacteria (Hausladen et al., 1998), and flavohemoglobin-like activity has been identified in mammalian cells (Gardner et al., 2001). Yet, an important non-enzymatic mechanism regulating nitric oxide concentration is its reaction with superoxide yielding peroxynitrite (Beckman et al., 1990).

Under pathophysiological conditions, excessive nitric oxide production can elicit nitrosative damage (Espey et al., 2000) *via* independent nitrosylation of protein heme sites (e.g. cytochrome *c*; Schonhoff et al., 2003) or through its reaction products with oxygen or other nitrogen oxides. Superoxide can cause oxidative damage of iron/sulfur clusters of aconitase (Gardner and Fridovich, 1991), an important enzyme in the

tricarboxylic acid cycle. The major oxidative stress produced by superoxide, however, is derived from its participation in peroxynitrite formation (Beckman et al., 1990) and its involvement in the iron-catalyzed Haber–Weiss reaction (superoxide-driven Fenton chemistry; Liochev and Fridovich, 2002), causing hydrogen peroxide to be converted to hydroxyl radical. Hydroxyl radical, peroxynitrite and peroxynitrite-derived products (hydroxyl radical, carbonate radical and nitrogen dioxide) all have the potential to react with and damage most cellular targets including lipids, proteins and DNA.

Direct measurement of reactive oxygen (ROS) and nitrogen (RNS) species concentrations in tissue subjected to ischemia/reperfusion is problematic (Tarpey and Fridovich, 2001). Low intracellular concentrations, short half-lives and the efficient and redundant systems that have evolved to scavenge ROS/RNS require that any detection technique must be sensitive and specific enough to compete with antioxidant defenses against the species in question (Fridovich, 2003; Glebska and Koppenol, 2003; Myhre et al., 2003; Zhao et al., 2003). Additionally, the methods applied must have intracellular access to monitor the intracellular milieu. This undoubtedly has contributed to confusion surrounding the roles of these species in disease. Most commonly, ROS/RNS have been tracked by measuring stable metabolites (e.g. nitrates/nitrites) or ‘footprints’ of the reactions of these molecules with lipids (e.g. thiobarbituric acid adducts, 4-hydroxynonenal), DNA (e.g. 8-hydroxyguanine) or proteins (e.g. nitrotyrosine). Electrochemical and microdialysis approaches have also proven useful in tracking superoxide (Fabian et al., 1995) and hydroxyl radical (Globus et al., 1995) concentrations.

An alternative approach to the study of ROS/RNS in ischemic brain is the use of either transgenic animals or pharmacological agents to alter antioxidant potential. For example, if targeted disruption of a specific SOD genetic coding sequence increases ischemic tissue damage, evidence is provided that the enzyme plays a beneficial role in the response of brain to oxidative stress. This is further supported if overexpression of the same gene results in increased tissue tolerance to ischemia. There are two major limitations to the use of transgenic mice in study of oxidative stress. First, compensatory mechanisms, perhaps developed during ontogeny so as to allow survival in the absence/overexpression of the gene, are rarely considered, particularly in the context of the experiment being performed (Ibrahim et al., 2000; Przedborski et al., 1992). Second, although progress is being made in the use of conditional ‘knock-outs’ and ‘overexpressors’, in which a selected gene’s expression is decreased/increased in response to a specific pharmacological stimulus, most work continues to be performed with animals that retain their knock-out (or overexpressing) status throughout the entire ischemia/reperfusion interval. This makes it difficult to determine when and how the gene product influences ischemic injury.

The ultimate goal for understanding the mechanism of

oxidative stress in brain ischemia is to develop therapeutic interventions. To this end, innumerable pharmacological antioxidants have been evaluated. Although these agents have received the greatest scrutiny for therapeutic potential, the same agents can also be used to dissect the role of oxidative stress in ischemic brain injury by assessing the impact of their purported mechanism of action on ischemia-induced intracellular cascades and outcome. On the other hand, the study of pharmacological agents is limited by bioavailability and undefined secondary effects when introduced into an *in vivo* environment. Thus, transgenic and pharmacological interventions can be viewed as complimentary tools to examine the role of oxidative stress in ischemic brain injury. This review will consider various possible contributions of oxidative stress to ischemic brain injury, with a focus on validation of the mechanism *via* either transgenic or pharmacological intervention (Fig. 1).

### Inhibition of lipid peroxidation

Free radical damage was one of the earliest mechanisms postulated to explain tissue demise after a cerebral ischemic insult (Flamm et al., 1978). Stroke research rapidly focused on lipid metabolism for good reason. During cerebral ischemia, free fatty acid concentrations are markedly increased, the largest increase being that of arachidonic acid (Bazan, 1970; Marion and Wolfe, 1979; Rao et al., 1999; Siesjo and Wieloch, 1983).  $Ca^{2+}$ -activated phospholipases C and  $A_2$  result in phospholipid hydrolysis, while resynthesis of phospholipids requires ATP. As a result, ischemia-induced  $Ca^{2+}$  influx and energy failure promote free fatty acid release and concomitant membrane damage. Free fatty acid metabolism has multiple other adverse effects including inhibition of oxidative phosphorylation (Wojtczak, 1976), oxidative conversion of free arachidonic acid *via* the cyclo-oxygenase pathway to eicosanoids (thromboxanes and prostaglandins) (Gaudet et al., 1980), free radical generation and lipid peroxidation-mediated chain reactions (Imaizumi et al., 1986; Watson et al., 1984), and cytotoxicity from lipid peroxidation products (e.g. 4-hydroxynonenal; Kruman et al., 1997), which may stimulate apoptosis (Mattson et al., 2000).

Increased nitric oxide concentrations associated with ischemia may have dual effects on lipid peroxidation. Reaction of nitric oxide with superoxide causes formation of peroxynitrite that initiates lipid peroxidation *via* reaction of lipids with its decomposition products hydroxyl radical and nitrogen dioxide (Brookes et al., 1998; Rubbo et al., 1994). In contrast, nitric oxide itself may directly inhibit lipid peroxidation by intercepting alkoxy and peroxy radical intermediates thereby terminating chain propagation reactions (Nicolescu et al., 2002; Niziolek et al., 2003; Rubbo et al., 1994).

Despite this, it has been difficult to confirm that lipid peroxidation is a primary and critical contributor to ischemic cell death as opposed to being a result of intracellular organelle dysfunction mediated by oxidative stress (Watson,

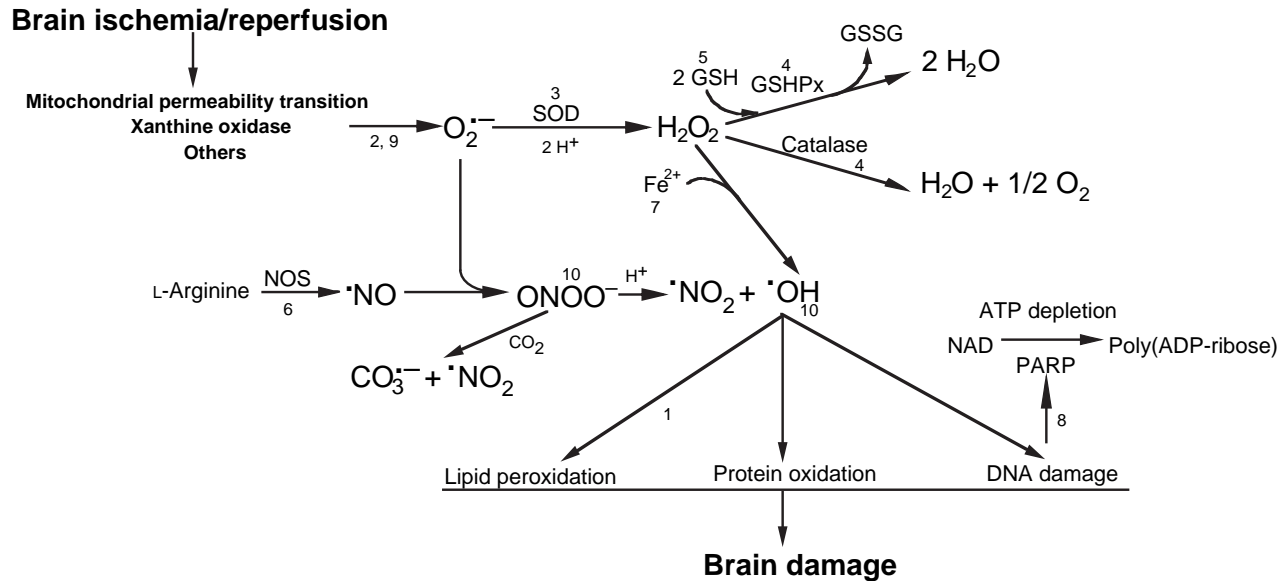


Fig. 1. Ischemia/reperfusion presents numerous opportunities for formation of reactive oxygen/nitrogen species and resultant tissue injury. Simultaneously, numerous site-specific targets for therapeutic intervention are presented. It quickly becomes clear that inhibition of a single pathway may be insufficient to provide persistent protection against oxidative stress. (1) Inhibition of lipid peroxidation; (2) inhibition of xanthine oxidase; (3) the superoxide dismutases (SOD) and their mimetics; (4) catalase and glutathione peroxidase (GSHPx); (5) glutathione (GSH) mimetics; (6) nitric oxide synthase (NOS) inhibition; (7) metal chelators; (8) poly(ADP-ribose) polymerase (PARP) inhibitors; (9) mitochondrial permeability transition inhibitors; (10) spin traps and peroxynitrite scavengers.  $O_2^{\bullet-}$ , superoxide;  $CO_3^{\bullet-}$ , carbonate radical;  $H_2O_2$ , hydrogen peroxide; GSSG, glutathione disulfide;  $\bullet OH$ , hydroxyl radical;  $\bullet NO_2$ , nitrogen dioxide;  $\bullet NO$ , nitric oxide;  $ONOO^-$ , peroxynitrite; NAD, nicotinamide adenine dinucleotide.

1998). Indeed, numerous pharmacological inhibitors of lipid peroxidation have been tested. The most notable is tirilazad, a non-glucocorticoid steroid. Despite abundant preclinical evidence that tirilazad improved ischemic outcome *via* its putative action as inhibitor of lipid peroxidation (Kavanagh and Kam, 2001), no effect on outcome from human stroke was observed (Haley, 1998). It should be noted that virtually all of the positive preclinical studies recorded only a short-term outcome (i.e. several days post-ischemia), while human trials measured the outcome after 3 months. Although it is clear that lipid peroxidation occurs in response to oxidative stress and that membrane disruption is disadvantageous to the cell, the available outcome data are insufficient to allow the conclusion that this mechanism is critical in defining ischemic outcome.

#### Inhibition of xanthine oxidase

Metabolism of ATP leads to accumulation of hypoxanthine (Morimoto et al., 1982). In non-ischemic tissue, xanthine oxidase exists as a nicotinamide adenine dinucleotide (NAD)-reducing hydrogenase. During ischemia,  $Ca^{2+}$ -stimulated proteases cause irreversible partial cleavage of xanthine dehydrogenase to xanthine oxidase, which in turn catalyzes oxidation of hypoxanthine to xanthine. Xanthine oxidase further oxidizes xanthine to produce uric acid, superoxide and hydrogen peroxide (Parks and Granger, 1986). Thus, xanthine oxidase inhibitors have been subjected to extensive scrutiny with respect to antioxidant potential. Most work has used either

allopurinol or oxypurinol. Allopurinol is oxidized by xanthine oxidase to oxypurinol, which binds to the active site of xanthine oxidase causing xanthine oxidase inhibition. Thus, either compound can be administered with the same net mechanistic effect.

Allopurinol decreases post-ischemic cerebral uric acid, xanthine and conjugated diene concentrations (Marro et al., 1994; Nihei et al., 1989), preserves ATP (Williams et al., 1992), and reduces edema (Patt et al., 1988). Despite this, studies employing the requisite physiological control and long-term outcome analysis of effects of xanthine oxidase inhibitors on post-ischemic behavior and histology have not been performed. The results from short-term outcome studies in adult rats have been mixed (Lindsay et al., 1991; Martz et al., 1989). More encouraging results have been observed in perinatal brain (Palmer et al., 1993, 1990; van Bel et al., 1998), but no long-term outcome studies have been reported. As a result, despite biochemical evidence of diminished oxidative stress from inhibition of hypoxanthine metabolism, evidence supporting xanthine dehydrogenase/oxidase activity as a major contributor to ischemic outcome is modest. This is not surprising because many other avenues for superoxide and hydrogen peroxide generation (e.g. inflammation) are unaffected by xanthine oxidase inhibitors.

#### The superoxide dismutases and their mimetics

As stated above, superoxide is a key constituent in oxidative

stress. It is derived from various sources at different stages of reperfusion. There are three major endogenous superoxide dismutases. Cu,Zn-SOD (SOD1) is principally found in the cytosolic and lysosomal fractions, but is also in the mitochondrial intermembrane space (Okado-Matsumoto and Fridovich, 2001). MnSOD (SOD2) is found in the mitochondrial matrix. Both Cu,Zn-SOD and MnSOD are abundant in neural tissue and for this reason have received greatest scrutiny. Knock-out and overexpressing mutants for both isozymes have been created, but direct comparison of the relative importance of the two enzymes has not been made. Cu,Zn-SOD overexpression reduces ischemic damage resulting from ischemia/reperfusion (Yang et al., 1994). However, neither Cu,Zn-SOD overexpression nor Cu,Zn-SOD targeted deletion alter the outcome from permanent focal ischemia (Chan et al., 1993; Fujimura et al., 2001), indicating the requirement of reperfusion for this enzyme to play a role. In contrast, MnSOD targeted deletion worsens the outcome from both temporary and permanent middle cerebral artery occlusion (Kim et al., 2002; Murakami et al., 1998). Cu,Zn-SOD overexpression has been shown to inhibit post-ischemic mitogen-activated protein kinase activation (Noshita et al., 2002), the Bad cell death signaling pathway (Saito et al., 2003), caspase activation (Sugawara et al., 2002b), early mitochondrial cytochrome *c* release (Fujimura et al., 2000), DNA fragmentation (Fujimura et al., 1999) and poly(ADP-ribose) polymerase (PARP) activation (Narasimhan et al., 2003). Cumulatively, these data indicate a potential pro-apoptotic role for superoxide in ischemia/reperfusion. This can be abated by SOD overexpression and potentially by treatment with SOD mimetic compounds. However, constitutive transgenic SOD overexpression prohibits prediction of the length of any potential pharmacological therapeutic window for treatment efficacy during reperfusion. Furthermore, no studies have evaluated effects of SOD overexpression on the long-term outcome from ischemia/reperfusion, and thus the stability of the protection afforded is unknown.

Extracellular SOD (SOD3) is also expressed in brain but in substantially lower concentrations than SOD1 or SOD2 (Marklund, 1984). EC-SOD, a tetrameric protein, is secreted into the extracellular compartment (Tibell et al., 1987). EC-SOD has a heparin binding domain that allows adherence to the glycocalyx (Sandstrom et al., 1992). EC-SOD is presumed to provide defense against superoxide present in the extracellular space (e.g. produced by membrane-bound NAD(P)H oxidase or secreted by inflammatory cells; Oury et al., 1992). The relatively low EC-SOD concentration in whole brain may be misleading with respect to its importance to ischemic events. The extracellular compartment is small and thus EC-SOD concentration in the extracellular compartment may be sufficient to provide biological relevance. Indeed, EC-SOD overexpressing mice have increased tolerance to both focal and global cerebral ischemia (Sheng et al., 1999a, 2000), while EC-SOD knock-outs exhibit enhanced damage (Sheng et al., 1999b). These data implicate an important role for extracellular superoxide in the pathogenesis of

ischemia/reperfusion and suggest a therapeutic role for SOD mimetics that localize in the extracellular compartment.

Recent pharmacological advances have allowed the advent of potent SOD mimetics. Although bovine SOD has shown some therapeutic potential (Liu et al., 1989), its short-half life in circulation, inability to penetrate the blood-brain barrier and potential antigenicity have limited its appeal. Several major classes of SOD mimetics have been reported to date (Sheng et al., 2002a): Mn(II) cyclic polyamines (Riley, 2000), Mn(III) salen derivatives (Baker et al., 1998), Mn(III) porphyrins (Batinic-Haberle, 2002; Batinic-Haberle et al., 2002) and stable cyclic nitroxides (Goldstein et al., 2003a; Kwon et al., 2003; Sugawara et al., 2001). All eliminate superoxide in catalytic fashion, with catalytic rate constants being in excess of  $10^6 \text{ M}^{-1} \text{ s}^{-1}$ , except in the case of nitroxides. With nitroxides the catalytic rate constant, involving nitroxide/oxoammonium cation redox couple, is limited by the very slow nitroxide oxidation with superoxide ( $<10^3 \text{ M}^{-1} \text{ s}^{-1}$ ) and is  $<10^6 \text{ M}^{-1} \text{ s}^{-1}$  at pH 7.4 (Goldstein et al., 2003a). The compounds variously have selective SOD-like properties [Mn cyclic(II) polyamines (Salvemini et al., 1999)], modest catalase-like activity [Mn(III) salen derivatives (Baker et al., 1998) and Mn(III) porphyrins (Day et al., 1997)], potential to oxidize nitric oxide [oxoMn(V) salen derivatives) (Sharpe et al., 2002) and Mn(III) porphyrins (Spasojevic et al., 2000) and oxidized nitroxides, i.e. oxoammonium cations (Goldstein et al., 2004)], and ability to eliminate peroxynitrite [Mn(III) salen derivatives (Sharpe et al., 2002), Mn(III) porphyrins (Ferrer-Sueta et al., 2003) and oxoammonium cations (Goldstein et al., 2004)] or peroxynitrite-derived products such as nitrogen dioxide radical (nitroxides; Goldstein et al., 2004, 2003b) and carbonate radical [Mn(III) porphyrins (Ferrer-Sueta et al., 2003) and nitroxides (Goldstein et al., 2004)]. Reactivity of antioxidants towards a wide range of ROS/RNS would make them more versatile antioxidants, i.e. protective in different cellular environments. Mn(III) porphyrins have been most intensively investigated in models of cerebral ischemia/reperfusion. The cationic Mn(III) porphyrins, *ortho* *N*-ethylpyridylporphyrin (MnTE-2-PyP<sup>5+</sup>, AEOL 10113) and di-*ortho* *N,N'*-diethylimidazolylporphyrin (MnTDE-2-ImP<sup>5+</sup>, AEOL 10150) have both been shown to provide potent protection against infarct formation when given as late as 6 h after onset of reperfusion from 90 min of temporary middle cerebral artery occlusion (Mackensen et al., 2001; Sheng et al., 2002b). This was associated with post-ischemic decreases in aconitase inactivation, 8-hydroxyguanine formation and cytokine expression (Bowler et al., 2002; Mackensen et al., 2001). Long-term outcome studies and effects on apoptotic responses have not yet been reported for these drugs.

#### Catalase and glutathione peroxidase

SOD dismutates superoxide to hydrogen peroxide and oxygen. Hydrogen peroxide has modest oxidative potential and can freely cross cell membranes. Through the iron-catalyzed Haber-Weiss reaction (superoxide-driven Fenton chemistry),

hydrogen peroxide can be converted to hydroxyl radical (Halliwell and Gutteridge, 1999). Elimination of hydrogen peroxide is therefore critical to the efficacy of SOD in reducing oxidative stress. Catalase and glutathione peroxidase serve this purpose. Both are present in the brain although glutathione peroxidase activity is sevenfold greater than that of catalase (Marklund et al., 1982). Further, while glutathione peroxidase is present in the cytosol, catalase is localized mainly in peroxisomes. As a result, the more ubiquitous presence of glutathione peroxidase predicts it to be the more important enzyme in responding to increased hydrogen peroxide.

Both glutathione peroxidase-overexpressing and knock-out mice have been studied in the context of focal cerebral ischemia/reperfusion. Overexpression reduces necrotic and apoptotic cell death, astrocytic/microglial activation and inflammatory cell infiltration (Ishibashi et al., 2002; Weisbrodt-Lefkowitz et al., 1998). In contrast, intracerebroventricular infusion of exogenous glutathione peroxidase failed to improve outcome from global forebrain ischemia/reperfusion (Yano et al., 1998). This difference might be attributable to differences in model type (focal *versus* global) or intracellular bioavailability of glutathione peroxidase when administered intracerebroventricularly. The progeny of cross-breeding a glutathione peroxidase knock-out and a Cu,Zn-SOD overexpressor caused a loss of protection that was otherwise afforded by overexpression of Cu,Zn-SOD (Crack et al., 2001). However, the glutathione peroxidase knockout alone was insufficient to worsen cerebral ischemia/reperfusion injury (Crack et al., 2001), consistent with overlap in function with catalase. Cumulatively, these data implicate an important role for glutathione peroxidase in brain ischemia/reperfusion, although the relative contributions of glutathione peroxidase and catalase have not been clarified.

Selective pharmacological antagonists of glutathione peroxidase have not been studied. Ebselen is a synthetic mimetic of glutathione peroxidase (Muller et al., 1984). It is not selective in that it also inhibits protein kinase C, 5-lipoxygenase, cyclooxygenase and NADPH oxidase (Schewe, 1995). Thus, inferences from the efficacy of this drug in the context of ischemia/reperfusion regarding the role of glutathione peroxidase must be limited. Ebselen has been shown to be protective in several ischemia models (Imai et al., 2003; Kondoh et al., 1999) and is currently being studied in ongoing clinical trials (Saito et al., 1998; Yamaguchi et al., 1998).

Although a catalase-overexpressing mouse strain exists (Chen et al., 2003), it has not been studied in the context of cerebral ischemia/reperfusion. An alternative method is to examine catalase deficiency. The developing brain provides a natural model for this in that both catalase and glutathione peroxidase are poorly expressed. Cu,Zn-SOD overexpression in neonatal mice worsens the outcome from ischemia/reperfusion (Fullerton et al., 1998). In contrast Cu,Zn-SOD overexpression in adult mice improves the outcome (Yang et al., 1994). This difference is probably attributable to inadequate catalase and glutathione peroxidase

enzymatic activity available to the developing brain for the conversion of superoxide-generated hydrogen peroxide to water and oxygen (Fullerton et al., 1998). The same argument suggests that endogenous concentrations of catalase and glutathione concentrations are sufficient in the adult brain to process superoxide, should its dismutation to hydrogen peroxide be enhanced by a SOD mimetic.

There has been some attempt to test efficacy of exogenously administered catalase in adult ischemia/reperfusion models with mixed results, possibly due to the question of bioavailability of proteins that must cross the blood-brain barrier (Forsman et al., 1988; Liu et al., 1989). Catalase inhibitors, such as 3-aminotriazole, have not been evaluated in the context of ischemia. Therefore, there is insufficient pharmacological information to conclude that catalase, particularly in the presence of normal glutathione peroxidase concentrations, plays a central role in the response of brain to ischemia. This, however, should be tempered by the possibility that the importance of catalase may increase if superoxide production and SOD activity are increased.

### Glutathione depletion

Glutathione is a tripeptide ( $\gamma$ -L-glutamyl-L-cysteinylglycine) that is the reductant for glutathione peroxidase. Oxidation of the cysteine sulfhydryl groups joins two glutathione (GSH) molecules with a disulfide bridge to form glutathione disulfide (GSSG). NADPH-dependent glutathione reductase catalyzes recovery of glutathione. Normally, the brain maintains a high ratio of GSH/GSSG for antioxidant defense. Depletion of total glutathione and a decreased GSH/GSSG ratio are markers for oxidative stress in ischemic brain and as long as 72 h may be required to restore concentrations to normal values following an ischemic insult (Namba et al., 2001; Park et al., 2000). Ischemic outcome is worsened by pharmacological depletion of glutathione (Vanella et al., 1993), but improved by administration of a glutathione mimetic, glutathione monoisopropyl ester, YM737 (Gotoh et al., 1994), or *N*-acetyl cysteine, a glutathione precursor. No study of glutathione reductase mutants in cerebral ischemia paradigms has been reported.

### Nitric oxide synthase inhibition

Since the original suggestion that nitric oxide synthesis plays a role in cerebral ischemia (Marshall and Kontos, 1990), over 800 research reports have addressed this issue. Nitric oxide is enzymatically synthesized from L-arginine and is massively increased by ischemia (Wei et al., 1999). Three nitric oxide synthases (NOS) have been reported (eNOS, nNOS and iNOS), so named because of their originally defined endothelial (eNOS) and neuronal (nNOS) localization, or ability to be upregulated when induced (iNOS). Initially, the field was confusing because NOS inhibitors were not selective and were given in large doses. Some ischemic outcome studies found improved outcome using NOS inhibitors, while others

found worsened outcome. It soon became apparent that the effect of NOS inhibition was dependent upon which isoform was being inhibited. Pharmacologic eNOS inhibition would be expected to worsen outcome, secondary to cerebral vasoconstriction and reduced blood flow. This is supported by studies of eNOS-deficient mice (Lo et al., 1996) that have worsened ischemic outcomes. In contrast, upregulation of eNOS activity by treatment with 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase inhibitors (e.g. simvastatin) caused increased intra-ischemic blood flow and reduced infarct size (Amin-Hanjani et al., 2001; Endres et al., 1998). Use of selective nNOS antagonists (O'Neill et al., 2000) and nNOS knockout mice (Huang et al., 1994), confirmed that neuronal production of nitric oxide contributes to ischemic cell death. iNOS has been associated with oxidative stress (Han et al., 2002), and modifying its activity may have therapeutic potential (Parmentier et al., 1999). However, nitric oxide may also serve as an antioxidant against products of the Fenton reaction (Chiueh, 1999). At the same time, iNOS expression has been implicated as a critical factor for promoting post-ischemic neurogenesis (Zhu et al., 2003). Further, iNOS expression may contribute to increased tolerance of brain to ischemia induced by preconditioning stimuli (Kapinya et al., 2002) as does eNOS upregulation (Hashiguchi et al., 2004). The fact that eNOS and nNOS are  $\text{Ca}^{2+}$ -dependent, while iNOS is not, can be used to distinguish among them for mechanistic purposes.

The relevance of nitric oxide was increased with the report that the diffusion-limited reaction between superoxide and nitric oxide gives rise to peroxynitrite (Beckman et al., 1990). The highly reactive peroxynitrite provided a mechanistic basis for oxidative stress derived from increased nitric oxide production caused by ischemia/reperfusion (Eliasson et al., 1999). Studies confirmed increased peroxynitrite formation occurring in parallel with upregulation of iNOS (Suzuki et al., 2002) and lack of peroxynitrite formation in nNOS knockouts (Eliasson et al., 1999). Nitric oxide has also been shown to inhibit mitochondrial respiration *via* competition with oxygen for cytochrome oxidase (Brown and Borutaite, 1999) and play a role in the initiation of apoptosis (Bonfoco et al., 1995). Although little has been reported on efforts to bring nitric oxide inhibitors to clinical investigation, there is no doubt that nitric oxide plays a pivotal role in mediating oxidative stress (Mikkelsen and Wardman, 2003).

### Metal chelators

Free iron is released from protein storage in the ischemic brain, providing substrate for the iron-catalyzed Haber–Weiss reaction, resulting in hydroxyl radical formation from hydrogen peroxide. Iron chelators such as deferoxamine are logical candidates to probe the role of these reactions in ischemic brain. Deferoxamine-treatment has been associated with reduced lipid peroxidation, improved post-ischemic vasoreactivity, cerebral perfusion and ATP recovery (Hurn et al., 1995; Liachenko et al., 2003; Nayini et al., 1985; Nelson

et al., 1992). Unfortunately, histological/behavioral outcome studies have failed to find consistent benefit from this strategy (Fleischer et al., 1987; Kumar et al., 1988), possibly due to its short-half-life. Further, deferoxamine does not chelate copper ion, which can also catalyze the Haber–Weiss reaction. There is an exception to this, however. Consistent observations of deferoxamine-mediated improvement in post-ischemic/hypoxic outcome have been made in perinatal brain (Palmer et al., 1994; Peeters-Scholte et al., 2003; Sarco et al., 2000). Perhaps this is attributable to low endogenous expression of catalase and glutathione peroxidase, which might make the developing brain particularly prone to hydrogen peroxide accumulation (Fullerton et al., 1998).

### Poly(ADP-ribose) polymerase inhibitors

PARP was first introduced to the ischemia literature with the report that PARP knock-out mice exhibited profoundly diminished cerebral infarct sizes when compared to wild-type counterparts (Eliasson et al., 1997). Poly(ADP-ribose) is synthesized from NAD by PARP and degraded by poly(ADP-ribose) glycohydrolase (PARG). PARP is activated in response to DNA damage as a repair mechanism but also causes NAD and ATP depletion, potentially exacerbating ischemic injury. A principal source of DNA damage is likely to be peroxynitrite formation from superoxide and nitric oxide, mediated by NMDA receptor activation (Giovannelli et al., 2002; Mandir et al., 2000). Cu,Zn-SOD overexpressing mice do not exhibit post-ischemic PARP activation (Narasimhan et al., 2003). Effects of pharmacological antioxidants on PARP activation have not been reported. Pharmacological PARP antagonists have provided protection in several ischemia models (Abdelkarim et al., 2001; Plaschke et al., 2000), one of which followed outcome for up to 30 days post-ischemia (Ding et al., 2001). Similarly, treatment with systemic NAD improved ischemic outcome (Yang et al., 2002). PARP activation remains a plausible mechanism to explain downstream effects of oxidative stress on ischemic outcome.

### Mitochondrial permeability transition inhibitors

The concept is relatively new that the mitochondrial permeability transition (MPT) pore plays an important role in response of brain to ischemia (Friberg and Wieloch, 2002; Kristian and Siesjo, 1996).  $\text{Ca}^{2+}$  overload causes translocation of cyclophilin-D from the matrix to the MPT pore that activates the pore allowing flux of solutes from the matrix to the intermembrane space (Tanveer et al., 1996). Persistent MPT allows mitochondrial swelling and disruption of the outer mitochondrial membrane, loss of the hydrogen ion gradient, and failure of oxidative phosphorylation. Other factors, including oxidative stress, open the MPT pore. Therefore, oxidative stress can initiate MPT which, in turn, potentiates oxidative stress. It is tempting to speculate that MPT allows release of proapoptotic factors (e.g. cytochrome *c*) into the cytosol (Brown and Borutaite, 1999). However, release of

proapoptotic factors has been shown to be MPT-independent (Kobayashi et al., 2003), albeit modulated by oxidative stress (Morita-Fujimura et al., 2001) and potentially corrected by Cu,Zn-SOD overexpression (Sugawara et al., 2002a). Of note, inhibition of the MPT by drugs that might interact with cyclophilin-D (e.g. cyclosporine A; Li et al., 2000; Waldmeier et al., 2003) or antibodies to MPT pore elements (Perez Velazquez et al., 2003) provide ischemic neuroprotection with reduced mitochondrial swelling and inhibition of cytochrome *c* release. Cumulatively, MPT provides a convergence between various oxidative and anti-oxidative forces that are likely to have major impact on ischemic outcome.

### Spin traps

Chemists have developed a variety of methods to 'capture' ROS allowing their detection and quantification. A classic application of this technology in the study of ischemic brain is use of salicylate, which reacts with hydroxyl radical to form a relatively stable adduct, 2,3-DHBA. This has been useful in microdialysis studies, allowing near-real time measurement of hydroxyl radical production (Globus et al., 1995; Zhang and Piantadosi, 1994). Taking a different approach, in addition to nitroxide spin probes, nitron spin traps were developed to capture ROS, allowing detection by electron paramagnetic spectroscopy. Recognizing the potential for nitrones to scavenge ROS, it was postulated that these compounds might present therapeutic potential (Britigan et al., 1991). Indeed, early rodent studies found consistent benefit from the spin trap  $\alpha$ -phenyl-*N*-*t*-butyl-nitron (PBN) against both global and focal ischemic insults (Yue et al., 1992; Zhao et al., 1994). More important, the second generation spin trap, NXY-059 (disodium 4-[(*tert*-butylimino)-methyl]benzene-1,3-disulfonate *N*-oxide), has been found to improve ischemic outcome in primates when measured at 10 weeks after permanent occlusion of the middle cerebral artery, even when treatment was begun as late as 4 h after onset of ischemia (Marshall et al., 2003). NXY-059 has been shown to maintain Akt activation and inhibit cytochrome *c* release after ischemia (Yoshimoto et al., 2002). There are no reports regarding its direct effect on oxidative damage to cellular constituents *in vivo*. The compound is in Phase III clinical trials after being found tolerable at proposed therapeutic concentrations in humans (Lees et al., 2003). The implications of this work at the clinical level could be substantial.

Mechanistically, in the presence of free radicals, nitrones undergo oxidation to nitroxide radicals. Goldstein et al. (2003a) have shown that stable nitroxides can be reduced to hydroxylamine and oxidized to oxammonium cation, and thus can act catalytically to eliminate superoxide. However, no data are presently available to justify the catalytic role of nitrones based on the formation of nitroxides. Based on its poor blood-brain barrier penetration, the protection afforded by NXY-059 against transient focal cerebral ischemia may be the result of the events occurring at the blood/endothelial interface (Kuroda et al., 1999), or indicate that the drug enters the brain

after blood-brain barrier breakdown. This distinction is important. More important is the implication that because commencement of treatment at 4 h after onset of ischemia was efficacious, only oxidative stress occurring more than 4 h after onset of ischemia has importance for ischemic outcome.

### Conclusions

The above outline presents data for several mechanisms of oxidative damage in ischemic and post-ischemic brain, leaving little doubt that oxidative stress is a major contributor to ischemic brain injury. The advent of transgenic mutants and relatively selective pharmacological antioxidants has allowed improved definition of the varied mechanisms of oxidative stress and potential targets for therapeutic intervention. Conspicuously absent from extant data, with the exception of NKY-059, are long-term outcome studies designed to assess the stability of protection from ischemia afforded by gene mutations and drugs having purported efficacy as antioxidants. Long-term studies are critical in predicting clinical efficacy. Although there is substantial evidence that many oxidative pathways contribute to damage resulting from ischemia/reperfusion, it seems unlikely that any one pathway is sufficiently critical to singularly define outcome. Because most interventions are targeted at specific mechanisms of oxidative damage, it seems likely that combined therapeutic mechanisms will be required to substantively and persistently alter outcome from an ischemic insult.

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### References

- Abdelkarim, G. E., Gertz, K., Harms, C., Katchanov, J., Dirnagl, U., Szabo, C. and Endres, M. (2001). Protective effects of PJ34, a novel, potent inhibitor of poly(ADP-ribose) polymerase (PARP) in *in vitro* and *in vivo* models of stroke. *Int. J. Mol. Med.* **7**, 255-260.
- Amin-Hanjani, S., Stagliano, N. E., Yamada, M., Huang, P. L., Liao, J. K. and Moskowitz, M. A. (2001). Mevastatin, an HMG-CoA reductase inhibitor, reduces stroke damage and upregulates endothelial nitric oxide synthase in mice. *Stroke* **32**, 980-986.
- Baker, K., Bucay Marcus, C., Huffman, K., Kruk, H., Malfroy, B. and Doctrow, S. R. (1998). Synthetic combined superoxide dismutase/catalase mimetics are protective as a delayed treatment in a rat stroke: a key role for reactive oxygen species in ischemic brain injury. *J. Pharmacol. Exp. Ther.* **284**, 215-221.
- Batinic-Haberle, I. (2002). Manganese porphyrins and related compounds as mimics of superoxide dismutase. *Methods Enzymol.* **349**, 223-233.
- Batinic-Haberle, I., Spasojevic, I., Stevens, R. D., Hambricht, P. and Fridovich, I. (2002). Manganese(III) meso-tetrakis(ortho-*N*-alkylpyridyl)porphyrins. Synthesis, characterization, and catalysis of O<sub>2</sub><sup>-</sup> dismutation. *J. Chem. Soc. Dalton Trans.* 2689-2696.
- Bazan, N. G., Jr (1970). Effects of ischemia and electroconvulsive shock on free fatty acid pool in the brain. *Biochim. Biophys. Acta* **218**, 1-10.
- Beckman, J. S., Beckman, T. W., Chen, J., Marshall, P. A. and Freeman, B. A. (1990). Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proc. Natl. Acad. Sci. USA* **87**, 1620-1624.
- Bonfoco, E., Krainc, D., Ankarcrona, M., Nicotera, P. and Lipton, S. A. (1995). Apoptosis and necrosis: two distinct events induced, respectively, by mild and intense insults with *N*-methyl-D-aspartate or nitric

- oxide/superoxide in cortical cell cultures. *Proc. Natl. Acad. Sci. USA* **92**, 7162-7166.
- Bowler, R. P., Sheng, H., Enghild, J. J., Pearlstein, R. D., Warner, D. S. and Crapo, J. D.** (2002). A catalytic antioxidant (AEOL 10150) attenuates expression of inflammatory genes in stroke. *Free Rad. Biol. Med.* **33**, 1141-1152.
- Britigan, B. E., Roeder, T. L. and Buettner, G. R.** (1991). Spin traps inhibit formation of hydrogen peroxide via the dismutation of superoxide: implications for spin trapping the hydroxyl free radical. *Biochim. Biophys. Acta* **1075**, 213-222.
- Brookes, P. S., Land, J. M., Clark, J. B. and Heales, S. J.** (1998). Peroxynitrite and brain mitochondria: evidence for increased proton leak. *J. Neurochem.* **70**, 2195-2202.
- Brown, G. C. and Borutaite, V.** (1999). Nitric oxide, cytochrome c and mitochondria. *Biochem. Soc. Symp.* **66**, 17-25.
- Chan, P. H., Kamii, H., Yang, G. Y., Gafni, J., Epstein, C. J., Carlson, E. and Reola, L.** (1993). Brain infarction is not reduced in SOD-1 transgenic mice after a permanent focal cerebral ischemia. *NeuroReport* **5**, 293-296.
- Chen, X., Mele, J., Giese, H., Van Remmen, H., Dolle, M. E., Steinhilber, M., Richardson, A. and Vijg, J.** (2003). A strategy for the ubiquitous overexpression of human catalase and CuZn superoxide dismutase genes in transgenic mice. *Mech. Ageing Dev.* **124**, 219-227.
- Chiueh, C. C.** (1999). Neuroprotective properties of nitric oxide. *Ann. NY Acad. Sci.* **890**, 301-311.
- Crack, P. J., Taylor, J. M., Flentjar, N. J., de Haan, J., Hertzog, P., Iannello, R. C. and Kola, I.** (2001). Increased infarct size and exacerbated apoptosis in the glutathione peroxidase-1 (Gpx-1) knockout mouse brain in response to ischemia/reperfusion injury. *J. Neurochem.* **78**, 1389-1399.
- Dawson, V. L., Dawson, T. M., London, E. D., Bretz, D. S. and Snyder, S. H.** (1991). Nitric oxide mediates glutamate neurotoxicity in primary cortical cultures. *Proc. Natl. Acad. Sci. USA* **88**, 6368-6371.
- Day, B. J., Fridovich, I. and Crapo, J. D.** (1997). Manganic porphyrins possess catalase activity and protect endothelial cells against hydrogen peroxide-mediated injury. *Arch. Biochem. Biophys.* **347**, 256-262.
- Ding, Y., Zhou, Y., Lai, Q., Li, J., Gordon, V. and Diaz, F. G.** (2001). Long-term neuroprotective effect of inhibiting poly(ADP-ribose) polymerase in rats with middle cerebral artery occlusion using a behavioral assessment. *Brain Res.* **915**, 210-217.
- Eliasson, M. J. L., Huang, Z. H. and Ferrante, R. J.** (1999). Neuronal nitric oxide synthase activation and peroxynitrite formation in ischemic stroke linked to neural damage. *J. Neurosci.* **19**, 5910-5918.
- Eliasson, M. J. L., Sampei, K., Mandir, A. S., Hurn, P. D., Traystman, R. J., Bao, J., Pieper, A., Wang, Z. Q., Dawson, T. M., Snyder, S. H. et al.** (1997). Poly(ADP-ribose) polymerase gene disruption renders mice resistant to cerebral ischemia. *Nature Med.* **3**, 1089-1095.
- Endres, M., Laufs, U., Huang, Z., Nakamura, T., Huang, P., Moskowitz, M. A. and Liao, J. K.** (1998). Stroke protection by 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase inhibitors mediated by endothelial nitric oxide synthase. *Proc. Natl. Acad. Sci. USA* **95**, 8880-8885.
- Espey, M. G., Miranda, K. M., Feelisch, M., Fukuto, J., Grisham, M. B., Vitek, M. P. and Wink, D. A.** (2000). Mechanism of cell death governed by the balance between nitrosative and oxidative stress. *Ann. NY Acad. Sci.* **899**, 209-221.
- Fabian, R. H., DeWitt, D. S. and Kent, T. A.** (1995). In vivo detection of superoxide anion production by the brain using a cytochrome c electrode. *J. Cereb. Blood Metab.* **15**, 242-247.
- Ferrer-Sueta, G., Vitturi, D., Batinic-Haberle, I., Fridovich, I., Goldstein, S., Czapski, G. and Radi, R.** (2003). Reactions of manganese porphyrins with peroxynitrite and carbonate radical anion. *Stroke* **34**, 27432-27438.
- Flamm, E. S., Demopoulos, H. B., Seligman, M. L., Poser, R. G. and Ransohoff, J.** (1978). Free radicals in cerebral ischemia. *Stroke* **9**, 445-447.
- Fleischer, J. E., Lanier, W. L., Milde, J. H. and Michenfelder, J. D.** (1987). Failure of deferoxamine, an iron chelator, to improve neurologic outcome following complete cerebral ischemia in dogs. *Stroke* **18**, 124-127.
- Forsman, M., Fleischer, J. E., Milde, J. H., Steen, P. A. and Michenfelder, J. D.** (1988). Superoxide dismutase and catalase failed to improve neurologic outcome after complete cerebral ischemia in the dog. *Acta Anaesthesiol. Scand.* **32**, 152-155.
- Friberg, H. and Wieloch, T.** (2002). Mitochondrial permeability transition in acute neurodegeneration. *Biochimie* **84**, 241-250.
- Fridovich, I.** (1995). Superoxide radical and superoxide dismutases. *Annu. Rev. Biochem.* **64**, 97-112.
- Fridovich, I.** (2003). Editorial commentary on 'superoxide reacts with hydroethidine but forms a fluorescent product that is distinctly different from ethidium: potential implications in intracellular fluorescence detection of superoxide' by H. Zhao et al. *Free Rad. Biol. Med.* **34**, 1357-1358.
- Fujimura, M., Morita-Fujimura, Y., Copin, J., Yoshimoto, T. and Chan, P. H.** (2001). Reduction of copper,zinc-superoxide dismutase in knockout mice does not affect edema or infarction volumes and the early release of mitochondrial cytochrome c after permanent focal cerebral ischemia. *Brain Res.* **889**, 208-213.
- Fujimura, M., Morita-Fujimura, Y., Narasimhan, P., Copin, J. C., Kawase, M. and Chan, P. H.** (1999). Copper-zinc superoxide dismutase prevents the early decrease of apurinic/aprimidinic endonuclease and subsequent DNA fragmentation after transient focal cerebral ischemia in mice. *Stroke* **30**, 2408-2415.
- Fujimura, M., Morita-Fujimura, Y., Noshita, N., Sugawara, T., Kawase, M. and Chan, P. H.** (2000). The cytosolic antioxidant copper/zinc-superoxide dismutase prevents the early release of mitochondrial cytochrome c in ischemic brain after transient focal cerebral ischemia in mice. *J. Neurosci.* **20**, 2817-2824.
- Fukui, S., Ookawara, T., Nawashiro, H., Suzuki, K. and Shima, K.** (2002). Post-ischemic transcriptional and translational responses of EC-SOD in mouse brain and serum. *Free Rad. Biol. Med.* **32**, 289-298.
- Fullerton, H. J., Ditelberg, J. S., Chen, S. F., Sarco, D. P., Chan, P. H., Epstein, C. J. and Ferriero, D. M.** (1998). Copper/zinc superoxide dismutase transgenic brain accumulates hydrogen peroxide after perinatal hypoxia ischemia. *Ann. Neurol.* **44**, 357-364.
- Gardner, P. R. and Fridovich, I.** (1991). Superoxide sensitivity of the *Escherichia coli* aconitase. *J. Biol. Chem.* **266**, 19328-19333.
- Gardner, P. R., Martin, L. A., Hall, D. and Gardner, A. M.** (2001). Dioxigen-dependent metabolism of nitric oxide in mammalian cells. *Free Rad. Biol. Med.* **31**, 191-204.
- Garthwaite, J., Charles, S. L. and Chess-Williams, R.** (1988). Endothelium-derived relaxing factor release on activation of NMDA receptors suggests role as intercellular messenger in the brain. *Nature* **336**, 385-388.
- Garthwaite, J., Garthwaite, G., Palmer, R. M. J. and Moncada, S.** (1989). NMDA receptor activation induces nitric oxide synthesis from arginine in rat brain slices. *Eur. J. Pharmacol.* **172**, 413-416.
- Gaudet, R. J., Alam, I. and Levine, L.** (1980). Accumulation of cyclooxygenase products of arachidonic acid metabolism in gerbil brain during reperfusion after bilateral common carotid artery occlusion. *J. Neurochem.* **35**, 653-658.
- Giovannelli, L., Cozzi, A., Guarnieri, I., Dolara, P. and Moroni, F.** (2002). Comet assay as a novel approach for studying DNA damage in focal cerebral ischemia: differential effects of NMDA receptor antagonists and poly(ADP-ribose) polymerase inhibitors. *J. Cereb. Blood Flow Metab.* **22**, 697-704.
- Glebska, J. and Koppenol, W. H.** (2003). Peroxynitrite-mediated oxidation of dihydrodichlorofluorescein and dihydrorhodamine. *Free Rad. Biol. Med.* **35**, 676-682.
- Globus, M. Y. T., Busto, R., Lin, B., Schnippering, H. and Ginsberg, M. D.** (1995). Detection of free radical activity during transient global ischemia and recirculation: Effects of intraschemic brain temperature modulation. *J. Neurochem.* **65**, 1250-1256.
- Goldstein, S., Merenyi, G., Russo, A. and Samuni, A.** (2003a). The role of oxoammonium cation in the SOD-mimic activity of cyclic nitroxides. *J. Am. Chem. Soc.* **125**, 789-795.
- Goldstein, S., Samuni, A. and Merenyi, G.** (2004). Reactions of nitric oxide, peroxynitrite, and carbonate radicals with nitroxides and their corresponding oxoammonium cations. *Chem. Res. Toxicol.* **17**, 250-257.
- Goldstein, S., Samuni, A. and Russo, A.** (2003b). Reaction of cyclic nitroxides with nitrogen dioxide the intermediacy of the oxoammonium cations. *J. Am. Chem. Soc.* **125**, 8364-8370.
- Gotoh, O., Yamamoto, M., Tamura, A. and Sano, K.** (1994). Effect of YM737, a new glutathione analogue, on ischemic brain edema. *Acta Neurochir. Suppl. (Wien)* **60**, 318-320.
- Haley, E. C., Jr** (1998). High-dose tirilazad for acute stroke (RANTTAS II). RANTTAS II Investigators. *Stroke* **29**, 1256-1257.
- Halliwell, B. and Gutteridge, J. M. C.** (1999). *Free Radicals in Biology and Medicine*. Oxford: Oxford University Press.
- Han, H. S., Qiao, Y., Karabiyikoglu, M., Giffard, R. G. and Yenari, M. A.** (2002). Influence of mild hypothermia on inducible nitric oxide synthase expression and reactive nitrogen production in experimental stroke and inflammation. *J. Neurosci.* **22**, 3921-3928.
- Hashiguchi, A., Yano, S., Moriko, M., Hamada, J., Ushio, Y., Takeuchi, Y. and Fukunaga, K.** (2004). Up-regulation of endothelial nitric oxide synthase via phosphatidylinositol 3-kinase pathway contributes to ischemic



- tolerance in the CA1 subfield of gerbil hippocampus. *J. Cereb. Blood Flow Metab.* **24**, 271-279.
- Hausladen, A., Gow, A. J. and Stamler, J. S. (1998). Nitrosative stress: Metabolic pathway involving the flavohemoglobin. *Proc. Natl. Acad. Sci. USA* **95**, 14100-14105.
- Huang, Z., Huang, P. L., Panahian, N., Dalkara, T., Fishman, M. C. and Moskowitz, M. A. (1994). Effects of cerebral ischemia in mice deficient in neuronal nitric oxide synthase. *Science* **265**, 1883-1885.
- Hurn, P. D., Koehler, R. C., Blizzard, K. K. and Traystman, R. J. (1995). Deferoxamine reduces early metabolic failure associated with severe cerebral ischemic acidosis in dogs. *Stroke* **26**, 688-694; Discussion 694-695.
- Ibrahim, W., Lee, U. S., Yen, H. C., St Clair, D. K. and Chow, C. K. (2000). Antioxidant and oxidative status in tissues of manganese superoxide dismutase transgenic mice. *Free Rad. Biol. Med.* **28**, 397-402.
- Ignarro, L. J., Byrns, R. E., Buga, G. M. and Wood, K. S. (1987). Endothelium-derived relaxing factor from pulmonary artery and vein possesses pharmacological and chemical properties identical to those of nitric oxide radical. *Circ. Res.* **61**, 866-879.
- Imai, H., Graham, D. I., Masayasu, H. and Macrae, I. M. (2003). Antioxidant ebselen reduces oxidative damage in focal cerebral ischemia. *Free Rad. Biol. Med.* **34**, 56-63.
- Imaizumi, S., Tominaga, T., Uenohara, H., Yoshimoto, T., Suzuki, J. and Fujita, Y. (1986). Initiation and propagation of lipid peroxidation in cerebral infarction models. Experimental studies. *Neurol. Res.* **8**, 214-220.
- Ishibashi, N., Prokopenko, O., Weisbrodt-Lefkowitz, M., Reuhl, K. R. and Mirochnitchenko, O. (2002). Glutathione peroxidase inhibits cell death and glial activation following experimental stroke. *Brain Res. Mol. Brain Res.* **109**, 34-44.
- Joshi, M. S., Ferguson, T. B., Jr, Han, T. H., Hyduke, D. R., Liao, J. C., Rassaf, T., Bryan, N., Feelisch, M. and Lancaster, J. R., Jr (2002). Nitric oxide is consumed, rather than conserved, by reaction with oxyhemoglobin under physiological conditions. *Proc. Natl. Acad. Sci. USA* **99**, 10341-10346.
- Kapinya, K. J., Prass, K. and Dirnagl, U. (2002). Isoflurane induced prolonged protection against cerebral ischemia in mice: a redox sensitive mechanism? *NeuroReport* **13**, 1431-1435.
- Kavanagh, R. J. and Kam, P. C. (2001). Lazaroids: efficacy and mechanism of action of the 21-aminosteroids in neuroprotection. *Br. J. Anaesth.* **86**, 110-119.
- Kim, G. W., Kondo, T., Noshita, N. and Chan, P. H. (2002). Manganese superoxide dismutase deficiency exacerbates cerebral infarction after focal cerebral ischemia/reperfusion in mice: implications for the production and role of superoxide radicals. *Stroke* **33**, 809-815.
- Kobayashi, T., Kuroda, S., Tada, M., Houkin, K., Iwasaki, Y. and Abe, H. (2003). Calcium-induced mitochondrial swelling and cytochrome *c* release in the brain: its biochemical characteristics and implication in ischemic neuronal injury. *Brain Res.* **960**, 62-70.
- Kondoh, S., Nagasawa, S., Kawanishi, M., Yamaguchi, K., Kajimoto, S. and Ohta, T. (1999). Effects of ebselen on cerebral ischemia and reperfusion evaluated by microdialysis. *Neurol. Res.* **21**, 682-686.
- Kristian, T. and Siesjö, B. K. (1996). Calcium-related damage in ischemia. *Life Sci.* **59**, 357-367.
- Kruman, I., Bruce-Keller, A. J., Bredesen, D., Waeg, G. and Mattson, M. P. (1997). Evidence that 4-hydroxynonenal mediates oxidative stress-induced neuronal apoptosis. *J. Neurosci.* **17**, 5089-5100.
- Kumar, K., White, B. C., Krause, G. S., Indrieri, R. J., Evans, A. T., Hoehner, T. J., Garritano, A. M. and Koestner, A. (1988). A quantitative morphological assessment of the effect of lidoflazine and deferoxamine therapy on global brain ischaemia. *Neurol. Res.* **10**, 136-140.
- Kuroda, S., Tsuchidate, R., Smith, M. L., Maples, K. R. and Siesjö, B. K. (1999). Neuroprotective effects of a novel nitron, NXY-059, after transient focal cerebral ischemia in the rat. *J. Cereb. Blood Flow Metab.* **19**, 778-787.
- Kwon, T. H., Chao, D. L., Malloy, K., Sun, D., Alessandri, B. and Bullock, M. R. (2003). Tempol, a novel stable nitroxide, reduces brain damage and free radical production, after acute subdural hematoma in the rat. *J. Neurotrauma* **20**, 337-345.
- Lees, K. R., Barer, D., Ford, G. A., Hacke, W., Kostulas, V., Sharma, A. K. and Odergren, T. (2003). Tolerability of NXY-059 at higher target concentrations in patients with acute stroke. *Stroke* **34**, 482-487.
- Li, P. A., Kristian, T., He, Q. P. and Siesjö, B. K. (2000). Cyclosporin A enhances survival, ameliorates brain damage, and prevents secondary mitochondrial dysfunction after a 30-minute period of transient cerebral ischemia. *Exp. Neurol.* **165**, 153-163.
- Liachenko, S., Tang, P. and Xu, Y. (2003). Deferoxamine improves early postresuscitation reperfusion after prolonged cardiac arrest in rats. *J. Cereb. Blood Flow Metab.* **23**, 574-581.
- Lindsay, S., Liu, T. H., Xu, J. A., Marshall, P. A., Thompson, J. K., Parks, D. A., Freeman, B. A., Hsu, C. Y. and Beckman, J. S. (1991). Role of xanthine dehydrogenase and oxidase in focal cerebral ischemic injury to rat. *Am. J. Physiol.* **261**, H2051-H2057.
- Liochev, S. I. and Fridovich, I. (2002). The Haber-Weiss cycle – 70 years later: an alternative view. *Redox Report* **7**, 55-57.
- Liu, T. H., Beckman, J. S., Freeman, B. A., Hogan, E. L. and Hsu, C. Y. (1989). Polyethylene glycol-conjugated superoxide dismutase and catalase reduce ischemic brain injury. *Am. J. Physiol.* **256**, H589-H593.
- Lo, E. H., Hara, H., Rogowska, J., Trocha, M., Pierce, A. R., Huang, P. L., Fishman, M. C., Wolf, G. L. and Moskowitz, M. A. (1996). Temporal correlation mapping analysis of the hemodynamic penumbra in mutant mice deficient in endothelial nitric oxide synthase gene expression. *Stroke* **27**, 1381-1385.
- Mackensen, G. B., Patel, M., Sheng, H., Calvi, C., Batinic-Haberle, I., Day, B. J., Liang, L. P., Fridovich, I., Crapo, J. D., Pearlstein, R. D. et al. (2001). Neuroprotection from delayed post-ischemic administration of a metalloporphyrin catalytic antioxidant. *J. Neurosci.* **21**, 4582-4592.
- Mandir, A. S., Poitras, M. F., Berliner, A. R., Herring, W. J., Guastella, D. B., Feldman, A., Poirier, G. G., Wang, Z. Q., Dawson, T. M. and Dawson, V. L. (2000). NMDA but not non-NMDA excitotoxicity is mediated by Poly(ADP-ribose) polymerase. *J. Neurosci.* **20**, 8005-8011.
- Marion, J. and Wolfe, L. S. (1979). Origin of the arachidonic acid released post-mortem in rat forebrain. *Biochim. Biophys. Acta* **574**, 25-32.
- Marklund, S. L. (1984). Extracellular superoxide dismutase in human tissues and human cell lines. *J. Clin. Invest.* **74**, 1398-1403.
- Marklund, S. L., Westman, N. G., Lundgren, E. and Roos, G. (1982). Copper- and zinc-containing superoxide dismutase, manganese-containing superoxide dismutase, catalase, and glutathione peroxidase in normal and neoplastic human cell lines and normal human tissues. *Cancer Res.* **42**, 1955-1961.
- Marro, P. J., McGowan, J. E., Razdan, B., Mishra, O. P. and Delivoria-Papadopoulos, M. (1994). Effect of allopurinol on uric acid levels and brain cell membrane Na<sup>+</sup>,K<sup>+</sup>-ATPase activity during hypoxia in newborn piglets. *Brain Res.* **650**, 9-15.
- Marshall, J. J. and Kontos, H. A. (1990). Endothelium-derived relaxing factors. A perspective from in vivo data. *Hypertension* **16**, 371-386.
- Marshall, J. W. B., Duffin, K. J., Green, R. and Ridley, R. M. (2003). NXY-059, a free radical-trapping agent, substantially lessens the functional disability resulting from cerebral ischemia in a primate species. *Stroke* **32**, 190-198.
- Martz, D., Rayos, G., Schielke, G. P. and Betz, A. L. (1989). Allopurinol and dimethylthiourea reduce brain infarction following middle cerebral artery occlusion in rats. *Stroke* **20**, 488-494.
- Mattson, M. P., Culmsee, C. and Yu, Z. F. (2000). Apoptotic and antiapoptotic mechanisms in stroke. *Cell Tissue Res.* **301**, 173-187.
- Mikkelsen, R. B. and Wardman, P. (2003). Biological chemistry of reactive oxygen and nitrogen and radiation-induced signal transduction mechanisms. *Oncogene* **22**, 5734-5754.
- Morimoto, K., Tagawa, K., Hayakawa, T., Watanabe, F. and Mogami, H. (1982). Cellular level of purine compounds in ischemic gerbil brain by high performance liquid chromatography. *J. Neurochem.* **38**, 833-835.
- Morita-Fujimura, Y., Fujimura, M., Yoshimoto, T. and Chan, P. H. (2001). Superoxide during reperfusion contributes to caspase-8 expression and apoptosis after transient focal stroke. *Stroke* **32**, 2356-2361.
- Muller, A., Cadenas, E., Graf, P. and Sies, H. (1984). A novel biologically active seleno-organic compound-I. Glutathione peroxidase-like activity in vitro and antioxidant capacity of PZ 51 (Ebselen). *Biochem. Pharmacol.* **15**, 3235-3239.
- Murakami, K., Kondo, T., Kawasr, M., Li, Y., Sato, S., Chen, S. F. and Chan, P. H. (1998). Mitochondrial susceptibility to oxidative stress exacerbates cerebral infarction that follows permanent focal cerebral ischemia in mutant mice with manganese superoxide dismutase deficiency. *J. Neurosci.* **18**, 205-213.
- Myhre, O., Andersen, J. A., Aarnes, H. and Fonnum, F. (2003). Evaluation of the probes 2',7'-dichlorofluorescein diacetate, luminol, and lucigenin as indicators of reactive species formation. *Biochem. Pharmacol.* **65**, 1575-1582.
- Namba, K., Takeda, Y., Sunami, K. and Hirakawa, M. (2001). Temporal profiles of the levels of endogenous antioxidants after four-vessel occlusion in rats. *J. Neurosurg. Anesthesiol.* **13**, 131-137.
- Narasimhan, P., Fujimura, M., Noshita, N. and Chan, P. H. (2003). Role

- of superoxide in poly(ADP-ribose) polymerase upregulation after transient cerebral ischemia. *Brain Res. Mol. Brain Res.* **113**, 28-36.
- Nayini, N. R., White, B. C., Aust, S. D., Huang, R. R., Indrieri, R. J., Evans, A. T., Bialek, H., Jacobs, W. A. and Komara, J. (1985). Post resuscitation iron delocalization and malondialdehyde production in the brain following prolonged cardiac arrest. *Free Rad. Biol. Med.* **1**, 111-116.
- Nelson, C. W., Wei, E. P., Powlislock, J. T., Kontos, H. A. and Moskowitz, M. A. (1992). Oxygen radicals in cerebral ischemia. *Am. J. Physiol.* **263**, H1356-H1362.
- Nicolescu, A. C., Zavorin, S. I., Turro, N. J., Reynolds, J. N. and Thatcher, G. R. (2002). Inhibition of lipid peroxidation in synaptosomes and liposomes by nitrates and nitrites. *Chem. Res. Toxicol.* **15**, 985-998.
- Nihei, H., Kanemitsu, H., Tamura, A., Oka, H. and Sano, K. (1989). Cerebral uric acid, xanthine, and hypoxanthine after ischemia: the effect of allopurinol. *Neurosurgery* **25**, 613-617.
- Niziolek, M., Korytowski, W. and Girotti, A. W. (2003). Nitric oxide inhibition of free radical-mediated lipid peroxidation in photodynamically treated membranes and cells. *Free Rad. Biol. Med.* **34**, 997-1005.
- Noshita, N., Sugawara, T., Hayashi, T., Lewen, A., Omar, G. and Chan, P. H. (2002). Copper/zinc superoxide dismutase attenuates neuronal cell death by preventing extracellular signal-regulated kinase activation after transient focal cerebral ischemia in mice. *J. Neurosci.* **22**, 7923-7930.
- O'Neill, M. J., Murray, T. K., McCarthy, D. R., Hicks, C. A., Dell, C. P., Patrick, K. E., Ward, M. A., Osborne, D. J., Wiernicki, T. R., Roman, C. R. et al. (2000). ARL 17477, a selective nitric oxide synthase inhibitor, with neuroprotective effects in animal models of global and focal cerebral ischaemia. *Brain Res.* **871**, 234-244.
- Okado-Matsumoto, A. and Fridovich, I. (2001). Subcellular distribution of superoxide dismutases (SOD) in rat liver. *J. Biol. Chem.* **276**, 38388-38393.
- Oury, T. D., Ho, Y. S., Piantadosi, C. A. and Crapo, J. D. (1992). Extracellular superoxide dismutase, nitric oxide, and central nervous system O<sub>2</sub> toxicity. *Proc. Natl. Acad. Sci. USA* **89**, 9715-9719.
- Palmer, C., Roberts, R. L. and Bero, C. (1994). Deferoxamine posttreatment reduces ischemic brain injury in neonatal rats. *Stroke* **25**, 1039-1045.
- Palmer, C., Towfighi, J., Roberts, R. L. and Heitjan, D. F. (1993). Allopurinol administered after inducing hypoxia-ischemia reduces brain injury in 7-day-old rats. *Pediatr. Res.* **33**, 405-411.
- Palmer, C., Vannucci, R. C. and Towfighi, J. (1990). Reduction of perinatal hypoxic-ischemic brain damage with allopurinol. *Pediatr. Res.* **27**, 332-336.
- Park, E. M., Choi, J. H., Park, J. S., Han, M. Y. and Park, Y. M. (2000). Measurement of glutathione oxidation and 8-hydroxy-2'-deoxyguanosine accumulation in the gerbil hippocampus following global ischemia. *Brain. Res. Brain Res. Protoc.* **6**, 25-32.
- Parks, D. A. and Granger, D. N. (1986). Xanthine oxidase: biochemistry, distribution and physiology. *Acta Physiol. Scand. Suppl.* **548**, 87-99.
- Parmentier, S., Bohme, G. A., Lerouet, D., Damour, D., Stutzmann, J. M., Margaille, I. and Plotkine, M. (1999). Selective inhibition of inducible nitric oxide synthase prevents ischaemic brain injury. *Br. J. Pharmacol.* **127**, 546-552.
- Patt, A., Harken, A. H., Burton, L. K., Rodell, T. C., Piermattei, D., Schorr, W. J., Parker, N. B., Berger, E. M., Horesh, I. R., Terada, L. S. et al. (1988). Xanthine oxidase-derived hydrogen peroxide contributes to ischemia reperfusion-induced edema in gerbil brains. *J. Clin. Invest.* **81**, 1556-1562.
- Peeters-Scholte, C., Braun, K., Koster, J., Kops, N., Blomgren, K., Buonocore, G., Van Buul-Offers, S., Hagberg, H., Nicolay, K., Van Bel, F. et al. (2003). Effects of allopurinol and deferoxamine on reperfusion injury of the brain in newborn piglets after neonatal hypoxia-ischemia. *Pediatr. Res.* **54**, 516-522.
- Perez Velazquez, J. L., Kokorotseva, L., Weisspapir, M. and Frantseva, M. V. (2003). Anti-porin antibodies prevent excitotoxic and ischemic damage to brain tissue. *J. Neurotrauma* **20**, 633-647.
- Plaschke, K., Kopitz, J., Weigand, M. A., Martin, E. and Bardenheuer, H. J. (2000). The neuroprotective effect of cerebral poly(ADP-ribose)polymerase inhibition in a rat model of global ischemia. *Neurosci. Lett.* **284**, 109-112.
- Przedborski, S., Jackson-Lewis, V., Kostic, V., Carlson, E., Epstein, C. J. and Cadet, J. L. (1992). Superoxide dismutase, catalase, and glutathione peroxidase activities in copper/zinc-superoxide dismutase transgenic mice. *J. Neurochem.* **58**, 1760-1707.
- Rao, A. M., Hatcher, J. F., Kindy, M. S. and Dempsey, R. J. (1999). Arachidonic acid and leukotriene C<sub>4</sub>: role in transient cerebral ischemia of gerbils. *Neurochem. Res.* **24**, 1225-1232.
- Riley, D. P. (2000). Rational design of synthetic enzymes and their potential utility as human pharmaceuticals. *Adv. Supramol. Chem.* **6**, 217-244.
- Rubbo, H., Radi, R., Trujillo, M., Telleri, R., Kalyanaraman, B., Barnes, S., Kirk, M. and Freeman, B. A. (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.
- Saito, A., Hayashi, T., Okuno, S., Ferrand-Drake, M. and Chan, P. H. (2003). Overexpression of copper/zinc superoxide dismutase in transgenic mice protects against neuronal cell death after transient focal ischemia by blocking activation of the Bad cell death signaling pathway. *J. Neurosci.* **23**, 1710-1718.
- Saito, I., Asano, T., Sano, K., Takakura, K., Abe, H., Yoshimoto, T., Kikuchi, H., Ohta, T. and Ishibashi, S. (1998). Neuroprotective effect of an antioxidant, ebsele, in patients with delayed neurological deficits after aneurysmal subarachnoid hemorrhage. *Neurosurgery* **42**, 269-277; Discussion 277-278.
- Salvemini, D., Wang, Z. Q., Zweier, J. L., Samouilov, A., Macarthur, H., Misko, T. P., Currie, M. G., Cuzzocrea, S., Sikorski, J. A. and Riley, D. P. (1999). A nonpeptidyl mimic of superoxide dismutase with therapeutic activity in rats. *Science* **286**, 209-210.
- Sandstrom, J., Carlsson, L., Marklund, S. L. and Edlund, T. (1992). The heparin-binding domain of extracellular superoxide dismutase C and formation of variants with reduced heparin affinity. *J. Biol. Chem.* **267**, 18205-18209.
- Sarco, D. P., Becker, J., Palmer, C., Sheldon, R. A. and Ferriero, D. M. (2000). The neuroprotective effect of deferoxamine in the hypoxic-ischemic immature mouse brain. *Neurosci. Lett.* **282**, 113-116.
- Schewe, T. (1995). Molecular actions of ebsele - an antiinflammatory antioxidant. *Gen. Pharmacol.* **26**, 1153-1169.
- Schonhoff, C. M., Gaston, B. and Mannick, J. B. (2003). Nitrosylation of cytochrome c during apoptosis. *J. Biol. Chem.* **278**, 18265-18270.
- Sharpe, M. A., Olsson, R., Stewart, V. C. and Clark, J. B. (2002). Oxidation of nitric oxide by oxomanganese-salen complexes: a new mechanism for cellular protection by superoxide dismutase/catalase mimetics. *Biochem. J.* **366**, 97-107.
- Sheng, H., Bart, R. D., Oury, T. D., Pearlstein, R. D., Crapo, J. D. and Warner, D. S. (1999a). Mice overexpressing extracellular superoxide dismutase have increased resistance to focal cerebral ischemia. *Neuroscience* **88**, 185-191.
- Sheng, H., Batinic-Haberle, I. and Warner, D. S. (2002a). Catalytic antioxidants as novel pharmacological approaches to treatment of ischemic brain injury. *Drug News Perspect.* **15**, 654-665.
- Sheng, H., Brody, T., Pearlstein, R. D., Crapo, J. and Warner, D. S. (1999b). Extracellular superoxide dismutase deficient mice have decreased resistance to focal cerebral ischemia. *Neurosci. Lett.* **267**, 13-17.
- Sheng, H., Enghild, J., Bowler, R., Patel, M., Batinic-Haberle, I., Calvi, C. L., Day, B. J., Pearlstein, R. D., Crapo, J. and Warner, D. S. (2002b). Effects of metalloporphyrin catalytic antioxidants in experimental brain ischemia. *Free Rad. Biol. Med.* **33**, 947-961.
- Sheng, H., Kudo, M., Mackensen, G. B., Pearlstein, R. D., Crapo, J. D. and Warner, D. S. (2000). Mice overexpressing extracellular superoxide dismutase have increased tolerance to global cerebral ischemia. *Exp. Neurol.* **163**, 392-398.
- Sies, H. (1991). *Oxidative Stress II: Oxidants and Antioxidants*. London: Academic Press.
- Siesjo, B. K. and Wieloch, T. (1983). Fatty acid metabolism and the mechanisms of ischemic brain damage. In *Cerebrovascular Diseases* (ed. M. Reivich and H. I. Hurtig), pp. 251-268. New York: Raven Press.
- Spasojevic, I., Batinic-Haberle, I. and Fridovich, I. (2000). Nitrosylation of manganese(III) tetrakis(N-ethylpyridinium-2-yl)porphyrin: A simple and sensitive spectrophotometric assay for nitric oxide. *Nitric Oxide: Biol. Chem.* **4**, 526-533.
- Sugawara, T., Lewen, A., Gasche, Y., Yu, F. and Chan, P. H. (2002a). Overexpression of SOD1 protects vulnerable motor neurons after spinal cord injury by attenuating mitochondrial cytochrome c release. *FASEB J.* **16**, 1997-1999.
- Sugawara, T., Noshita, N., Lewen, A., Gasche, Y., Ferrand-Drake, M., Fujimura, M., Morita-Fujimura, Y. and Chan, P. H. (2002b). Overexpression of copper/zinc superoxide dismutase in transgenic rats protects vulnerable neurons against ischemic damage by blocking the mitochondrial pathway of caspase activation. *J. Neurosci.* **22**, 209-217.
- Sugawara, T., Yu, F., Ma, L., Hsia, C. J. and Chan, P. H. (2001). Delayed

- treatment with polynitroxyl albumin reduces infarct size after stroke in rats. *NeuroReport* **12**, 3609-3612.
- Suzuki, M., Tabuchi, M., Ikeda, M. and Tomita, T.** (2002). Concurrent formation of peroxynitrite with the expression of inducible nitric oxide synthase in the brain during middle cerebral artery occlusion and reperfusion in rats. *Brain Res.* **951**, 113-120.
- Tanveer, A., Virji, S., Andreeva, L., Totty, N. F., Hsuan, J. J., Ward, J. M. and Crompton, M.** (1996). Involvement of cyclophilin D in the activation of a mitochondrial pore by  $Ca^{2+}$  and oxidant stress. *Eur. J. Biochem.* **238**, 166-172.
- Tarpey, M. and Fridovich, I.** (2001). Methods of detection of vascular reactive species: nitric oxide, superoxide, hydrogen peroxide, and peroxynitrite. *Circ. Res.* **89**, 224-236.
- The RANTTAS Investigators** (1996). A randomized trial of tirilazad mesylate in patients with acute stroke (RANTTAS). *Stroke* **27**, 1453-1458.
- Tibell, L., Hjalmarsson, K., Edlund, T., Skogman, G., Engstom, A. and Marklund, S. L.** (1987). Expression of human extracellular superoxide dismutase in Chinese hamster ovary cells and characterization of the product. *Proc. Natl. Acad. Sci. USA* **84**, 6634-6638.
- van Bel, F., Shadid, M., Moison, R. M., Dorrepaal, C. A., Fontijn, J., Monteiro, L., Van De Bor, M. and Berger, H. M.** (1998). Effect of allopurinol on postasphyxial free radical formation, cerebral hemodynamics, and electrical brain activity. *Pediatrics* **101**, 185-193.
- Vanella, A., Di Giacomo, C., Sorrenti, V., Russo, A., Castorina, C., Campisi, A., Renis, M. and Perez-Polo, J. R.** (1993). Free radical scavenger depletion in post-ischemic reperfusion brain damage. *Neurochem. Res.* **18**, 1337-1340.
- Waldmeier, P. C., Zimmermann, K., Qian, T., Tintelnot-Blomley, M. and Lemasters, J. J.** (2003). Cyclophilin D as a drug target. *Curr. Med. Chem.* **10**, 1485-1506.
- Watson, B. D.** (1998). Usual and unusual methods for detection of lipid peroxides as indicators of tissue injury in cerebral ischemia: what is appropriate and useful? *Cell. Mol. Neurobiol.* **18**, 581-598.
- Watson, B. D., Busto, R., Goldberg, W. J., Santiso, M., Yoshida, S. and Ginsberg, M. D.** (1984). Lipid peroxidation in vivo induced by reversible global ischemia in rat brain. *J. Neurochem.* **42**, 268-274.
- Wei, G., Dawson, V. L. and Zweier, J. L.** (1999). Role of neuronal and endothelial nitric oxide synthase in nitric oxide generation in the brain following cerebral ischemia. *Biochim. Biophys. Acta* **1455**, 23-34.
- Weisbrodt-Lefkowitz, M., Reuhl, K., Perry, B., Chan, P. H., Inouye, M. and Mirochnitchenko, O.** (1998). Overexpression of human glutathione peroxidase protects transgenic mice against focal cerebral ischemia/reperfusion damage. *Brain Res. Mol. Brain Res.* **53**, 333-338.
- Williams, G. D., Palmer, C., Heitjan, D. F. and Smith, M. B.** (1992). Allopurinol preserves cerebral energy metabolism during perinatal hypoxia-ischemia: a 31P NMR study in unanesthetized immature rats. *Neurosci. Lett.* **144**, 103-106.
- Wojtczak, L.** (1976). Effect of long-chain fatty acids and acyl-CoA on mitochondrial permeability, transport, and energy-coupling processes. *J. Bioenerg. Biomembr.* **8**, 293-311.
- Yamaguchi, T., Sano, K., Takakura, K., Saito, I., Shinohara, Y., Asano, T. and Yasuhara, H.** (1998). Ebselen in acute ischemic stroke: a placebo-controlled, double-blind clinical trial. Ebselen Study Group. *Stroke* **29**, 12-17.
- Yang, G., Chan, P. H., Chen, J., Carlson, E., Chen, S., Weinstein, P., Epstein, C. J. and Kamii, H.** (1994). Human copper-zinc superoxide dismutase transgenic mice are highly resistant to reperfusion injury after focal cerebral ischemia. *Stroke* **25**, 165-170.
- Yang, J., Klaidman, L. K., Chang, M. L., Kem, S., Sugawara, T., Chan, P. and Adams, J. D.** (2002). Nicotinamide therapy protects against both necrosis and apoptosis in a stroke model. *Pharmacol. Biochem. Behav.* **73**, 901-910.
- Yano, T., Ushijima, K. and Terasaki, H.** (1998). Failure of glutathione peroxidase to reduce transient ischemic injury in the rat hippocampal CA1 subfield. *Resuscitation* **39**, 91-98.
- Yoshimoto, T., Kristian, T., Hu, B., Ouyang, H.-B. and Siesjö, B. K.** (2002). NYX-059 maintains Akt activation and inhibits release of cytochrome c after focal cerebral ischemia. *Brain Res.* **947**, 191-198.
- Yue, T. L., Gu, J. L., Lysko, P. G., Cheng, H. Y., Barone, F. C. and Feuerstein, G.** (1992). Neuroprotective effects of phenyl-t-butyl-nitron in gerbil global brain ischemia and in cultured rat cerebellar neurons. *Brain Res.* **574**, 193-197.
- Zhang, J. and Piantadosi, C. A.** (1994). Prolonged production of hydroxyl radical in rat hippocampus after brain ischemia-reperfusion is decreased by 21-aminosteroids. *Neurosci. Lett.* **177**, 127-130.
- Zhao, H., Kalivendi, S., Zhang, H., Joseph, J., Nithipatikom, K., Vasquez-Vivar, J. and Kalyanaraman, B.** (2003). Superoxide reacts with hydroethidine but forms a fluorescent product that is distinctly different from ethidium: potential implications in intracellular fluorescence detection of superoxide. *Free Rad. Biol. Med.* **34**, 1359-1368.
- Zhao, Q., Pahlmark, K., Smith, M. L. and Siesjö, B. K.** (1994). Delayed treatment with the spin trap alpha-phenyl-N-tert-butyl nitron (PBN) reduces infarct size following transient middle cerebral artery occlusion in rats. *Acta Physiol. Scand.* **152**, 349-350.
- Zhu, D. Y., Liu, S. H., Sun, H. S. and Lu, Y. M.** (2003). Expression of inducible nitric oxide synthase after focal cerebral ischemia stimulates neurogenesis in the adult rodent dentate gyrus. *J. Neurosci.* **23**, 223-229.