Oxygen radical mechanisms of brain injury following ischemia and reperfusion

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TRAYSTMAN, RICHARD J., JEFFREY R. KIRSCH, AND RAYMOND C. KOEHLER. Oxygen radical mechanisms of brain injury following ischemia and reperfusion. J. Appl. Physiol. 71(4): 1185-1195, 1991.—This review addresses current understanding of oxygen radical mechanisms as they relate to the brain during ischemia and reperfusion. The mechanism for radical production remains speculative in large part because of the difficulty of measuring radical species in vivo. Breakdown of lipid membranes during ischemia leads to accumulation of free fatty acids. Decreased energy stores during ischemia result in the accumulation of adenine nucleotides. During reperfusion, metabolism of free fatty acids via the cyclooxygenase pathway and metabolism of adenine nucleotides via the xanthine oxidase pathway are the most likely sources of oxygen radicals. Although leukocytes have been found to accumulate in some models of ischemia and reperfusion, their mechanistic role remains in question. Therapeutic strategies aimed at decreasing brain injury have included administration of radical scavengers at the time of reperfusion. Efficacy of traditional oxygen radical scavengers such as superoxide dismutase and catalase may be limited by their inability to cross the blood-brain barrier. Lipid-soluble antioxidants appear more efficacious because of their ability to cross the blood-brain barrier and because of their presence in membrane structures where peroxidative reactions can be halted.

cerebral ischemia; superoxide; oxygen radical scavengers; cerebral resuscitation; mechanisms of cerebral ischemic damage; lipid peroxidation

THE OVERALL MORTALITY and morbidity in patients who have been “resuscitated” from cardiac arrest or stroke are contingent on reducing brain damage. Although significant brain damage occurs during an ischemic episode (20), new cerebral damage can occur after the restitution of blood flow (81, 114). Treatment designed to improve recovery from cerebral ischemia is usually possible only after the onset of ischemia or after reperfusion. One proposed mechanism for brain damage that occurs during reperfusion involves generation of oxygen radicals. Radicals are molecules containing an unpaired electron in their outer orbital ring. The concept that oxygen radicals may be important mediators of brain damage related to ischemia and reperfusion is not new (39); yet validation has been difficult, and questions regarding mechanism remain unanswered. This review attempts to synthesize the considerable body of literature that has appeared over the last several years on brain reperfusion injury and to provide a critical assessment of the methodological limitations in delineating radical mechanisms of injury in vivo.

Models of Ischemia and Reperfusion

It is clear from evaluating different animal models of ischemia and reperfusion that a number of factors are important in determining the extent of neurological damage that occurs as a direct result of ischemia. These include but are not limited to the amount of blood flow reduction, the duration of time that flow is reduced, the regional location of the flow reduction, and the metabolic state of the brain before the ischemic period. Multiple mechanisms may contribute to the injury, and the relative contribution of each mechanism depends on the specific situation. For example, calcium-induced damage from the release of excitatory neurotransmitters is gen-
eraly most prominent with moderate reductions of blood flow in regions with high N-methyl-d-aspartate (NMDA) receptors (98) and high excitatory amino acid innervation. With severe or complete reduction of blood flow, calcium entry through non-NMDA receptor-operated channels and voltage-sensitive channels becomes prominent (127). With reduced blood flow over prolonged periods, continued anaerobic glycolysis enhances the contribution of acidosis to ischemic injury (117). Therefore, when different potential mechanisms of ischemia and reperfusion injury in brain are evaluated, it is important to recognize that the mechanism of injury will depend on the degree, duration, and localization of reduced blood flow and other aspects of the experimental model.

Furthermore the issue of radical-mediated injury is complicated because the various potential mechanisms are interrelated with radical injury. For example, as discussed more fully below, acidosis can potentiate lipid peroxidation (127); calcium accumulation stimulates phospholipase activity, which may generate radicals via arachidonic acid metabolism; and NMDA receptor activation generates nitric oxide (19, 47), which is capable of reacting with superoxide to form other cytotoxic oxidants (11). Thus it is an oversimplification to consider an array of parallel pathways that can be independently blocked for assessing the individual contribution of these purported mechanisms of injury in brain.

Detection of Radical Production

Detection of radical production has been difficult because of the short half-lives of radicals. Several investigators have administered radical scavengers and extrapolated the presence of neurological protection afforded by these compounds as a measure of radical production. The details of these experiments are described below (see Therapeutic Efficacy of Radical Scavengers).

Other investigators have extrapolated changes that occur in endogenous radical scavengers as a measure of radical production. α-Tocopherol, ascorbic acid, and reduced ubiquinones are endogenous substances that are thought to play important roles as chain breakers in radical reactions in brain. During ischemia there is a reduced concentration of ascorbic acid in brain parenchyma (39). However, this decrease may be attributable to a decrease in NADH-dependent recycling of ascorbic acid rather than increased utilization by radical processes. During reperfusion, changes in lipid-soluble endogenous antioxidants (e.g., α-tocopherol and reduced ubiquinones) (149) but not in water-soluble antioxidants (e.g., ascorbate) (33) are consistent with lipid peroxidation as an important mechanism of radical damage during reperfusion.

More recently, several investigators have attempted to measure production of radicals more directly. Four different techniques have been described for measurement of radicals in brain: nitroblue tetrazolium (NBT) reduction (5, 79), chemiluminescence (61), electron spin resonance (ESR) spectroscopy (69, 83, 132), and salicylate trapping (24).

With the NBT technique, the yellow soluble dye is placed on the surface of the brain via a cranial window. Superoxide produced by the brain exits cells via anion channels and reacts with the NBT (79). In the presence of reducing agents including superoxide, NBT turns from yellow to blue and precipitates. The precipitated blue formazin is measured in vitro with a spectrophotometer. The amount of NBT reduction that can be attributed to the production of superoxide is determined by measuring the difference in NBT reduction between one window containing NBT alone and another contralateral window containing NBT and superoxide dismutase (SOD). It is important that both windows be constructed in the same animal because of the considerable variability among animals in ability to reduce NBT. The limitations of this technique are that it is semiquantitative, only the superoxide species is measured, and only extracellular levels are detected.

Chemiluminescence techniques depend on capturing photons of light that are released during radical reactions. In vivo chemiluminescence techniques are difficult in brain because capturing emitted photons is hindered by bone, dura, arachnoid, and pia, which separate brain tissue from the atmosphere. However, in vitro chemiluminescence techniques have been useful to detect radical production in brain homogenate exposed to hypoxia and reoxygenation (61). The advantage of this technique is that it provides the time course of radical generation. The limitations of the technique for use in vivo are low sensitivity even with the use of lucigenin or luminol to amplify photon generation and lack of specificity for radical generation.

ESR techniques utilize compounds called spin traps to stabilize more reactive radicals after they are formed. For in vivo use, spin trap compounds need to be administered systemically or into the cerebrospinal fluid so that enough radical adduct can accumulate in quantities that can be extracted and measured. α-Phenyl 1 butyl nitrone is a lipid-soluble spin trap compound that has been used to measure lipid-soluble radical adducts in brain (83). There are several disadvantages of using spin-trapping techniques for measurement of oxygen radical production in brain. For example, most superoxide spin adducts are relatively unstable and decay rapidly to nonradical species, and the rate constants for the spin trapping of superoxide are usually low ($k_r \approx 10^{-4} M^{-1} s^{-1}$). To counter this low rate constant, relatively high concentrations of spin traps must be given, which may cause systemic toxicity. Use of spin trap compounds in vivo is also hampered by the finding that blood itself may cause production of radicals unrelated to ischemia and reperfusion (97). In addition, attempts to measure radical production in tissues like brain require homogenization or sonication, either of which will artifactually result in production of radical species. Although ESR offers the ability to differentiate oxygen-, carbon-, and nitrogen-based radicals, the technique presently is not sufficiently sensitive for quantitative analysis.

Others have used salicylates as an in vivo hydroxyl radical trap (24). Upon scavenging hydroxyl radical, salicylate forms the stable adducts 2,5- and 2,3 dihydroxy benzoic acid (DHBA) by a hydroxylation reaction that can be quantified by high-performance liquid chromatog-
raphy (41, 42). After salicylate administration, DHBA levels in brain are unchanged by ischemia alone but are increased substantially by combination of ischemia and reperfusion (24). A potential disadvantage to this technique is that salicylates may have other effects (e.g., on platelet function) that could independently alter responses to ischemia and reperfusion.

**Potential Mechanisms for Production of Radicals During Ischemia**

**Electron transport chain.** During normal mitochondrial respiration, cytochrome c is involved in a four-electron transfer to reduce oxygen to water without production of oxygen radicals. During electron transport, the presence of oxygen at the terminus of the chain favors the maintenance of the members of the carrier system in an oxidized state. During ischemia, when oxygen supply is limited, the electron transport chain of the inner mitochondrial membrane becomes highly reduced. In this reduced state, when oxygen availability is decreased by ischemia, oxygen radical production may result. Studies in brain implicate the ubiquinone-cytochrome b region as the major site of oxygen radical production when mitochondria are in a maximally reduced state (31).

**Potential Mechanisms for Radical Production During Reperfusion**

**Free fatty acid metabolites.** Severe cerebral ischemia is associated with failure of ATP dependent ionic pumps, which results in the rapid loss of cellular potassium and influx of sodium, chloride, and calcium (126). One of the postulated consequences of calcium influx during ischemia is in initiating pathways involved in breakdown of lipid membrane constituents and accumulation of free fatty acids. In particular, with the onset of ischemia, there is a decrease in brain content of phosphatidylethanolamine (151). The exact mechanism for breakdown of lipid membranes is controversial. In an ATP-poor environment, calcium influx may be responsible for activation of phospholipase C with breakdown of phospholipids in the cell membrane and liberation of free fatty acids (130, 146). Alternatively, ischemia may induce an adenosine 3',5'-cyclic monophosphate- (cAMP)- dependent activation of phospholipase A (37). cAMP is increased by release of potassium, adenosine, and catecholamines, all of which increase with ischemia (10, 22, 72).

Ischemia leads to a rapid (~30 s) increase in free fatty acids (9, 113), and the increase in arachidonic acid, in particular, directly correlates with the duration of ischemia (125). Arachidonic acid accumulation is higher in brain regions that are more prone to damage after ischemia and reperfusion (141). Free fatty acids have also been correlated with direct tissue damage. Arachidonic acid readily intercalates into membranes and produces changes in packing of lipid molecules (70, 71, 136). Arachidonic acid itself produces cerebral edema (27, 28). This edema may be due to a direct result of arachidonic acid inhibition of Na⁺-K⁺-adenosinetriphosphatase. This inhibition has been observed in brain tissue (17) but not in microvessels (74). In addition, high concentrations of arachidonic acid cause release of neurotransmitters from neurons and reduction of reuptake mechanisms for these toxic neurotransmitters (29, 153). Pharmacological agents (102) and hypothermia (101), which have been found to be protective in brain exposed to ischemia and reperfusion, have also been shown to decrease brain free fatty acid accumulation during ischemia. The mechanism for decreased free fatty acid accumulation has been hypothesized to be related to inhibition of calcium entry into ischemic cells (143). For example, pentobarbital, which decreases ischemia-induced free fatty acid accumulation (102), has also been found to be a weak calcium entry blocker in arterioles (4) and neurons (16, 38).

Successful resuscitation from ischemia allows for return of oxygen to the brain, which, although necessary for neurological recovery, may lead to additional neurological injury. During recirculation there is rapid utilization of free fatty acids, in particular arachidonic acid (151). Arachidonic acid accumulated during ischemia is metabolized upon reperfusion via the lipoxygenase and cyclooxygenase pathways to produce prostaglandins, thromboxanes, (48, 66), and superoxide (5). The prostaglandins may be responsible for vascular sludging and altered vascular reactivity (80, 134). Oxygen radicals may cause brain injury (35) directly by lipid peroxidation (151) and indirectly by vascular paralysis (140). Pretreatment with cyclooxygenase inhibitors can block the postischemic accumulation of arachidonic acid metabolites (48), superoxide generation (5), the delayed decrease in cerebral blood flow (57), and neurological deterioration (28).

Superoxide has also been detected on the brain surface with the NBT technique after concussive head injury (77) and with seizures (6). Prevention of the systemic hypertensive response or inhibition of cyclooxygenase activity blocked superoxide appearance (78). By analogy to ischemia-reperfusion, with rapid establishment of normal cerebral perfusion pressure after ischemia, the cerebral microcirculation presumably experiences rapid increase in transmural pressure because large arterioles are dilated. Thus it is important to consider that, in addition to cyclooxygenase-dependent superoxide generation on the cortical surface during reperfusion (5), oxygen radical production may also be related to the increase in pial arteriolar pressure, which occurs with restitution of blood flow.

**Purine metabolites.** Toxic oxygen metabolites may also be produced during reperfusion through a mechanism that depends on the degradation of ATP. With the onset of ischemia, brain adenine nucleotides are metabolized to nucleosides and purine bases. Therefore, cerebral ischemia has been associated with a rapid rise in interstitial concentration of adenosine (137) and hypoxanthine (103). Tissue adenosine concentration increases to >200% of control by 5 s of ischemia and reaches a plateau at ~500% of control by 60 s of ischemia (147). The increase in brain adenosine makes it available for further metabolism to inosine, hypoxanthine, and ultimately as a substrate for the xanthine oxidase pathway. In the presence of oxygen, as occurs with recirculation, metabolism via the xanthine oxidase pathway results in production of oxygen radicals. In order for this pathway to be considered as an important source of oxygen radicals, the brain...
must have adequate xanthine oxidase activity during reperfusion. Under normal circumstances, whole brain has a low xanthine oxidase activity (3). However, with ischemia and reperfusion there is an increase in the conversion of xanthine dehydrogenase to xanthine oxidase (12, 68) by calcium-activated proteases (93). In addition, xanthine oxidase/dehydrogenase activity found in brain microvessels is approximately fourfold higher than that found in cerebral cortex (15). Thus the xanthine oxidase pathway may be important in endothelial cells for superoxide production during reperfusion.

Aldehyde oxidase is an enzyme that closely resembles xanthine oxidase; both enzymes contain molybdenum, iron, and flavin adenine dinucleotide (FAD) and have oxygen reduction sites that are very similar (115). Although aldehyde oxidase has been located in brain (59), it is not known whether the distribution or activity of aldehyde oxidase in brain changes in response to transient cerebral ischemia or whether it contributes to radical generation.

Because xanthine oxidase may be an important step in formation of oxygen radicals during reperfusion in brain, several investigators have determined the effect of blocking the activity of this enzyme. For example, gerbils that have been fed a diet enriched with tungsten have decreased cerebral xanthine oxidase activity and improved recovery from cerebral ischemia and reperfusion (108). Likewise, allopurinol, which has the ability to cross the blood-brain barrier (67), has been effective when given in sufficient doses for inhibiting xanthine oxidase. Allopurinol pretreatment improved survival of gerbils (12) and spontaneously hypertensive rats (64) exposed to transient forebrain ischemia. Allopurinol also decreased infarct volume (89) and improved neurological outcome (60) after permanent focal ischemia. However, allopurinol also has the ability to scavenge hydroxyl radicals (99), and its mechanism of protection may not be due to its effect on xanthine oxidase.

Nitric oxide. Another potential pathway for production of radicals is the metabolism of arginine with the production of nitric oxide (107, 122). Production of nitric oxide from arginine in brain is stimulated by an excitatory amino acid mechanism. Ischemia causes an increase in extracellular concentration of excitatory amino acids (14). Once released, excitatory amino acids bind to and stimulate NMDA receptors within brain and cause the formation of nitric oxide (19, 47). In normal brain, nitric oxide appears to be nontoxic and an important mediator of endothelial-derived relaxation (46). However, in the presence of superoxide during reperfusion, nitric oxide can potentially lead to the formation of hydroxyl radical-like reactive species (11).

Polymorphonuclear leukocytes. There is some evidence that leukocytes contribute to reperfusion injury in brain. It has been proposed that ischemia leads to activation of leukocytes trapped in the cerebral vasculature, which may result in release of chemotactic factors (e.g., leukotrienes) during reperfusion. Likewise, during reperfusion, leukocytes may interact with platelets to metabolize arachidonic acid and produce lipoxygenase and cyclooxygenase byproducts, including oxygen radicals (56, 73). Leukocyte accumulation is noted in damaged brain 60 min after cerebral ischemia produced by incrementally introducing air emboli into the cerebral circulation (56, 73), and leukocyte accumulation appears to correlate with brain regions having low blood flow (56).

Consistent with the hypothesis that leukocyte accumulation may alter the distribution of postischemic blood flow is the finding that rats made neutropenic before being exposed to transient forebrain ischemia had amelioration of hypoperfusion during reperfusion (52). Activated leukocytes may cause mechanical obstruction of capillaries. However, treatment with antineutrophil serum at the time of reperfusion is not associated with improved postischemic blood flow in rats (52) or improved neurological recovery in dogs (123) despite a reduction in circulating leukocytes. These data suggest that there is activation of leukocytes trapped in brain during ischemia, whereas postischemic leukocyte depletion has no protective effect. In addition, although reducing the number of circulating leukocytes before ischemia improves recovery of electrical function after focal ischemia (36), treatment with indomethacin, prostacyclin, and heparin eliminates severe hypoperfusion without altering early postischemic leukocyte accumulation (73). These data together suggest that leukocyte accumulation in brain may impair microvascular circulation, but the presence of leukocyte accumulation is not necessarily the major cause of injury from ischemia and reperfusion. It is also important to recognize that most of the positive evidence of early leukocyte accumulation is based on models using air embolism, which produces significant endothelial damage. The role of leukocyte activation in other models of cerebral ischemia is less clear.

Mononuclear cells. Two types of mononuclear phagocytes may be important in ischemic injury to brain, the microglia and blood-borne macrophages. Both classes of mononuclear phagocytes have cytotoxic activity within brain (49, 104). Systemically administered chloroquine in combination with colchicine blocks endocytotic, secretory, and phagocytic activities of monocytes and microglia and, therefore, can be used as a tool to determine the role of monocytes in cerebral reperfusion injury. In a model of transient spinal cord ischemia, even when administered after the period of ischemia, intravenous administration of chloroquine in combination with colchicine reduced the likelihood of delayed functional deterioration (50, 51). These studies suggest that therapy designed to disable phagocytes in general may be more efficacious than therapy designed to decrease leukocyte numbers.

Role of Calcium in Production of Oxygen Radicals

Calcium appears to play an important role in release of oxygen radicals in several of the pathways that have been proposed as predominant for radical production in brain. Calcium influx, such as that promoted by a calcium ionophore, and activation of protein kinase C have synergistic effects on superoxide release by endothelial cells (90). As discussed above, during ischemia there is an increase in intracellular calcium that activates phospholipases and liberates free fatty acids (e.g., arachidonic acid) (130, 140). During reperfusion, arachidonic acid can be metab-
olized by the lipoxygenase and cyclooxygenase pathways with the production of oxygen radicals and vasoconstricting prostanoids. Calcium entry into vascular smooth muscle may also have an additive effect with vasoconstricting prostanoids and contribute to postschismic hypoperfusion.

Calcium may also play a central role in activation of the protease calpain, which cleaves a peptide bond in xanthine dehydrogenase to form xanthine oxidase in endothelium of cerebral blood vessels (68, 93). As discussed above, elevated xanthine oxidase activity during reperfusion may contribute to increased oxygen radical formation as a result of the metabolism of xanthine to uric acid.

At least two mechanisms of activating oxygen radical production from leukocytes exist. One of these is dependent on a calcium-related mechanism and the other is not. As with endothelium, calcium influx, such as that promoted by a calcium ionophore, and activation of protein kinase C have synergistic effects on superoxide release by neutrophils (119). Although oxygen radical production is calcium dependent, leukocytes are stimulated by complement components (23), the role of calcium in production of oxygen radicals by leukocytes after ischemia and reperfusion in brain is not known.

### Potential Sites of Radical-Induced Injury

Although both parenchyma and vascular endothelium have potential pathways for radical production, it is unclear which of these sites is the main source of radical production during reperfusion. Evidence for radical production in each of these locations is presented.

Oxygen radical production has been demonstrated in cultured endothelial cells treated with menadione or nitrazepam (120). Likewise, the increase in alumin permeability across pulmonary endothelial monolayers in response to hypoxia and reoxygenation is completely blocked by treatment with oxypurinol or SOD (63). In brain, microvessels appear to have a significant amount of xanthine oxidase available for production of oxygen radicals during reperfusion (15). In addition, there is increased prostanoid pathway activity in cerebral blood vessels during recirculation from ischemia (105). Oxygen radicals produce arteriolar dilation, reduce reactivity to the vasoconstrictive effects of hypocapnia, produce discrete lesions of endothelium and vascular smooth muscle (140), and impair capillary endothelial cell mechanisms that help maintain homeostasis of electrolytes and water in brain (88, 106).

If radical production by endothelial cells is an important step in the pathogenesis of reperfusion injury in brain, one would predict that systemic administration of oxygen radical scavengers would alter postschismic blood flow changes. Treatment with indomethacin and scavengers of oxygen radicals has been demonstrated to prevent neuronal injury from transient focal ischemia (1, 62, 65, 87). In the setting of transient global ischemia, treatment with SOD1 plus deferoxamine produced modest improvements in recovery of cerebral blood flow and evoked potentials (26). However, recovery of cerebral blood flow was not improved by treatment with SOD alone (124), nor was neurological recovery improved even when SOD was administered in combination with catalase (43).

The difference in efficacy of oxygen radical scavengers for focal and global cerebral ischemia may be explained by a difference in blood-brain barrier permeability or in the half-life of conjugated SOD used in the focal but not global ischemia experiments. Alternatively, other extraparenchymal mechanisms of brain injury may be important in the setting of transient focal but not global ischemia. Indeed, leukocyte accumulation is noted in damaged brain 60 min after cerebral ischemia produced by multifocal ischemia (56, 73), and leukocyte accumulation appears to correlate with brain regions having low blood flow (56).

Injury may also result from production of radicals within the parenchyma itself. Evidence for parenchymal production of radicals comes predominantly from studies of brain homogenates (61, 133, 150) and mitochondrial preparations (31) in which the contribution from endothelium is presumed to be negligible on the basis of its small proportion of total brain mass. Furthermore, brain synaptosomes have the capability of generating oxygen radicals when they are appropriately stimulated in vitro (145).

Finally, the cell type injured during ischemia and reperfusion may not necessarily correspond precisely to the source of oxygen radicals for several reasons. First, superoxide is able to exit cells via anion channels (79) and thereby affect neighboring cell types. Second, primary damage to endothelium and vascular smooth muscle can result in secondary damage to neurons and astrocytes by impairing oxygen and substrate supply and by producing vasogenic edema. Third, primary radical damage to neurons and astrocytes can result in cytotoxic edema, which can have secondary effects by causing vascular compression.

### Mediators of Radical-Induced Injury During Reperfusion

**Superoxide.** Superoxide has been detected with the NBT technique at the onset of reperfusion (5). It may be considered that because superoxide is not as reactive as other radical species, it is not the mediator of radical injury in brain. However, if this were the case, one could not explain the apparent therapeutic efficacy of SOD when administered by itself (45). For example, superoxide-mediated inactivation of catalase can be blocked by SOD (118). Likewise, SOD but not catalase prevents accumulation of granulocytes at the site of developing inflammation (95). Evidence for primary superoxide-induced injury in brain comes from the finding that polyethylene glycol-conjugated SOD (PEG-SOD) by itself decreases caudate injury volume when administered before transient focal ischemia (91). Likewise, liposomal-entrapped SOD decreases cerebral infarction in rats exposed to permanent focal ischemia (62) and decreases brain edema after head trauma (29). However, the finding that superoxide produces injury by itself does not exclude the likelihood that other more reactive radicals also contribute to brain injury after ischemia and reperfusion.

**Hydroxyl radicals.** The hydroxyl radical reacts at ex-
tremely high rate constants with almost every type of molecule found in living cells: sugars, amino acids, phospholipids, DNA bases, and organic acids (58). Thus hydroxyl radicals are more reactive than superoxide, but they are less specific in the type of molecules that they attack. It is generally assumed that, in the presence of ferrous iron, superoxide-dependent Fenton chemistry can occur and result in production of hydroxyl radicals. The relevance of hydroxyl radical formation in brain has been extrapolated from experiments that have tested the benefit of decreasing the availability of iron for Fenton chemistry. On the basis of these data, which are described below, iron-catalyzed formation of hydroxyl radical has generally been considered as the common final pathway for brain damage from radical production. Normally, the brain has efficient scavenging systems that maintain a low concentration of the factors necessary for this reaction to occur. Likewise, the rate for the Fenton chemistry may be limited in vivo by reduction of iron by cellular compounds like ascorbate. However, in some models of ischemia there is a decreased concentration of ascorbic acid in brain (39) and there is an increase in low-molecular-weight iron available in brain for Fenton chemistry (25). In addition to Fenton chemistry, hydroxyl radical-like reactive species may be generated via decomposition of protonated peroxynitrite formed by superoxide and nitric oxide at acidic pH independent of iron (11).

Mechanism of Brain Injury From Radical Production

Within brain, oxygen radicals impair capillary endothelial cell mechanisms that help maintain homocostasis of electrolytes and water in brain (88) and alter membrane fluidity characteristics (112). Oxidative mechanisms also appear to contribute to synaptic damage within brain (109). Changes in membrane fluidity have been detected in synaptosomes isolated from cortex after reperfusion, but not after ischemia alone (138).

Because of a high concentration of polyunsaturated fatty acids, brain is very susceptible to radical injury. Since neuronal function appears to depend to a significant degree on structural integrity of cellular and subcellular membranes, many investigators have centered their investigation on determining the detrimental effects of radical mechanisms on phospholipids, a major constituent of biologic membranes. Once peroxidative reactions have begun in brain, they are chain propagating in the presence of sufficient concentrations of oxygen.

When cerebral ischemia is followed by reperfusion, evidence for lipid peroxidation with accumulation of both conjugated dienes and thiobarbiturate-reactive material is consistently found (75, 139, 151). Likewise, a delayed increase in lipid peroxides occurs in selectively vulnerable regions during reperfusion, but not in permanently ischemic infarcted tissue (21). Brain lipid peroxidation through Fenton chemistry depends both on adequate production of oxygen radicals and the presence of a transitional metal catalyst (iron) at the site where radicals are produced (58). During reperfusion, whereas iron is elevated only transiently, lipid peroxidation progresses for many hours (82). Lipid peroxidation is accentuated when excess oxygen is available during reperfusion (96) and with tissue acidosis (116). Lactic acidosis presumably increases the production of oxygen radicals because of its ability to dissociate protein-bound iron (7), making more available for production of hydroxyl radicals.

Treating animals with deferoxamine decreases iron available for Fenton chemistry and, therefore, presumably decreases production of hydroxyl radical. Deferoxamine may also serve as a competitive inhibitor of peroxynitrite-initiated oxidation (11). When animals are treated with deferoxamine before ischemia, there is a decreased production of lipid peroxides (75) and decreased brain edema during reperfusion (108). Associated with the effect of deferoxamine on lipid peroxidation and brain edema formation during reperfusion is the finding that in some studies (76) deferoxamine pretreatment improves overall survival rate of animals exposed to transient ischemia without improving the neurological examination in the survivors. However, this finding could not be confirmed by others (40).

Therapeutic Efficacy of Radical Scavengers

α-Tocopherol. Several different radical scavengers and antioxidants have been tested for their efficacy in preventing biochemical, histological, physiological, or neurological abnormalities after transient ischemia. Administration of α-tocopherol, an antioxidant in membranes (92), before onset of ischemia attenuates lipid peroxidation during reperfusion (148). Likewise, if rats are raised on a diet deficient in α-tocopherol, exposure to ischemia and reoxygenation results in enhanced lipid peroxidation (150). When α-tocopherol is given to dogs in combination with phenytoin and mannitol before ischemia, there is significant improvement in recovery of electrical activity and decreased brain swelling compared with dogs treated with phenytoin alone (131). Whereas both deferoxamine and α-tocopherol are effective alone in reducing brain injury during reperfusion, only α-tocopherol has been demonstrated to be effective in improving neurological outcome (40, 148).

Radical scavengers. The enzymes SOD and catalase are present naturally in brain; however, in the setting of ischemia and reperfusion their capacity may be inadequate (142). The role of SOD is to scavenge superoxide, and that of catalase is to scavenge hydrogen peroxide. In addition to preventing direct toxicity of superoxide, SOD may also decrease substrate available for Fenton chemistry and formation of peroxynitrite anion (11). In very high doses, intravenous administration of SOD has been demonstrated to provide marked improvement in neurological recovery from acute hypertension (154) and transient spinal cord ischemia (84). However, intravenous administration of SOD is not associated with improvement in neurological recovery in animals exposed to cerebral ischemia and reperfusion (43). When SOD is administered in combination with deferoxamine after cardiac arrest in dogs, some improvement in recovery of cerebral blood flow, somatosensory evoked potential (25), and electroencephalogram (EEG) (26) were observed, although neurological deficit was not improved.

The enzyme SOD has two drawbacks as a therapeutic
agent. First, it is readily cleared by the kidney and has a circulatory half-life of only 8 min in rats (135). Second, copper-zinc SOD is a large water-soluble molecule (molecular mass 32 kDa) (95) and therefore cannot readily penetrate cell membranes (13, 44) or cross the blood-brain barrier in significant quantities after intravenous administration (111). Thus with the limited access of SOD in brain, it is not surprising that SOD appears to have only limited efficacy for reperfusion injury in brain.

Conjugation of polyethylene glycol (PEG) monomers to SOD increases its circulatory half-life to almost 40 h in rats (144) and improves its access into cultured endothelial cells over approximately a 4-h period, presumably because of pinocytosis (13). If oxygen radical production by endothelium is an important mechanism for reperfusion injury, increased uptake of PEG-SOD may result in cerebroprotection. When PEG-SOD is administered intravenously alone (91) or in combination with PEG-catalase (87) before transient focal ischemia, infarct volume is decreased. A vascular mechanism permitting increased collateral blood flow may be involved in this experimental paradigm. Likewise, PEG-SOD in combination with PEG-catalase improves recovery of cerebral blood flow and oxygen consumption after asphyxia in newborn lambs (121).

Liposomal entrapment has also been used as a means to decrease plasma clearance of SOD and increase delivery of SOD to brain. Liposomal entrapped SOD has increased uptake into cultured endothelial cells (44), and intravenous administration results in a more than threefold increase in brain SOD activity within 1 h (62). In association with increased brain SOD activity, intravenously administered liposomal entrapped SOD protects the brain from brain edema after trauma (30) and decreases cerebral infarction after permanent focal ischemia (62).

Dimethyl sulfoxide (DMSO), an excellent hydroxyl radical scavenger (rate constant \( \sim 10^{10} \text{ M}^{-1} \text{ s}^{-1} \)), improves neurological outcome and microscopic evidence of damage when given to animals just before reperfusion from spinal cord ischemia (32). However, DMSO may exert other effects not related to hydroxyl radical scavenging (34). With permanent focal ischemia, DMSO was not effective in improving EEG or decreasing cerebral edema or changes in blood-brain barrier permeability in cats (85) or neurological injury in baboons (86). However, its efficacy has not been tested when it is administered during reperfusion from transient focal cerebral ischemia when production of hydroxyl radical would be expected to be accelerated.

Other more novel radical scavengers have also been tested for efficacy after ischemia and reperfusion. Pre-treatment with one such scavenger, ONO-3144 (2-amino- methyl-4-tert-butyl-6-propionylphenol hydrochloride, ONO Pharmaceutical, Osaka, Japan) (2), significantly improved recovery of blood flow and decreased cerebral edema after transient focal ischemia in cats (65). However, this agent also has inhibitory effects on thromboxane A2. Another potent agent is MCI-186 (Mitsubishi Chemical Industries, Tsukuba, Japan). This agent inhibits both nonenzymatic lipid peroxidation and lipoygenase activity but has little effect on cyclooxygenase activity. In cats pretreated with MCI-186 before the onset of transient focal ischemia, development of brain edema has decreased (1).

The 21-aminosteroids are a group of compounds that lack classic steroidal activities but are potent inhibitors of lipid peroxidation in vitro. The most extensively studied agent in this group in the setting of ischemia and reperfusion in brain is tirilazad [21-{4(2,6-di-1-pyrrrolidinyl-4-pyrimidinyl)-1-piperazinyl}-16a-methylpregna-1,4,9-(11) - triene - 3,20 - dionemonomethane sulfonate, U74006F, Upjohn, Kalamazoo, MI]. Tirilazad is a potent scavenger of lipid peroxyl radicals (18), it is a scavenger of oxygen radicals, it has an \( \alpha \)-tocopherol-sparing effect, and it has membrane-stabilizing action (53). Tirilazad is effective in improving recovery of cerebral blood flow and neurological function after transient complete global ischemia in cats (55) and dogs (100, 110). Tirilazad is also associated with improved survival and decreased histological injury after transient focal ischemia in gerbils (54) and decreased brain edema after permanent (152) but not transient focal (8) ischemia in rats.

**Summary**

Several different pathways are available in brain for production of radicals. These include electron transport chain dissociation and excitatory amino acid stimulation of arginine to nitric oxide formation during ischemia. Furthermore, there is accumulation of fatty acids and adenine nucleotides during ischemia, which sets the stage for production of superoxide during reperfusion. In the presence of ferrous iron, superoxide-dependent Fenton chemistry may result in production of the highly reactive hydroxyl radical. In addition, superoxide ion may react with nitric oxide to form highly reactive radicals derived from peroxynitrite anions. Regardless of the exact mechanism, there is substantial indirect evidence that radical production appears to be an important mechanism of brain injury after exposure to ischemia and reperfusion. Literature on therapeutic efficacy suggests that drugs which are able to cross the blood-brain barrier may have the greatest potency. These agents may also be more efficacious because of their lipophilicity in neuronal membranes where they can more effectively inhibit lipid peroxidation. However, definitive evidence of radical-mediated injury awaits more sensitive techniques for measuring radical presence during reperfusion and demonstration that their presence can be inhibited in vivo by purported antioxidants and scavengers with known therapeutic efficacy.

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**REFERENCES**


BRIEF REVIEW

1195


130. Sun, G. Y., T. Yang, S. F. L. Huang, and L. Foudin. Is phos


140. Wei, E. P., C. H. Chekstman, H. A. Kuntun, and J. T. Puvils-


142. White, B. C., J. F. Hilderrandt, A. T. Evans, I. Aronson, R. J. Indrierei, T. Hoeinier, L. Fox, R. Huang, and D. Johns. Prolonged cardiac arrest and resuscitation in dogs: brain mito-


