Review Article
http://dx.doi.org/10.5115/acb.2014.47.3.149
pISSN 2093-3665 eISSN 2093-3673
Anatomy & Cell Biology acb

Neuroprotection of antioxidant enzymes against transient global cerebral ischemia in gerbils

Jae-Chul Lee, Moo-Ho Won
Department of Neurobiology, School of Medicine, Kangwon National University, Chuncheon, Korea

Abstract: Experimentally transient global cerebral ischemia using animal models have been thoroughly studied and numerous reports suggest the involvement of oxidative stress in the pathogenesis of neuronal death in ischemic lesions. In animal models, during the reperfusion period after ischemia, increased oxygen supply results in the overproduction of reactive oxygen species (ROS), which are involved in the process of cell death. ROS, such as superoxide anions, hydroxyl free radicals, hydrogen peroxide and nitric oxide are produced as a consequence of metabolic reactions and central nervous system activity. These reactive species are directly involved in the oxidative damage of cellular macromolecules such as nucleic acids, lipids and proteins in ischemic tissues, which can lead to cell death. Antioxidant enzymes are believed to be among the major mechanisms by which cells counteract the deleterious effect of ROS after cerebral ischemia. Consequently, antioxidant strategies have been long suggested as a therapy for experimental ischemic stroke; however, clinical trials have not yet been able to promote the translation of this concept into patient treatment regimens. This article focuses on the contribution of oxidative stress or antioxidants to the post-ischemic neuronal death following transient global cerebral ischemia by using a gerbil model.

Key words: Mongolian gerbil, Global cerebral ischemia, Neuronal death, Reactive oxygen species, Antioxidants

Received July 21, 2014; Accepted August 20, 2014

Introduction

Global cerebral ischemia occurs when the blood supply to the entire brain or a large part of the brain is disrupted, resulting in the tissue deprivation of oxygen and glucose that may give permanent brain damage. In adult humans, global cerebral ischemic injury occurs in some conditions like cardiac arrest, coronary artery bypass surgery, cardio-respiratory failure, and others leading to the drastic reduction of the blood flow to the brain [1]. Global cerebral ischemia, for a short period of time, results in selective neuro-degeneration in vulnerable brain areas, such as the CA1 region of the hippocampus [2, 3]. The underlying mechanisms of CA1 neuronal loss following global cerebral ischemia may involve excitotoxicity, reactive oxygen species (ROS), inflammation, and apoptosis [4]. However, the exact mechanisms underlying neuronal damage including delayed neuronal death after ischemia-reperfusion injury have not been elucidated yet. Among these, cerebral ischemia-reperfusion in particular are responsible for oxidative stress due to the generation of free radicals [5], which culminates into deleterious effects during pathogenesis [6]. Therefore, oxidative stress has emerged as an important underlying factor in the delayed neuronal death induced by global cerebral ischemia [6, 7].

Corresponding author:
Moo-Ho Won
Department of Neurobiology, School of Medicine, Kangwon National University, 1 Gangwondaehak-gil, Chuncheon 200-701, Korea
Tel: +82-33-250-8891, Fax: +82-33-256-1614, E-mail: mhwon@kangwon.ac.kr

Animal Models Inducing Global Cerebral Ischemia

Various methods have been used to induce global cerebral ischemia in experimental animal models including decapitation without recirculation [8], neck tourniquet [9], bilateral common carotid artery occlusion (2-VO) [10] and four-vessel occlusion (4-VO) [11]. Cardiac arrest achieved through ventricular fibrillation has been also used to produce global cerebral ischemia [12, 13]. One of the various animal models to produce global cerebral ischemia, experimentally induced transient cerebral ischemia has been widely induced by the occlusion of related artery or arteries. Pulsinelli and Brierley [14] developed a 4-VO model to provide a method of reversible global cerebral ischemia in rats. This model has been also utilized for morphological and metabolic studies in anesthetized rats [15, 16]. The 4-VO model involves a two-stage procedure to produce global cerebral ischemia. In the first stage, on the day before the experiment, non-traumatic clasps are placed loosely around each common carotid artery and exteriorized in the neck of the animal. The vertebral arteries are then electro-cauterized via the alar foramina of the first cervical vertebra. On the second day, the common carotid arteries are occluded while the animal is awake, and ischemia is produced. This procedure must result in a complete loss of the righting reflex for the animal to be included in the study. It can be utilized in either awake or anesthetized animals, which makes it extremely useful. However, it is not an easy model to use, and there has been much variability in results between laboratories. The success rate of the model is approximately 50% to 75%; however, the effects of ischemia are quite variable between rat strains. It is believed that this variability may be the result of variability of collateral circulation present in each strain [11]. As alternative to the 4-VO model, global cerebral ischemia has been widely induced by the occlusion of the two common carotid arteries, i.e., by 2-VO together with the induction of hypotension for a limited time period. In this forebrain ischemia model, selective injury in the CA1 of the hippocampus, the caudate putamen and neocortex is observed [17]. Mongolian gerbils have a unique and convenient vascular anatomy, thus, they have been used for global ischemia studies. The unique anatomical feature of the Mongolian gerbil is that they do not have the posterior communicating artery, which connects the carotid and vertebrobasilar arterial system [18]. Thus, the Mongolian gerbil has been used as a good 2-VO animal model to investigate the mechanisms of selective neuronal death following transient global cerebral ischemia. In this animal, bilateral common carotid arteries occlusion reduces cerebral blood flow to almost zero in most gerbils [10]. When both the common carotid arteries are transiently occluded for 5 minutes, neuronal death in the hippocampus is observed [19]. These changes are also accompanied by various behavioral impairments as a spatial learning and memory deficits that vary with the degree and the duration of occlusion. The major advantages of the gerbil model are relative simplicity of the surgical procedure that allows for the study regarding transient global cerebral ischemia, and are easily suitable for long-term studies because of long survival after ischemic injury. An important feature of global cerebral ischemic damage is the vulnerability of specific neuronal populations. Especially, pyramidal neurons in the hippocampal CA1 region do not die immediately but rather survive over several days. One clear aspect is that the model can be physiologically controlled so that the resulting injury is reproducible and variability is as limited as possible. In the Mongolian gerbil, prolonged global cerebral ischemia kills about 96% of the CA1 pyramidal neurons after 4 days [20]. Delayed and rapid neuronal death appears to follow a similar sequence of cellular events. The lag between an ischemic episode and neuronal death depends upon the severity and/or duration of ischemia [21]. Finally, it is important to mention the Mongolian gerbil because this animal has been widely used as a good animal model of transient global cerebral ischemia [19].

Generation of Oxygen Free Radicals

Oxygen free radicals are molecular species that contain one or more unpaired valence electrons and they are highly active with other molecules, such as DNA and lipids, pairing with their single electrons and causing oxidation of those molecules [22]. Several oxygen free radicals, including superoxide anions ($O_2\bullet^-$), hydroxyl radical (•OH), nitric oxide (NO•) and hydrogen peroxide (H_2O_2) have been implicated in the development of many neurological disorders and brain dysfunctions [23, 24]. These radicals are known to initiate lipid peroxidation and to cause protein oxidation and DNA damage in cells.

$O_2\bullet^-$ is formed when oxygen acquires an additional electron, leaving the molecule with only one unpaired electron [25]. This process is mediated by pro-oxidant enzymes such as nicotine adenine dinucleotide phosphate (NAD(P)H)

oxidase and xanthine oxidase. The major site for producing $O_2\bullet^-$ is mitochondria, which are the machinery of the cells to produce adenosine triphosphate. Normally, electrons are transferred through mitochondrial electron transport chain for the reduction of oxygen to water; however, approximately 2% to 5% of electron flow is used to produce them [6]. $O_2\bullet^-$ is converted into H_2O_2 by the action of superoxide dismutases (SODs). SODs detoxify $O_2\bullet^-$ to H_2O_2 ($O_2\bullet^- + 2\ H^+ \rightarrow H_2O_2$), which is further converted to H_2O by catalase or glutathione peroxidase (GPx) ($2H_2O_2 \rightarrow 2H_2O + O_2$) [6]. $O_2\bullet^-$ itself can also react with H_2O_2 and generate OH^- [26].

On the other hand, •OH is a neutral form of hydroxide ion and has a high reactivity, making it a very dangerous radical with a very short *in vivo* half-life [27]. Highly reactive •OH is not produced as a by-product of any known enzymatic reaction; however, it is produced from H_2O_2 through Fenton reaction ($H_2O_2 + Fe^{2+} \rightarrow OH^- + Fe^{3+} + \bullet OH$) and Haber-Weiss reaction ($O_2\bullet^- + H_2O_2 \rightarrow \bullet OH + HO^- + O_2$) or by peroxynitrite ($ONOO^-$) [28, 29]. Once formed, •OH reacts almost instantaneously with many cellular components, including polyunsaturated fatty acids of membrane lipids. The initial reaction of •OH with polyunsaturated fatty acids produces an alkyl radical, which in turn reacts with molecular oxygen to form a peroxyl radical (ROO•). The ROO• can abstract hydrogen from an adjacent fatty acid to produce a lipid hydroperoxide (ROOH), propagating a chain reaction of lipid oxidation [30].

NO• is formed by the enzymatic oxidation of L-arginine to citrulline by nitric oxide synthases and serves as an important regulator of vascular response and neuronal signaling [31]. NO• has a half-life of only a few seconds in an aqueous environment. NO• has greater stability in an environment with a lower oxygen concentration. However, it readily diffuses through the cytoplasm and plasma membranes, since it is soluble in both aqueous and lipid media [32]. NO• has effects on neuronal transmission as well as on synaptic plasticity in the central nervous system. $O_2\bullet^-$ does not directly induce lipid peroxidation; however, it can react with NO• to produce $ONOO^-$, which is a strong oxidative radical that causes protein nitration and lipid peroxidation, which leads to cell dysfunction [29, 33]. Lipid peroxidation is not the sole route of cellular damage initiated by •OH and $ONOO^-$ as these species also oxidize proteins and DNA [30].

H_2O_2 easily diffuses across the plasma membrane. H_2O_2 is also produced by xanthine oxidase, amino acid oxidase, and NAD(P)H oxidase [34, 35]. In the presence of chloride ion, H_2O_2 is converted to hypochlorous acid (HOCl). The HOCl is highly oxidative and plays an important role in killing pathogens in the airways [36]. However, HOCl can react with DNA, induce DNA-protein interactions, produce pyrimidine oxidation products and add chloride to DNA bases [37]. The accumulation of H_2O_2 is reported to impair mitochondrial function. H_2O_2 is a longer-lasting reactive species, electrically neutral, able to pass through cell membranes and more stable than $O_2\bullet^-$, •OH, and other ROS. Therefore, H_2O_2 may persist for longer time after reperfusion to produce neuronal injury.

Oxidative Stress by Global Cerebral Ischemia

It has been well established that oxidative stress displays an important role in the pathophysiology of cerebral ischemia [38] and that the excessive production of ROS occurs during cerebral ischemia and reperfusion [39]. Generally, alteration in Ca^{2+} homeostasis in the brain due to an increase in ROS has been suggested to explain the adverse effects of cerebral ischemia and reperfusion. The activation of N-methyl-D-aspartate receptor by glutamate increases Ca^{2+} influx as well as the activation of neuronal nitric oxide synthase, which generates nitric oxide (NO) from l-arginine. Under these conditions, the oxidation of xanthine and hypoxanthine by xanthine oxidase is accompanied with the generation of $O_2\bullet^-$ and H_2O_2 [40, 41]. NO reacts with $O_2\bullet^-$ to generate the harmful radical $ONOO^-$ that contributes to neuronal injury during reperfusion. Oxidative stress and increased Ca^{2+} cause the opening of mitochondrial permeability transition pore. This allows an entry of water and solutes from the cytoplasm resulting in mitochondrial swelling and damage. The generation of ROS also occurs in the cytoplasm through the action of NAD(P)H oxidase. The resulting oxidative stress can outweigh antioxidant defenses and lead to cell death because oxidative stress can cause a widespread damage to cellular components such as DNA, lipids, and proteins that ultimately promote cellular damage and death during cerebral ischemia and reperfusion [6, 39].

Antioxidants in Global Cerebral Ischemic Injury

Global cerebral ischemic injury is associated with the oxidative stress caused by the overproduction of ROS and other free radicals [42, 43]. Excessive ROS production is followed by dysfunctions of important redox-sensitive enzymes, membrane receptors and ion channels, DNA da-

http://dx.doi.org/10.5115/acb.2014.47.3.149

mage, membrane lipid peroxidation, and cytochrome *c* release from mitochondria, which activate caspases that aggravate cell death after ischemia and reperfusion injury [44]. Thus, neuronal defense mechanisms against oxidative stress have focused on antioxidant systems. Antioxidant system may be classified into enzymatic and non-enzymatic antioxidants. Several enzymatic antioxidants exist that convert ROS into less noxious compounds, for example, SODs, GPx, and catalase. Collectively, these enzymes provide a first line of defense against $O_2\bullet^-$ and •OH. Amongst these, copper, zinc-superoxide dismutase (CuZn-SOD, also called SOD1) provides a defense system against oxidative stress by catalyzing the dismutation of $O_2\bullet^-$ into O_2 and H_2O_2 [45]. It has been reported that SOD1 overexpression prevents neuronal injury in the hippocampal CA1 region in a murine cardiac arrest model. In addition, Kim et al. [46] have reported that increased SOD1 reduces oxidative DNA damage and subsequent DNA-fragmented cell death after photothrombotic ischemia in SOD1 transgenic mice. However, some investigators have obtained various degrees in success and failure when free non-modified SOD1 is used to ameliorate ischemic brain injury [47]. The extremely short half-life of SOD1 in circulating blood and its failure in passing the blood-brain barrier make it difficult to use enzyme therapy in cerebral ischemia. To overcome this shortness, substantial progress has been made in the development of cell-penetrating peptide-based extracellular and intracellular limitation. Polyethylene glycol-conjugated SOD1 has been successfully used to reduce infarct volume in animals that have been subjected to global cerebral ischemia [48]. Liposome-entrapped SOD1 has an increased half-life (4.2 hours), blood-brain barrier permeability, and cellular uptake, and it has proved to be an effective treatment in reducing the severity of global ischemic brain injuries [49]. Especially, it is reported that that the administration of PEP-1 SOD1 fusion protein significantly inhibits neuronal death in the gerbil hippocampal CA1 region induced by transient global cerebral ischemia [50, 51]. Similarly, manganese superoxide dismutase (Mn-SOD2, called SOD2) in mitochondria converts superoxide produced in the mitochondrial matrix into H_2O_2 [52]. Increased SOD2 immunoreactivity is consistently observed with an antioxidant response to the increased superoxide formation caused by hyperglycemic cerebral ischemia [53, 54]. Increased SOD2 immunereactivity is also demonstrated in a study by Bidmon et al. [55]; a large region with the increased SOD2 immunoreactivity is observed following a small cortical ischemic lesion, indicating a widespread response to a well-localized insult. In addition, transgenic mice that over-express SOD2 suffer a less ischemic brain damage compared with the wild-type mice [56]. Therefore, SOD2 has been demonstrated to be a key enzyme in protecting the brain from ischemia and reperfusion injury. Recently, it has been reported that increased and longer maintained SODs levels in the young hippocampal CA1 region may provide an evidence to explain more delayed and less neuronal death compared with those in the adult CA1 region after 5 min of transient cerebral ischemia [57, 58]. Furthermore, the regulation of SOD2 activity displays a neuroprotective effect against cerebral ischemic damage [59]. Thus, there is a clear need for a systematic study to determine the exact role of SODs in protection against ischemia and reperfusion injury. In consistent to this, an increasing number of investigators have demonstrated the importance of SODs in protecting the brain from global ischemia and reperfusion injury using a gerbil model [60-66].

GPx is another important enzyme contributing to H_2O_2 scavenging. In several *in vitro* studies, GPx confers a greater protection against oxidative stress than SOD. Antioxidant treatment should prevent the loss of GPx activity by effectively scavenging the excess ROS. Many researchers have reported that the regulation of GPx activity displays neuroprotective effects in the gerbil brain following global cerebral ischemia and it is involved in the control of cellular damage after ischemic insults [60, 62, 65, 67, 68].

On the other hand, catalase is a membrane bound enzyme that is present in peroxisomes; however, its activity has been observed in mitochondrial matrix [69]. Catalase is an important enzyme for the maintenance of intracellular concentration of reduced glutathione and has a crucial role as a free radical scavenger [70]. Decrease in the level of catalase is noted in the brain of ischemic rats. However, the role of catalase enzyme during global brain ischemia is controversial; a few studies have suggested the decrease of catalase activity in the brain induced by global brain ischemia [62, 63, 71, 72], while others have suggested the increase of catalase activity in ischemic brain tissue [73]. Recently, it has been reported that neuronal damage in the young gerbil hippocampal CA1 region induced by transient cerebral ischemia is much more delayed and less severe than that in the adult, showing that CAT immunoreactivity and its protein level are increased in the young CA1 region after ischemia and reperfusion [58]. Especially, the administration of PEP-1-catalase fusion

protein displays significant neuroprotection in the CA1 region of the hippocampus after transient cerebral ischemia [74]. Similarly, several studies have demonstrated that an enhanced expression of catalase significantly attenuates the injury of brain tissues after global ischemia in gerbils [60, 62, 68, 75-77].

In addition to these major enzymes, thioredoxin (Trx) and peroxiredoxins (Prxs) redox system exerts important roles in antioxidant regeneration and the regulation of intracellular ROS level [78-80]. In particular, Trx/Prx redox system is a major route for removing H_2O_2 in cellular organs [81]. Recent studies have reported that Trx/Prx redox system is a strong neuroprotective effect in cerebral ischemia [82, 83]. Trx is well known as a radical scavenger [84, 85]. A recent study has shown that Trx is a neuroprotective factor against neuronal damage subsequent to ischemia and oxidative stress [86] and that Trx is a trophic factor for neuronal homeostasis [87]. Among Trx subtypes, TrxR2 plays a key role in the mitochondrial Trx2/Prx3 redox system via a reduction of oxidized Trx2 utilizing NADPH electron to protect cells from an oxidative injury [79, 88]. Recently, it has been reported that Trx2 immunoreactivity in CA1 pyramidal neurons is increased 30 minutes after ischemia and reperfusion, decreased 6 hours after ischemia and reperfusion and increased again 1 day after ischemia and reperfusion [82]. This finding suggests that the decrease of Trx2 in the CA1 pyramidal neurons at 6 hours after ischemia and reperfusion may be associated with the depletion of antioxidants because of distinct increases in ROS in the CA1 region after ischemia and reperfusion. However, the increase of Trx2 in the CA1 pyramidal neurons at 1 day after post-ischemia may be associated with compensatory mechanisms against ischemic damage. Prxs are also considered efficient enzymes in removing low levels of H_2O_2 because they have a high affinity toward H_2O_2 [89, 90]. Prx family is divided into six subtypes: Prx1–4 have two conserved cysteine residues, whereas Prx5 and Prx6 have one of the cysteine residues involved in peroxidase activity [90, 91]. It has been reported that Prx3 has a potential to play a major role in mitochondrial dependent antioxidant effects [92, 93]. Recently, it has been reported that Prx3 immunoreactivity and protein level significantly increase in the pyramidal neurons of the hippocampal CA1 region 1 day after ischemia and reperfusion [82]. This indicates that Prx3 increases to remove ROS in the ischemic CA1 region because Prx3 can scavenge not only H_2O_2 in cooperation with thiol, but also $ONOO^-$ by itself [94]. In addition, it is reported that mitochondrial Trx2 interacts closely with Prx3 in mitochondria, and the increase of interaction between Trx2 and Prx3 protects cells from oxidative stress [79]. Furthermore, it is demonstrated that the co-treatment of Prx3 and Trx2 more efficiently protects CA1 pyramidal neurons from ischemia and reperfusion injury in the gerbil hippocampus induced by transient global cerebral ischemia [82].

Conclusion

Oxidative stress can arise from the overproduction of ROS that shift the balance between oxidant/antioxidant statuses leading to potential cellular damage, and plays a central role in the initiation of neuronal damage in global cerebral ischemia. The development of novel antioxidant treatments is based on the complete understanding of the role of oxidative stress in global cerebral ischemic injury. However, little is known about the formation of ROS in brain ischemia due to innate difficulty in studies on oxidative stress; however, knowledge toward blocking the sources of ROS will be extremely useful in the design of effective therapies against global cerebral ischemia.

Acknowledgements

This research was supported by the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0010580).

References

1. Llinas R, Barbut D, Caplan LR. Neurologic complications of cardiac surgery. Prog Cardiovasc Dis 2000;43:101-12.
2. Akai F, Yanagihara T. Identity of the dorsal hippocampal region most vulnerable to cerebral ischemia. Brain Res 1993;603:87-95.
3. Pulsinelli WA. Selective neuronal vulnerability: morphological and molecular characteristics. Prog Brain Res 1985;63:29-37.
4. Dirnagl U, Iadecola C, Moskowitz MA. Pathobiology of ischaemic stroke: an integrated view. Trends Neurosci 1999;22:391-7.
5. Nita DA, Nita V, Spulber S, Moldovan M, Popa DP, Zagrean AM, Zagrean L. Oxidative damage following cerebral ischemia depends on reperfusion: a biochemical study in rat. J Cell Mol Med 2001;5:163-70.
6. Chan PH. Reactive oxygen radicals in signaling and damage in the ischemic brain. J Cereb Blood Flow Metab 2001;21:2-14.
7. Wang Q, Sun AY, Simonyi A, Jensen MD, Shelat PB, Rottinghaus GE, MacDonald RS, Miller DK, Lubahn DE, Weisman GA, Sun GY. Neuroprotective mechanisms of curcumin against cerebral

http://dx.doi.org/10.5115/acb.2014.47.3.149

ischemia-induced neuronal apoptosis and behavioral deficits. J Neurosci Res 2005;82:138-48.
8. Lowry OH, Passonneau JV, Hasselberger FX, Schulz DW. Effect of ischemia on known substrates and cofactors of the glycolytic pathway in brain. J Biol Chem 1964;239:18-30.
9. Siemkowicz E, Gjedde A. Post-ischemic coma in rat: effect of different pre-ischemic blood glucose levels on cerebral metabolic recovery after ischemia. Acta Physiol Scand 1980;110:225-32.
10. Crockard A, Iannotti F, Hunstock AT, Smith RD, Harris RJ, Symon L. Cerebral blood flow and edema following carotid occlusion in the gerbil. Stroke 1980;11:494-8.
11. Pulsinelli WA, Buchan AM. The four-vessel occlusion rat model: method for complete occlusion of vertebral arteries and control of collateral circulation. Stroke 1988;19:913-4.
12. Kofler J, Hattori K, Sawada M, DeVries AC, Martin LJ, Hurn PD, Traystman RJ. Histopathological and behavioral characterization of a novel model of cardiac arrest and cardiopulmonary resuscitation in mice. J Neurosci Methods 2004;136:33-44.
13. Deng G, Yonchek JC, Quillinan N, Strnad FA, Exo J, Herson PS, Traystman RJ. A novel mouse model of pediatric cardiac arrest and cardiopulmonary resuscitation reveals age-dependent neuronal sensitivities to ischemic injury. J Neurosci Methods 2014;222:34-41.
14. Pulsinelli WA, Brierley JB. A new model of bilateral hemispheric ischemia in the unanesthetized rat. Stroke 1979;10:267-72.
15. Yoshida S, Abe K, Busto R, Watson BD, Kogure K, Ginsberg MD. Influence of transient ischemia on lipid-soluble antioxidants, free fatty acids and energy metabolites in rat brain. Brain Res 1982;245:307-16.
16. Globus MY, Busto R, Dietrich WD, Martinez E, Valdes I, Ginsberg MD. Intra-ischemic extracellular release of dopamine and glutamate is associated with striatal vulnerability to ischemia. Neurosci Lett 1988;91:36-40.
17. Smith ML, Bendek G, Dahlgren N, Rosén I, Wieloch T, Siesjö BK. Models for studying long-term recovery following forebrain ischemia in the rat. 2. A 2-vessel occlusion model. Acta Neurol Scand 1984;69:385-401.
18. Kirino T, Sano K. Fine structural nature of delayed neuronal death following ischemia in the gerbil hippocampus. Acta Neuropathol 1984;62:209-18.
19. Kirino T. Delayed neuronal death in the gerbil hippocampus following ischemia. Brain Res 1982;239:57-69.
20. Colbourne F, Sutherland GR, Auer RN. Electron microscopic evidence against apoptosis as the mechanism of neuronal death in global ischemia. J Neurosci 1999;19:4200-10.
21. Rosenblum WI. Histopathologic clues to the pathways of neuronal death following ischemia/hypoxia. J Neurotrauma 1997; 14:313-26.
22. Dröge W. Free radicals in the physiological control of cell function. Physiol Rev 2002;82:47-95.
23. Siesjö BK, Agardh CD, Bengtsson F. Free radicals and brain damage. Cerebrovasc Brain Metab Rev 1989;1:165-211.
24. Chan PH. Oxygen radicals in focal cerebral ischemia. Brain Pathol 1994;4:59-65.
25. Miller DM, Buettner GR, Aust SD. Transition metals as catalysts of "autoxidation" reactions. Free Radic Biol Med 1990;8:95-108.
26. Weiss J. The reaction between hydrogen peroxide and iron salts. Experientia 1951;7:135-6.
27. Pastor N, Weinstein H, Jamison E, Brenowitz M. A detailed interpretation of OH radical footprints in a TBP-DNA complex reveals the role of dynamics in the mechanism of sequence-specific binding. J Mol Biol 2000;304:55-68.
28. Chan PH. Role of oxidants in ischemic brain damage. Stroke 1996;27:1124-9.
29. Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proc Natl Acad Sci U S A 1990;87:1620-4.
30. Beal MF. Mitochondria, free radicals, and neurodegeneration. Curr Opin Neurobiol 1996;6:661-6.
31. Iadecola C. Bright and dark sides of nitric oxide in ischemic brain injury. Trends Neurosci 1997;20:132-9.
32. Chiueh CC. Neuroprotective properties of nitric oxide. Ann N Y Acad Sci 1999;890:301-11.
33. Radi R, Beckman JS, Bush KM, Freeman BA. Peroxynitrite-induced membrane lipid peroxidation: the cytotoxic potential of superoxide and nitric oxide. Arch Biochem Biophys 1991;288:481-7.
34. Dupuy C, Virion A, Ohayon R, Kaniewski J, Dème D, Pommier J. Mechanism of hydrogen peroxide formation catalyzed by NADPH oxidase in thyroid plasma membrane. J Biol Chem 1991;266:3739-43.
35. Granger DN. Role of xanthine oxidase and granulocytes in ischemia-reperfusion injury. Am J Physiol 1988;255(6 Pt 2): H1269-75.
36. Klebanoff SJ. Myeloperoxidase: friend and foe. J Leukoc Biol 2005;77:598-625.
37. Kulcharyk PA, Heinecke JW. Hypochlorous acid produced by the myeloperoxidase system of human phagocytes induces covalent cross-links between DNA and protein. Biochemistry 2001;40:3648-56.
38. Clemens JA. Cerebral ischemia: gene activation, neuronal injury, and the protective role of antioxidants. Free Radic Biol Med 2000;28:1526-31.
39. Sugawara T, Chan PH. Reactive oxygen radicals and pathogenesis of neuronal death after cerebral ischemia. Antioxid Redox Signal 2003;5:597-607.
40. Beetsch JW, Park TS, Dugan LL, Shah AR, Gidday JM. Xanthine oxidase-derived superoxide causes reoxygenation injury of ischemic cerebral endothelial cells. Brain Res 1998;786:89-95.
41. Nishino T, Tamura I. The mechanism of conversion of xanthine dehydrogenase to oxidase and the role of the enzyme in reperfusion injury. Adv Exp Med Biol 1991;309A:327-33.
42. Jung JE, Kim GS, Chen H, Maier CM, Narasimhan P, Song YS, Niizuma K, Katsu M, Okami N, Yoshioka H, Sakata H, Goeders CE, Chan PH. Reperfusion and neurovascular dysfunction in stroke: from basic mechanisms to potential strategies for neuroprotection. Mol Neurobiol 2010;41:172-9.

43. Kim GS, Jung JE, Niizuma K, Chan PH. CK2 is a novel negative regulator of NADPH oxidase and a neuroprotectant in mice after cerebral ischemia. J Neurosci 2009;29:14779-89.
44. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007;39:44-84.
45. Kofler J, Hurn PD, Traystman RJ. SOD1 overexpression and female sex exhibit region-specific neuroprotection after global cerebral ischemia due to cardiac arrest. J Cereb Blood Flow Metab 2005;25:1130-7.
46. Kim GW, Lewen A, Copin J, Watson BD, Chan PH. The cytosolic antioxidant, copper/zinc superoxide dismutase, attenuates blood-brain barrier disruption and oxidative cellular injury after photothrombotic cortical ischemia in mice. Neuroscience 2001;105:1007-18.
47. Chan PH, Kinouchi H, Epstein CJ, Carlson E, Chen SF, Imaizumi S, Yang GY. Role of superoxide dismutase in ischemic brain injury: reduction of edema and infarction in transgenic mice following focal cerebral ischemia. Prog Brain Res 1993;96:97-104.
48. Kirsch JR, Helfaer MA, Haun SE, Koehler RC, Traystman RJ. Polyethylene glycol-conjugated superoxide dismutase improves recovery of postischemic hypercapnic cerebral blood flow in piglets. Pediatr Res 1993;34:530-7.
49. Stanimirovic DB, Markovic M, Micic DV, Spatz M, Mrsulja BB. Liposome-entrapped superoxide dismutase reduces ischemia/reperfusion 'oxidative stress' in gerbil brain. Neurochem Res 1994;19:1473-8.
50. Eum WS, Kim DW, Hwang IK, Yoo KY, Kang TC, Jang SH, Choi HS, Choi SH, Kim YH, Kim SY, Kwon HY, Kang JH, Kwon OS, Cho SW, Lee KS, Park J, Won MH, Choi SY. In vivo protein transduction: biologically active intact pep-1-superoxide dismutase fusion protein efficiently protects against ischemic insult. Free Radic Biol Med 2004;37:1656-69.
51. Hwang IK, Eum WS, Yoo KY, Cho JH, Kim DW, Choi SH, Kang TC, Kwon OS, Kang JH, Choi SY, Won MH. Copper chaperone for Cu,Zn-SOD supplement potentiates the Cu,Zn-SOD function of neuroprotective effects against ischemic neuronal damage in the gerbil hippocampus. Free Radic Biol Med 2005;39:392-402.
52. De Vos KJ, Chapman AL, Tennant ME, Manser C, Tudor EL, Lau KF, Brownlees J, Ackerley S, Shaw PJ, McLoughlin DM, Shaw CE, Leigh PN, Miller CC, Grierson AJ. Familial amyotrophic lateral sclerosis-linked SOD1 mutants perturb fast axonal transport to reduce axonal mitochondria content. Hum Mol Genet 2007;16:2720-8.
53. Noack H, Lindenau J, Rothe F, Asayama K, Wolf G. Differential expression of superoxide dismutase isoforms in neuronal and glial compartments in the course of excitotoxically mediated neurodegeneration: relation to oxidative and nitrergic stress. Glia 1998;23:285-97.
54. Ste-Marie L, Hazell AS, Bémeur C, Butterworth R, Montgomery J. Immunohistochemical detection of inducible nitric oxide synthase, nitrotyrosine and manganese superoxide dismutase following hyperglycemic focal cerebral ischemia. Brain Res 2001;918:10-9.
55. Bidmon HJ, Kato K, Schleicher A, Witte OW, Zilles K. Transient increase of manganese-superoxide dismutase in remote brain areas after focal photothrombotic cortical lesion. Stroke 1998;29:203-10.
56. Keller JN, Kindy MS, Holtsberg FW, St Clair DK, Yen HC, Germeyer A, Steiner SM, Bruce-Keller AJ, Hutchins JB, Mattson MP. Mitochondrial manganese superoxide dismutase prevents neural apoptosis and reduces ischemic brain injury: suppression of peroxynitrite production, lipid peroxidation, and mitochondrial dysfunction. J Neurosci 1998;18:687-97.
57. Li H, Park JH, Lee JC, Yoo KY, Hwang IK, Lee CH, Choi JH, Kim JD, Kang IJ, Won MH. Neuroprotective effects of Alpinia katsumadai against experimental ischemic damage via control of oxidative stress. Pharm Biol 2013;51:197-205.
58. Yan BC, Park JH, Lee CH, Yoo KY, Choi JH, Lee YJ, Cho JH, Baek YY, Kim YM, Won MH. Increases of antioxidants are related to more delayed neuronal death in the hippocampal CA1 region of the young gerbil induced by transient cerebral ischemia. Brain Res 2011;1425:142-54.
59. Jung JE, Kim GS, Narasimhan P, Song YS, Chan PH. Regulation of Mn-superoxide dismutase activity and neuroprotection by STAT3 in mice after cerebral ischemia. J Neurosci 2009;29:7003-14.
60. Yan BC, Park JH, Ahn JH, Kim IH, Park OK, Lee JC, Yoo KY, Choi JH, Lee CH, Hwang IK, Park JH, Her S, Kim JS, Shin HC, Cho JH, Kim YM, Kwon SH, Won MH. Neuroprotection of posttreatment with risperidone, an atypical antipsychotic drug, in rat and gerbil models of ischemic stroke and the maintenance of antioxidants in a gerbil model of ischemic stroke. J Neurosci Res 2014;92:795-807.
61. Dekanski D, Selaković V, Piperski V, Radulović Z, Korenić A, Radenović L. Protective effect of olive leaf extract on hippocampal injury induced by transient global cerebral ischemia and reperfusion in Mongolian gerbils. Phytomedicine 2011;18:1137-43.
62. Zhang YB, Kan MY, Yang ZH, Ding WL, Yi J, Chen HZ, Lu Y. Neuroprotective effects of N-stearoyltyrosine on transient global cerebral ischemia in gerbils. Brain Res 2009;1287:146-56.
63. Sharma SS, Gupta S. Neuroprotective effect of MnTMPyP, a superoxide dismutase/catalase mimetic in global cerebral ischemia is mediated through reduction of oxidative stress and DNA fragmentation. Eur J Pharmacol 2007;561:72-9.
64. Selakovic V, Janac B, Radenovic L. MK-801 effect on regional cerebral oxidative stress rate induced by different duration of global ischemia in gerbils. Mol Cell Biochem 2010;342:35-50.
65. Cao Y, Mao X, Sun C, Zheng P, Gao J, Wang X, Min D, Sun H, Xie N, Cai J. Baicalin attenuates global cerebral ischemia/reperfusion injury in gerbils via anti-oxidative and anti-apoptotic pathways. Brain Res Bull 2011;85:396-402.
66. Park JH, Joo HS, Yoo KY, Shin BN, Kim IH, Lee CH, Choi JH, Byun K, Lee B, Lim SS, Kim MJ, Won MH. Extract from Terminalia chebula seeds protect against experimental ischemic neuronal damage via maintaining SODs and BDNF levels.

http://dx.doi.org/10.5115/acb.2014.47.3.149

Neurochem Res 2011;36:2043-50.
67. Li DQ, Duan YL, Bao YM, Liu CP, Liu Y, An LJ. Neuroprotection of catalpol in transient global ischemia in gerbils. Neurosci Res 2004;50:169-77.
68. Li L, Shen YM, Yang XS, Wu WL, Wang BG, Chen ZH, Hao XJ. Effects of spiramine T on antioxidant enzymatic activities and nitric oxide production in cerebral ischemia-reperfusion gerbils. Brain Res 2002;944:205-9.
69. Steare SE, Yellon DM. The protective effect of heat stress against reperfusion arrhythmias in the rat. J Mol Cell Cardiol 1993;25:1471-81.
70. Temsah RM, Netticadan T, Chapman D, Takeda S, Mochizuki S, Dhalla NS. Alterations in sarcoplasmic reticulum function and gene expression in ischemic-reperfused rat heart. Am J Physiol 1999;277(2 Pt 2):H584-94.
71. Homi HM, Freitas JJ, Curi R, Velasco IT, Junior BA. Changes in superoxide dismutase and catalase activities of rat brain regions during early global transient ischemia/reperfusion. Neurosci Lett 2002;333:37-40.
72. Ozerol E, Bilgic S, Iraz M, Cigli A, Ilhan A, Akyol O. The protective effect of erdosteine on short-term global brain ischemia/reperfusion injury in rats. Prog Neuropsychopharmacol Biol Psychiatry 2009;33:20-4.
73. Işlekel S, Işlekel H, Güner G, Ozdamar N. Alterations in superoxide dismutase, glutathione peroxidase and catalase activities in experimental cerebral ischemia-reperfusion. Res Exp Med (Berl) 1999;199:167-76.
74. Kim DW, Kim DS, Kim MJ, Kwon SW, Ahn EH, Jeong HJ, Sohn EJ, Dutta S, Lim SS, Cho SW, Lee KS, Park J, Eum WS, Hwang HS, Choi SY. Imipramine enhances neuroprotective effect of PEP-1-Catalase against ischemic neuronal damage. BMB Rep 2011;44:647-52.
75. Yoo KY, Lee CH, Park JH, Hwang IK, Park OK, Kwon SH, Choi JH, Kim DJ, Kwon YG, Kim YM, Won MH. Antioxidant enzymes are differently changed in experimental ischemic hippocampal CA1 region following repeated restraint stress. J Neurol Sci 2011;302:33-42.
76. Kim DH, Li H, Yoo KY, Lee BH, Hwang IK, Won MH. Effects of fluoxetine on ischemic cells and expressions in BDNF and some antioxidants in the gerbil hippocampal CA1 region induced by transient ischemia. Exp Neurol 2007;204:748-58.
77. Cao DH, Xu JF, Xue RH, Zheng WF, Liu ZL. Protective effect of chronic ethyl docosahexaenoate administration on brain injury in ischemic gerbils. Pharmacol Biochem Behav 2004;79:651-9.
78. Drechsel DA, Patel M. Respiration-dependent H2O2 removal in brain mitochondria via the thioredoxin/peroxiredoxin system. J Biol Chem 2010;285:27850-8.
79. Zhang H, Go YM, Jones DP. Mitochondrial thioredoxin-2/peroxiredoxin-3 system functions in parallel with mitochondrial GSH system in protection against oxidative stress. Arch Biochem Biophys 2007;465:119-26.
80. Nordberg J, Arnér ES. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. Free Radic Biol Med 2001;31:1287-312.
81. Klein JA, Ackerman SL. Oxidative stress, cell cycle, and neurodegeneration. J Clin Invest 2003;111:785-93.
82. Hwang IK, Yoo KY, Kim DW, Lee CH, Choi JH, Kwon YG, Kim YM, Choi SY, Won MH. Changes in the expression of mitochondrial peroxiredoxin and thioredoxin in neurons and glia and their protective effects in experimental cerebral ischemic damage. Free Radic Biol Med 2010;48:1242-51.
83. Wang L, Jiang DM. Neuroprotective effect of Buyang Huanwu Decoction on spinal ischemia/reperfusion injury in rats. J Ethnopharmacol 2009;124:219-23.
84. Kong L, Zhou X, Li F, Yodoi J, McGinnis J, Cao W. Neuroprotective effect of overexpression of thioredoxin on photoreceptor degeneration in Tubby mice. Neurobiol Dis 2010;38:446-55.
85. Turoczi T, Chang VW, Engelman RM, Maulik N, Ho YS, Das DK. Thioredoxin redox signaling in the ischemic heart: an insight with transgenic mice overexpressing Trx1. J Mol Cell Cardiol 2003;35:695-704.
86. Munemasa Y, Kim SH, Ahn JH, Kwong JM, Caprioli J, Piri N. Protective effect of thioredoxins 1 and 2 in retinal ganglion cells after optic nerve transection and oxidative stress. Invest Ophthalmol Vis Sci 2008;49:3535-43.
87. Masutani H, Bai J, Kim YC, Yodoi J. Thioredoxin as a neurotrophic cofactor and an important regulator of neuroprotection. Mol Neurobiol 2004;29:229-42.
88. Mustacich D, Powis G. Thioredoxin reductase. Biochem J 2000;346 Pt 1:1-8.
89. Chae HZ, Kim HJ, Kang SW, Rhee SG. Characterization of three isoforms of mammalian peroxiredoxin that reduce peroxides in the presence of thioredoxin. Diabetes Res Clin Pract 1999;45:101-12.
90. Rhee SG, Chae HZ, Kim K. Peroxiredoxins: a historical overview and speculative preview of novel mechanisms and emerging concepts in cell signaling. Free Radic Biol Med 2005;38:1543-52.
91. Fujii J, Ikeda Y. Advances in our understanding of peroxiredoxin, a multifunctional, mammalian redox protein. Redox Rep 2002;7:123-30.
92. Chen L, Na R, Gu M, Salmon AB, Liu Y, Liang H, Qi W, Van Remmen H, Richardson A, Ran Q. Reduction of mitochondrial H2O2 by overexpressing peroxiredoxin 3 improves glucose tolerance in mice. Aging Cell 2008;7:866-78.
93. Godoy JR, Oesteritz S, Hanschmann EM, Ockenga W, Ackermann W, Lillig CH. Segment-specific overexpression of redoxins after renal ischemia and reperfusion: protective roles of glutaredoxin 2, peroxiredoxin 3, and peroxiredoxin 6. Free Radic Biol Med 2011;51:552-61.
94. Bryk R, Griffin P, Nathan C. Peroxynitrite reductase activity of bacterial peroxiredoxins. Nature 2000;407:211-5.