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Cerebral tissue oxygenation and oxidative brain injury during ischemia and reperfusion

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1. ABSTRACT

The brain requires glucose and oxygen to maintain neuronal metabolism and function. Cerebral ischemia causes heterogeneous changes in tissue oxygenation and cellular metabolism, with a region of decreased blood flow, the penumbra, surrounding a severely damaged ischemic core. Because oxygenation is central in ischemic neuronal death, it is critical to understand exactly what actual changes occur in interstitial oxygen tension (pO_2) in ischemic regions during stroke, particularly the penumbra and ischemic core. Cerebral ischemia induces a complex series of molecular pathways involving signaling mechanisms, gene transcription, and protein formation. Free radicals and oxidative stress have been suggested to be involved in each of the steps in the injury cascade. The goal of this review paper is to summarize the current literature concerning our understanding about cerebral tissue oxygenation changes after cerebral ischemia and reperfusion, the subsequent cellular and physiological changes in response to alteration in tissue oxygenation, and treatment strategies utilized to minimize the detrimental effects caused by stroke.

2. INTRODUCTION

Oxygen is critical to human brain. Although the brain is only 2% of the body's weight, it uses 20% of the oxygen supply and accounts for approximately 20% of aerobic metabolism. The importance of maintaining oxygen supply to the brain is underscored by the fact that even reductions of blood flow that affect only a small region of the brain will impair the function of that area, and can even be life threatening. If a brain does not get adequate oxygen for 3 to 5 minutes, brain cells begin to die. Cerebral ischemia, a phenomenon of reduction in cerebral blood flow (CBF), accounts for approximately 80% of all strokes (1), the third leading cause of death and the leading cause of adult disability.

The consequences of cerebral ischemia on the structure and function of the brain depend largely on the degree and duration of reduced CBF. In rodent models, a 20-30% decrease in CBF results in decreased protein synthesis. A 50% decrease in CBF results in increased lactate production and concomitant glutamate increase. When CBF reaches 20% of its normal rate, brain cells

begin to lose their ionic gradients and undergo depolarization, which coincides with irreversible neuronal damage (2). Cerebral ischemia manifests itself in two distinctly different pathological areas in the brain referred to as the ischemic core and penumbra. The ischemic core is an area that has the greatest reduction in CBF and undergoes severe irreversible damage. The penumbra is the surrounding area of the ischemic core and is characterized by reduced CBF and O_2 metabolism, but an increased O_2-extraction fraction, which reflects an attempt to maintain oxygen-dependent high energy metabolism (3-5).

After a disrupted CBF such as those in cerebral ischemia, a series of metabolic processes ensue. Due to the depletion of high-energy phosphate, cells in the ischemic area are subjected to waves of anoxic depolarization with hypoxic injury. Restoration of cerebral blood flow with reoxygenation stimulates expression of adhesion molecules and chemokines (6, 7), resulting in an inflammatory reaction that involves recruitment of polymorphonuclear leukocytes, microvessel endothelial damage, hypoperfusion, and the "no-reflow" phenomena, as well as apoptosis.

Despite the considerable amount of information available so far, the mechanism of ischemic injury remains largely unknown. Free radical generation accounts for 3-4% of cellular oxygen metabolism. It is known that changes in tissues oxygen supplies and cellular metabolism causes abnormal free radical metabolism. Many of the cellular responses to cerebral ischemia have been linked to free radical intermediates. Free radicals play a significant role in cell signaling and the induction and activation of multiple genes. For example, there is growing evidence that free radicals influence the action of proteases at multiple levels, including transcription and processing of mRNA and activation of latent proteases, induction of hypoxia inducible factor 1, and caspase-involved apoptosis.

Oxygen and glucose supply are critical in maintaining neuronal metabolism and function. In order to better understand the mechanism of cerebral ischemic injury and to design reliable pharmacological regimens with the goal of the reduction or elimination of brain infarction, fundamental issues such as tissue oxygenation and cellular responses to the oxygen changes need to be addressed. In this review, we will focus on our current understanding concerning changes of oxygenation in the ischemic brain and the subsequent cellular and physiological changes (especially the involvement of free radicals), that occur in response to the changes in tissue oxygenation and to related treatments.

3. CHANGES OF TISSUE OXYGEN AFTER CEREBRAL ISCHEMIA AND REPERFUSION

Although the cerebral oxygen level is a critical issue in cerebral ischemia, monitoring oxygen levels in cerebral tissue *in vivo* and in real-time remains very challenging technically, especially in deeper tissue when repetitive measurements are needed. Several techniques are available that can be used to measure tissue oxygen, including Clark-type electrodes (8), fluorescence quenching (9, 10), phosphorescence quenching (11), near infrared spectroscopy (12), MRI (13), and electron paramagnetic resonance (EPR) oximetry (14). However, each of these techniques has its own particular advantages and limitations. For example, Clark-type electrode techniques cause traumatic lesions that do not heal when the electrode is inserted in to the brain. Optical measuring techniques for brain tissue pO_2 have the limitation of measurement depth. Although they hold some promise, fiberoptic technologies are not generally suitable for deep tissue measurement.

EPR oximetry is a well-established technique that has been used to measure interstitial pO_2 in living animals in a variety of organs/tissues, including brain, heart, liver, kidney, and tumor (15-23). It has several advantages (especially for the repetitive and highly accurate measurement of localized interstitial pO_2) over the above-mentioned techniques. EPR oximetry with the particulate probe LiPc implanted in the brain is ideal for directly following the changes in pO_2 in brain tissue during cerebral ischemia and reperfusion, as well as for monitoring the effect on tissue pO_2 by various treatments, such as hyperoxia. A detailed description of EPR oximetry is available in the literature (16). Briefly, EPR oximetry is based on the principle that the interaction with molecular oxygen can change the EPR spectrum of certain stable paramagnetic materials, e.g., the broadening of EPR linewidth, and that this oxygen-dependent change can be calibrated and used to measure tissue oxygen levels quantitatively (16, 20). The EPR oximetry measurement itself is non-invasive, similar to *in vivo* NMR spectroscopy. This implantation procedure is well characterized, and importantly, is known not to result in any localized long- or short-term changes in structure or function at the implantation site (16, 20). These recent developments have established EPR oximetry as a versatile technique for measurement of tissue oxygen *in vivo*, sensitively, repetitively, and reproducibly. In addition, EPR oximetry presents the possibility of three-dimensional pO_2 maps (24). For oxygen mapping of brain, a soluble imaging agent is administered to the animal, and a three-dimensional spectral–spatial EPR image is obtained. If the agent is administrated before the onset of cerebral ischemia, typically achieved through the middle cerebral artery occlusion (MCAO), it will stay in the whole brain, including the would-be ischemic region and reflect oxygen fluctuation there when MCAO occurs. Oxygen mapping is achieved through the conversion of the signal line-width to a pO_2 value at each single pixel (14).

Focal cerebral ischemia causes heterogeneous changes in tissue oxygenation, with a region of decreased blood flow, the penumbra, surrounding a severely damaged ischemic core. In ischemia, the decrease in tissue partial pressure of oxygen (pO_2) in the occluded vascular territory is most likely due to a combination of reduced O_2-delivery and an enhancement of O_2-extraction. Experimental efforts are hampered by the inherent difficulty of measuring cerebral blood flow (CBF) and O_2-delivery to the tissue at the microvascular level. By using the technique of EPR oximetry to non-invasively measure tissue pO_2 in brain

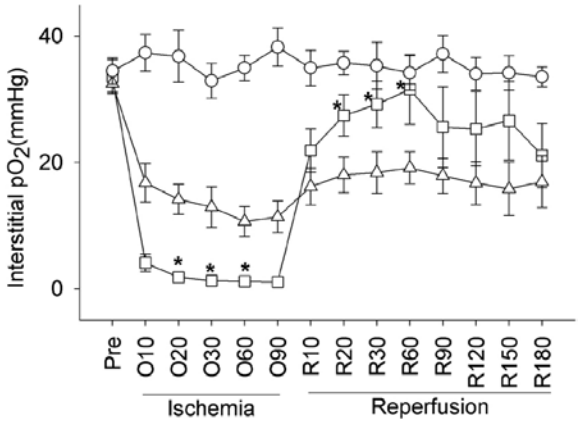

Figure 1. Interstitial pO_2 levels at different positions during cerebral ischemia and reperfusion. *Pre*, pre-ischemia; *O10-O90*, 10 to 90 minutes after MCAO; *R10-R180*, 10 to 180 minutes after reperfusion. (O), contralateral side; (Δ), penumbra; (□), core. Asterisk indicates significant ($P < 0.05$) difference when compared with penumbra. Reproduced with permission from 15.

with transient focal ischemia, we have measured both absolute values, and temporal changes of pO_2 in ischemic penumbra and core during ischemia and reperfusion in a rat model (Figure 1) (15). Pre-ischemic pO_2 values in the core and penumbra of the anesthetized rats were 33.4 mmHg. Interstitial pO_2 in the penumbra and core are differentially affected during ischemia and reperfusion. Ischemia rapidly decreased interstitial pO_2 to 32% and 4% of pre-ischemic values in penumbra and core, respectively, 1 hour after ischemia. The interstitial pO_2 values in the penumbra were significantly higher than the corresponding values in the core. Importantly, whilst reperfusion restored core pO_2 close to its pre-ischemic value, penumbral pO_2 only partially recovered. In contrast to the values in the occluded hemisphere, pO_2 values in the contralateral hemisphere remained stable during the entire experiment. Furthermore, it was shown that normobaric hyperoxia treatment could effectively increase the pO_2 in the penumbra during the ischemic phase. Throughout the ischemic period, no change of pO_2 in the core was observed with hyperoxia (70% oxygen). These divergent, important changes in pO_2 in the penumbra and core were explained by combined differences in cellular oxygen consumption rates and microcirculation conditions. These results demonstrate that interstitial pO_2 in the penumbra and core is differentially affected during ischemia and reperfusion, providing new insights into the pathophysiology of stroke. In addition, these studies show that EPR oximetry can make accurate and repeated measurements of cerebral pO_2 during cerebral ischemia, providing an important tool that can be used to study the role of oxygen in the pathophysiology of cerebral ischemia.

4. MOLECULAR, CELLULAR, AND PHYSIOLOGICAL RESPONSE TO CHANGES IN TISSUE OXYGENATION

Disruption of oxygen and glucose supply results in abnormal cellular metabolisms and various molecular, cellular, and physiological responses in cells in affected brain regions. Experimental evidence has suggested that abnormal free radical metabolism contributes, at least in part, to the damage that occurs after brain ischemia (25). Free radical consists of two categories: reactive oxygen species (ROS) and reactive nitrogen species (RNS). One of the major RNS in cerebral ischemia is nitric oxide (NO), a water and lipid soluble free radical, by the action of nitric oxide synthases (NOS). There are three isoforms of NOS in brain cells, neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS). Neurons produce NO mostly from nNOS activation. Glial cells generate NO mainly from iNOS activation. Endothelial cells produce NO by activation of eNOS (26). NO is a double-edge sword in cerebral ischemia and reperfusion: it can be both protective and deleterious, depending on where and when it is generated. Immediately after brain ischemia, NO release from eNOS is protective mainly by promoting vasodilation. However, NO produced by nNOS and iNOS after ischemia may causes brain damage (see review (27) for detail). One of the main ROS is superoxide radical anion, which can be generated through the action of NOS, xanthine oxidase, leakage from the mitochondrial electron transport chain, and other mechanisms. Nitric oxide and superoxide radical anions are themselves reactive, but can also combine to form a highly toxic anion, peroxynitrite.

Besides the direct increases of free radical generation in cerebral ischemia/reperfusion (25, 28, 29), there are two other factors that can contribute to the increase. First, cerebral ischemia changes the activities of antioxidant enzymes such as SOD, catalase, and glutathione peroxidase and the levels of small antioxidant molecules such as GSH and vitamin C (30-38). Second, cerebral ischemia changes the expression of redox-regulated genes such as SOD, thioredoxin, and redox-factor 1 (39-41). Importantly, the physiological mechanisms that generate free radical and alter antioxidant and redox protein levels do not occur homogeneously in ischemic tissue, because the central region (ischemic core) has little or no blood flow, whereas the peripheral region (ischemic penumbra) experiences a restricted blood flow during which partial energy metabolism continues (28). These heterogeneous changes of free radical generation and antioxidant levels indicate different redox statuses in different ischemic regions. For example, ROS production in the ischemic core was significantly enhanced during both ischemia and reperfusion, whereas in the striatal penumbra ROS levels remained low during ischemia (42). In addition, increased free radical generation occurs not only during the reperfusion phase because of a sudden increase in oxygen levels (43, 44), but also during the ischemic phase as well (42).

The toxicity of the abnormal free radical metabolism in cerebral ischemia/reperfusion results from their modification of macromolecules, from their effect on signal transduction pathways, and from the resulting induction of apoptotic and necrotic pathways. Their involvement in blood brain barrier (BBB) disruption, apoptosis, and cellular response to hypoxia are discussed as follows.

Free radicals have been implicated in BBB disruption during stroke, as demonstrated by the fact that mice lacking copper/zinc-superoxide dismutase are highly susceptible to focal cerebral ischemia–reperfusion, with exacerbated vasogenic edema and a higher mortality rate than in wild-type animals (45). More recently, free radicals have been shown to mediate BBB disruption through metalloproteinase activation in experimental ischemic stroke (46). Spin trapping agents reduce infarct size in ischemia and also reduce the incidence of hemorrhage (47). Polynitroxylated human serum albumin has been shown to reduce infarct size in the rat brain (48). A NO scavenger has been shown to reduce the disruption of BBB and matrix metalloproteinase (MMP) -9 expression (49). nNOS is associated with MMP-9 activation in the ischemic cortex after MCAO (50). These results strongly suggest a role for free radicals in the activation of MMPs during cerebral ischemia and reperfusion.

Mitochondrial alterations are critical in the cascade of oxidative cell death in cerebral ischemia. The mitochondria are the primary intracellular source of ROS. Thus, mitochondria are both the initiator and the first target of oxidative stress. Mitochondrial damage can lead to cell death, given the role for mitochondria in energy metabolism and calcium homeostasis, as well as the ability of mitochondria to release pro-apoptotic factors such as cytochrome C and apoptosis-inducing factor (AIF) (51). Mitochondria are very sensitive to ischemia. Damage and dysfunction can happen even after periods of moderately reduced cerebral blood flow without immediate changes in levels of ATP or phosphocreatine (52, 53). The reduced blood supply in the penumbra can result in mitochondrial alterations even during relatively normal energy states. Mitochondrial injury can also be triggered by reperfusion (also termed reperfusion-dependent alterations) and delayed or secondary mitochondria dysfunction. Apoptosis-related alterations in mitochondrial function, including membrane depolarization and release of cytochrome C, have been documented in neurons after focal cerebral ischemia (54-56). Mitochondria alterations play an important role in activations of caspases.

It has become clear that caspases play an important role in cell death after cerebral ischemia. Activation of caspases has been shown in animal ischemic models (57, 58). In global ischemia models, expression of caspase-3 mRNA was up-regulated (59). Activation of caspase-3 protein was detected in neurons (57, 60, 61). It has been reported that a time-dependent evolution of focal ischemic injury was characterized by the close correspondence between caspase-like enzyme activation and an associated increase in immunoreactive caspase cleavage products beginning 1 to 2 hours after severe ischemia and 9 to 12 hours after mild ischemia, followed several hours later by DNA laddering and morphological features of apoptosis (60, 62). Furthermore, studies using caspase inhibitors and genetically modified mice demonstrated that caspases are one of the major factors inducing cell death in cerebral ischemia (57, 63, 64). Substantial evidence has shown that ROS cause apoptosis through activation of caspases. ROS can induce a series of specific cell death signaling events, including release of cytochrome C into the cytosol, activation of caspase-9 and caspase-3, proteolytic cleavage of PKCδ, and nuclear DNA breakdown (65, 66). For ROS-mediated apoptotic mechanisms, caspase-3-dependent proteolytic cleavage of PKCδ plays a critical role in neurons (67). The roles of ROS in neuronal death during ischemia through the activation of caspases provide a potential approach to minimize the ischemic injury in brain. However, the complexity of free radical metabolism and our limited understanding of the complexity have hindered the progress of stroke therapy.

Poly(ADP-ribose) polymerase (PARP)-1 is a DNA nick sensor that transforms ADP-ribose from NAD+ in the form of polymer to over 40 nuclear proteins. PARP-1 activated by DNA breaks facilitates transcription, replication, and DNA base excision repair. Experimental evidence supports that PARP-1 activation follows DNA damage induced by ROS and RNS (68). Yu et al. reported that PARP-1 activation signals AIF release from mitochondria, resulting in a caspase-independent pathway of programmed cell death (69). Excessive activation of PARP causes depletion of nicotinamide-adenine dinucleotide and adenosine triphosphate, which ultimately leads to cellular energy failure and death. PARP-1 hyperactivity is causative in post-ischemic brain damage (70, 71). It has been found that PARP-1 activation is reduced in the ischemic brain of mice with nNOS deficiency or inhibition (60, 72). In addition, PARP-1 is a key regulator of numerous transcription factors including NF-kB (73, 74), AP1 (75, 76) and p53 (77, 78). The PARP-1 through interaction with NF-kB, p53, and other transcription factors might significantly modulate cell survival and death (79). Researches aimed at identifying mechanisms of neuroprotection by inhibition of poly(ADP-ribosyl)ation may discover novel players involved in post-ischemic brain damage and provide innovative targets of therapeutic relevance to treatment of cerebral ischemia.

Another area of our limited understanding is how brain cells response to ischemia. Ischemia results in not only hypoxia but also disrupted nutrient supply. Extensive research in cancer in the last fifteen years has proven that hypoxia inducible factor -1 (HIF-1) is a key regulator in cell's response to hypoxia. HIF-1 is a heterodimer of HIF-1α and HIF-1β subunits containing basic helix-loop-helix PAS domains (80, 81). HIF-1α is a unique subunit tightly regulated in response to hypoxia (80, 82), whereas HIF-1β is constitutively expressed in cells and is not affected by hypoxia (82, 83). The expression of HIF-1 subsequently regulates genes that generally increase blood flow, glucose delivery, and maintenance of energy after hypoxia (84-88). Although the exact mechanism of HIF-1 stabilization in hypoxia is not known, free radial and cellular redox status has been implied in the stabilizing process.

Studies have provided evidence that HIF-1 is induced in cerebral ischemia. In 1999, Bergeron et al. first reported that after focal ischemia in adult rat brain, mRNA encoding HIF-1α was up-regulated in the peri-infarct penumbra. This up-regulation was observed by 7.5 h after

the onset of ischemia and increased further at 19 and 24 h (89). HIF-1α was expressed in the rat brainstem in vivo under physiological hypoxia (90). Another study showed that HIF-1α was maximally expressed after 5 h of continuous hypoxia and declines to basal levels by 12 h (91). HIF-1 expression was also shown to increase after hypoxic and $CoCl_2$ preconditioning in newborn rat brain (92). In an OGD model, the activation of HIF-1 DNA binding was reported in primary cultured neurons (93). The above results show that HIF-1 is indeed induced in neurons under hypoxia.

Generally, there are two main downstream effects resulting from the induction of HIF-1. First, genes induced by HIF-1 and other hypoxia responsive transcription factors generally tend to increase blood flow, glucose delivery, and maintenance of energy after chronic hypoxia. In relation to this aspect of the activity of HIF-1, increased glucose transporter expression or erythropoietin expression might subsequently protect the brain. For example, vascular endothelial growth factor (VEGF) is a potential HIF-1 target gene that is induced by focal ischemia. Induction of VEGF could mediate the formation of new vessels, and thus protect brain tissue. It was reported that increased expression of HIF-1 target genes as a result of HIF-1 activation by hypoxia might contribute to tissue viability in the hypoxic/ischemic penumbra by increasing glucose transport and glycolysis (89). Nevertheless, HIF-1 may have a very different role in cells and tissues under hypoxia. First, increased nitric oxide from inducible NOS (94), dopamine from tyrosine hydroxylase (95), and lactate from lactate dehydrogenase (96) may worsen ischemia. Second, many research groups have reported that HIF-1 mediates apoptosis during hypoxia in various cells, although similar experiments have not been done with neurons. HIF-1-induced apoptosis has been reported in embryonic stem cells (ES) and MCF-7 cells (97). These studies indicate that in response to hypoxia, HIF-1α accumulates and then directly associates with and stabilizes the active wild-type p53. It is therefore conceivable that this increase in p53 protein is in fact responsible for the apoptosis reported in hypoxia ES cells. A significant correlation between HIF-1 expression, apoptosis, and the pro-apoptotic factors caspase-3, Fas, and Fas ligand was observed in human lung cancer (98). In a traumatic brain injury model, Yu et al. showed that HIF-1 could prompt apoptotic cell death after experimental traumatic brain injury (48). HIF-1 signaling in ischemic primary cortical neurons elicits delayed death involving the participation of p53 (99). From experimental evidence gathered over the past several years, it is clear that HIF-1 is involved in *both* cellular survival and death in cerebral ischemia. However, the exact mechanism and the particular roles that HIF-1 induction plays in these processes still remains to be determined for cerebral ischemia.

5. HYPEROXIA TREATMENT AND ANTIOXIDANTS AS THERAPY FOR STROKE

Since lack of oxygen and increased ROS are involved in the mechanism of brain injury after ischemia, oxygen and antioxidant therapy are potentially useful approaches. Oxygen therapy aimed at increasing tissue pO_2 has been used as a potential treatment modality for ischemic stroke (100). There are two different types of oxygen therapy, namely, hyperbaric hyperoxia and normobaric hyperoxia. In ischemic stroke, hyperbaric oxygen therapy has been proven successful in several animal and human stroke studies (100-103), but some other studies have failed to show benefits (104-106). The diversity of outcomes in regard to the therapeutic effects of oxygen therapy may result from differing experimental designs including differences in oxygen dosage (107), which may have resulted in a great variety of tissue oxygen levels. Several recent animal studies have shown that short duration normobaric hyperoxic treatment can be highly neuroprotective and does not increase oxidative stress if started early after stroke onset (15, 108, 109), although the neuroprotective effect was not observed in permanent cerebral ischemia (110). On the other hand, oxygen therapy in animals has been known to produce tissue damage, with toxicity increasing along with the increase in oxygen concentration and exposure pressure. End-organ damage from hyperoxia depends on both the concentration of oxygen administered and the oxygen pressure during exposure (111).

Unfortunately, due to technical challenges, few, if any, trials of oxygen therapy were conducted while monitoring the actual interstitial pO_2 level. Therefore, it is not known whether the oxygen therapy actually increased the interstitial pO_2 in the target tissue or not. If the therapy does not substantially increase pO_2 in the ischemic tissue, the beneficial effect of oxygen therapy cannot be achieved. On the other hand, if the therapy increases tissue pO_2 well above the physiological value, tissue damage may be the outcome of the therapy, possibly through increased oxidative stress. Using *in vivo* EPR oximetry to measure localized interstitial pO_2, we measured both absolute values, and temporal changes of pO_2 in the ischemic penumbra in a rat model of 90-min transient ischemia while normobaric hyperoxia was given during ischemia or reperfusion (Figure 2) (15). Our results showed that penumbral pO_2 level could be modulated by changing the percentage of oxygen content in the breathing gas, and that 95% O_2 given to rats was able to raise penumbral interstitial pO_2 close to the physiological (pre-ischemic) value during ischemia. However, 95% O_2 also caused an increase in penumbra pO_2 to a level that was twice as high as the pre-ischemic level when given during reperfusion. Oxygen therapy, which began immediately after ischemia and continued for 90-min, significantly reduced infarction volume by 40%. Neurological function improved by 1 point in an 8-point scale. Oxygen therapy given upon reperfusion also reduced infarction volume by 15%, but it was not statistically significant. This improved penumbral oxygenation status leads to reduced ROS production, decreased expression of MMP-9, and decreased cleavage of caspase-8 in the penumbra (112). These results demonstrate that maintaining penumbral oxygenation by normobaric oxygen treatment during ischemia is a potentially promising strategy for the treatment of ischemic stroke.

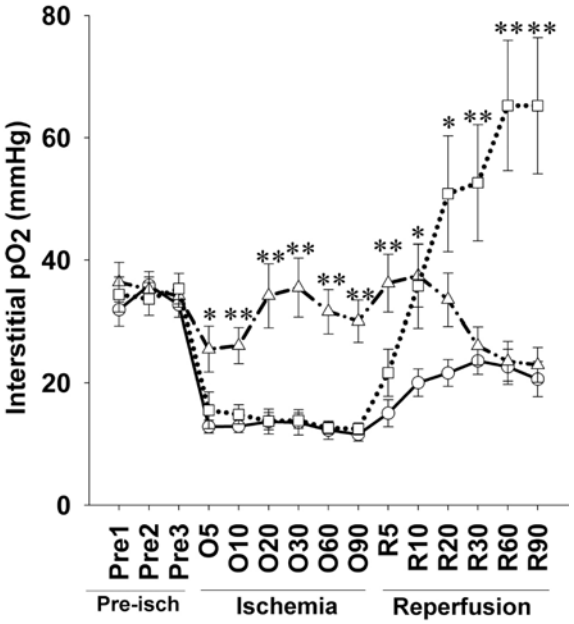

Figure 2. Penumbral interstitial pO_2 level in normoxia and hyperoxia rats during cerebral ischemia and reperfusion. Circle (o), group of normoxia. Triangle (Δ), group of normobaric hyperoxia treatment during 90-min ischemia. Square (□), group of normobaric hyperoxia treatment during 90-min reperfusion. Pre1-Pre3, three pre-ischemic measurement points. O5-O90, 5 to 90 min after occlusion of middle cerebral artery. R5-R90, 5-90 min after reperfusion. Single asterisk indicates significant difference ($p<0.05$) when compared with normoxia group. Double asterisks indicate significant difference ($p<0.05$) when compared with the other two groups. Data is expressed as Mean ± SD, n = 11 in each group. Reproduced with permission from 112

The important role of free radicals in cerebral damage associated with stroke is underscored by the fact that even delayed treatment with free radical scavengers can be effective in experimental focal cerebral ischemia. Several different reactive species have been shown to be generated after cerebral ischemia in animal stroke models, including superoxide, hydroxyl radicals, nitric oxide, and peroxynitrite. A variety of antioxidants and scavengers of free radicals have been tested, and many have shown neuroprotective effects (25, 113-116). Superoxide dismutase conjugated to polyethylene glycol reduces infarct size in permanent focal ischemia (21, 117). Spin trapping agents have been shown to reduce neuronal damage (118-120). In addition, the overproduction of radical-scavenging enzymes protects against stroke, and animals that lack radical-scavenging enzymes are more susceptible to cerebral ischemic damage (121). Most recently, it was reported that antioxidant-rich diets reduce brain damage from stroke(122). These results suggest that reducing free radical level is effective in protecting brain from ischemic injury. However, the mechanism of the neuroprotective action of antioxidants is not clear. Do antioxidants exert neuroprotection through inhibiting caspase activity, inhibiting MMP activity, inhibiting HIF-1 function or enhancing HIF-1 function? Further research is required to provide a clear answer to these questions.

6. PERSPECTIVE

Based on the wealth of evidence that has accumulated over the past decade, it is clear that free radicals, and the resulting oxidative stress, are involved in cell death and brain injury after stroke. So far, experimental results from manipulating antioxidants and free radical production as potential therapeutic tools seem quite promising. Although both tissue oxygenation and free radicals have been recognized as being integral in the pathophysiology of ischemic/reperfusion injury, many important and fundamental questions remain unanswered. For example, cerebral ischemia and reperfusion causes acute changes in tissue pO_2, and dramatically increases free radical generation. However, we still do not clearly know what the temporal and spatial distribution of free radical generation is, and whether there is a direct association between tissue pO_2 levels, free radical generation, MMP induction, caspase activation, and HIF-1 induction. Therefore, the challenge that remains is to expand upon our understanding of the mechanisms of free radical induced brain injury, thereby taking advantage of the opportunity to develop and design increasingly effective stroke-treatment drugs that are based on a more complete understanding of the mechanisms involved.

7. ACKNOWLEDGMENT

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