Abstract—The sequential univalent reduction of oxygen generates superoxide, hydrogen peroxide, and hydroxyl radical. The generation of hydroxyl radical is dependent on catalysis by ferrous iron. In addition, superoxide and nitric oxide produce peroxynitrite, which spontaneously generates hydroxyl radical independently of iron-mediated catalysis. These agents have a variety of cellular actions, which render them suitable candidates as mediators of tissue destruction and cellular death. In the intact brain, superoxide and its derivatives cause vasodilation, mediated by opening of potassium channels, altered vascular reactivity, breakdown of the blood-brain barrier, and focal destructive endothelial lesions. These abnormalities are also seen in early reperfusion following brain ischemia. During reperfusion there is a marked transient increase in superoxide production. Vasodilation, abnormal vascular reactivity, and blood-brain barrier breakdown are inhibited by eliminating superoxide. Superoxide production during reperfusion may be initiated by glutamate via activation of \( \alpha \)-amino-3-hydroxy-5-methylisoxasolepropionic acid (AMPA) receptors. These experimental findings have important implications for human cerebral ischemia. Agents directed at eliminating oxygen radicals must be administered before or in the early stages of reperfusion following ischemia. The therapeutic window appears to be narrow and limited to, at most, a few hours. The inhibition of AMPA receptors may be a promising approach to inhibit the production of oxygen radicals during ischemia-reperfusion of the brain. (Stroke. 2001;32:2712-2716.)

Key Words: glutamates ■ hydrogen peroxide ■ hydroxyl radical ■ nitrites ■ superoxides ■ receptors, AMPA ■ receptors, NMDA ■ vasodilation

There is considerable evidence that oxygen radicals are important mediators of tissue injury in cerebral ischemia.\(^1-4\) I review below the evidence implicating oxygen radicals in experimental cerebral ischemia and consider some important implications of these experimental studies for human brain ischemia and stroke.

Chemistry and Biology of Oxygen Radicals
The sequential univalent reduction of oxygen produces superoxide, hydrogen peroxide, hydroxyl radical, and water.\(^5,6\) Superoxide and hydroxyl radical have an unpaired electron in their molecules; hence, they are true radicals in the chemical sense. Hydrogen peroxide does not, but it is quite reactive and hence is considered together with the other 2 compounds.

Superoxide is the primary radical. It generates hydrogen peroxide by dismutation. Although the dismutation of superoxide is the main source of hydrogen peroxide in tissues, the latter can also be produced directly by several enzymatic reactions. Hydroxyl radical is generated from hydrogen peroxide in the presence of ferrous iron or other transition metals in the metal-catalyzed Haber-Weiss reaction. In this reaction, superoxide is essential because it serves to reduce the transition metal, which is then oxidized in the reaction that produces hydroxyl radical. As a result, the cycle can be repeated. Hydroxyl radical is extremely reactive and very short lived.

Superoxide is produced in tissues via a variety of enzymatic reactions or by auto-oxidation of tissue components. Common sources of superoxide are listed in Table 1. The concentration of superoxide in tissues is determined by multiple factors. The rate of production obviously is a major determinant. This is in turn influenced by the activity of the various enzymatic processes that generate the radical and by the availability of substrate. The rate of production of superoxide by autooxidation is influenced by the concentration of the oxidizable substrates and the availability of oxygen. A major factor determining the concentration of superoxide in tissues is the activity of antioxidant enzymes. Hydrogen peroxide and hydroxyl radical are oxidizing agents, while superoxide can act as an oxidizing as well as a reducing agent.

Interaction of oxygen radical with other tissues components produces a variety of other radicals. Of particular importance is the interaction of superoxide with nitric oxide, which produces peroxynitrous acid.\(^7\) This decomposes spontaneously to produce hydroxyl radical.\(^7\) The interaction of...
with nitric oxide is a source of hydroxyl radical independent of catalysis by iron.

Since oxygen radicals are produced mostly in the interior cells and since their life span is relatively short, it is important to consider whether their effects are limited to the cell in which they are produced or can extend to adjacent cells. Hydrogen peroxide is lipid soluble and readily crosses cell membranes. Similarly, superoxide traverses the cell membrane via the anion channel and exits into the extracellular space. Therefore, remote effects from these 2 agents are possible. On the other hand, hydroxyl radical is extremely reactive and does not survive beyond a few molecular diameters from its site of production. Therefore, its actions are likely to be limited to the site of the catalytic metal responsible for its production. However, it is possible that this local action may initiate a chain reaction that would extend the range of its action beyond the narrow limits predicted from its short life span.

Oxygen radicals have pronounced and wide-ranging cellular effects. Their most important actions relevant to cerebral ischemia are listed in Table 2. In large concentrations they are capable of causing cellular death and tissue destruction. Most of these deleterious effects are seen with high concentrations of radicals, which exceed the ability of antioxidant cellular defenses to eliminate them.

Oxygen radicals have important vascular effects. These merit special consideration for 2 reasons. First, because the effects of radicals on cerebral vascular caliber and vascular reactivity have important consequences for cerebral blood flow, and also because the vascular effects of radicals offer convenient methods for monitoring their presence and action.

Table 3 lists the cerebral vascular effects of oxygen radicals. Superoxide, hydrogen peroxide, and peroxynitrite are strong cerebral vasodilators. They dilate reversibly cerebral arterioles at low concentrations. In cats and rats, hydrogen peroxide and peroxynitrite cause cerebral arteriolar dilation by opening ATP-sensitive potassium channels. Their effects are completely inhibited by blocking these channels with glyburide. Superoxide dilates cerebral arterioles by opening calcium-activated potassium channels. Its effects are eliminated by inhibitors of these channels such as tetraethylammonium chloride, charybdotoxin, and iberiotoxin. Vasodilation from hydrogen peroxide is inhibited by deferoxamine, which scavenges iron, suggesting that the vascular effects of hydrogen peroxide are mediated by hydroxyl radical. In our hands, guanylate cyclase and its products do not contribute to the vasodilation from oxygen radicals. In fact, in the presence of oxygen radicals, guanylate cyclase is inactivated.

Oxygen radicals interfere with endothelium-dependent relaxation because of the interaction between nitric oxide and superoxide. For example, in the presence of oxygen radicals, acetylcholine causes slight vasoconstriction rather than the vasodilation seen under normal circumstances, presumably because the elimination of the dominant endothelium-dependent relaxation uncovers a direct vasoconstrictor effect of the agent. This alteration in reactivity as well as the vasodilation are useful in identifying the presence of radicals and following their time course.

### Production and Effects of Oxygen Radicals in Cerebral Ischemia

Complete cerebral ischemia followed by reperfusion results in production of superoxide. Superoxide under these conditions is generated during reperfusion. Its concentration is maximum in the early phases of reperfusion and subsides over the next 2 hours. Superoxide production is not detectable during complete ischemia because of the absence of oxygen. Partial cerebral ischemia also results in superoxide production during reperfusion, which follows the same time course as with complete ischemia, but the concentrations are lower. While the concentration of superoxide is sensitive to the duration of the preceding ischemia, the duration of the production of superoxide is less sensitive to the duration of ischemia.

Production of oxygen radicals during reperfusion following ischemia is accompanied by vasodilation and abolition of endothelium-dependent responses. For example, the response to acetylcholine becomes vasoconstrictor in the early period of reperfusion. The time course of the vasodilation and of the abnormal vascular response to acetylcholine corresponds closely to the time course of the production of superoxide. The blood-brain barrier permeability is increased and allows the extravasation of albumin and other high-molecular-weight compounds. This results in edema and increased intracranial pressure. The blood vessels under these conditions exhibit, in addition to the abnormal blood-brain barrier permeability, focal endothelial lesions in the form of...
SOD (Figure 2) or by blockade of NOS with nitroarginine, glutamate caused dose-dependent vasodilation. Pretreatment of the tissue with scavengers of oxygen radicals such as superoxide dismutase (SOD) and catalase inhibits the vasodilation, the abnormal endothelium-dependent responses, and the breakdown of the blood-brain barrier and reduces the density of the focal endothelial lesions, showing that oxygen radicals are important mediators of these abnormalities. Similarly, topical application of deferoxamine to scavenge iron has the same protective effects, suggesting that hydroxyl radical may be the immediate cause of these abnormalities.

Oxygen radicals are also important in determining the extent of tissue injury and death following cerebral ischemia. For example, treatment with SOD and catalase reduces substantially the infarct size seen following cerebral ischemia. Also, transgenic animals, which overexpress SOD, suffer cerebral infarcts from ischemia, which are severely reduced in size.

It is well known that the release of glutamate is an important mechanism that determines tissue injury following cerebral ischemia. Recent unpublished studies in cats and rats by Dr. Enoch Wei and myself have identified mechanisms that link the release of glutamate and the generation of oxygen radicals. Topical application of N-methyl-d-aspartate (NMDA) causes vasodilation, which is due to activation of NMDA receptors in neurons, and subsequent activation of nitric oxide synthase (NOS) with generation of nitric oxide. Surprisingly, glutamate caused little or no vasodilation, despite the fact that it is also a strong activator of NMDA receptors. Accordingly, we explored the possibility that glutamate may be initiating competitive mechanisms, which counteract the effect of NMDA receptor stimulation and NOS activation. In the presence of blockade of NMDA receptors with MK801 or inactivation of NOS with nitroarginine, glutamate caused dose-dependent vasodilation (Figures 1 and 2). This vasodilation was blocked by SOD (Figure 2) or by blockade of α-amino-3-hydroxy-5-methylisoxasolepropionic acid (AMPA) receptors with 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo-(F)-quinoxaline (NBQX) (Figure 3). This evidence suggested that glutamate through stimulation of NMDA receptors activates NOS, which produces nitric oxide, but through stimulation of AMPA receptors generates superoxide (Figure 4). The interaction between superoxide and nitric oxide inside the cell may generate peroxynitrite, which can cause injury. Peroxynitrite is inactivated quickly and evidently does not escape into the extracellular environment, because no vasodilation is seen under these conditions. Inactivation of one or the other mechanism results in vasodilation because it allows the escape of either nitric oxide when AMPA receptors are inactivated or superoxide and its derivatives when NMDA receptors or NOS are inactivated.

In contrast to the absence of consistent vasodilation from glutamate in cats and rats, glutamate induced significant vasodilation in cerebral arterioles of newborn pigs and induced marked hyperemia in the cerebellum of rats. In both of these studies the vasodilation was in part inhibited by inhibition of NOS, suggesting that it was mediated by nitric oxide. It is not known whether these differences reflect species differences or regional differences in the brain. In the absence of studies with AMPA receptor blockade, it is not possible to identify whether these differences in results reflect fundamental differences in responsiveness to glutamate.

**Clinical Implications for Human Cerebral Ischemia**

The experimental evidence cited above provides a strong basis for concluding that oxygen radicals are important mediators of tissue injury in cerebral ischemia. Accordingly, it may be productive to devise therapeutic approaches for human cerebral ischemia that are directed at either diminishing the production of oxygen radicals, scavenging them, or protecting the tissue from their effects.
The evidence cited above suggests strongly that inhibition of AMPA receptors may be an effective mechanism for diminishing the production of radicals in cerebral ischemia. Accordingly, this approach is worthy of testing in human cerebral ischemia. It must be remembered, however, that there are multiple sources of oxygen radicals, and any attempt to target specific enzymatic mechanisms may provide incomplete protection.

Scavenging of radicals is a popular approach to minimize radical mediated damage in cerebral ischemia. Since hydroxyl radical is the most reactive oxygen radical and the most probable immediate cause of tissue injury in cerebral ischemia, it is tempting to target this radical with scavengers. However, this approach has important practical limitations. Hydroxyl radical is extremely reactive, and although its reactivity with potential scavengers is very high, its reactivity with important tissue components is also high, so that the difference in reactivity between the scavenger and the tissue components is relatively low. Accordingly, very high concentrations of the scavenger are necessary to afford significant protection.7,22 Achieving such high concentrations may be impractical, and in fact may have limiting side effects. Targeting the less-reactive superoxide with an effective scavenger, such as SOD, is much more likely to succeed because the reactivity of superoxide with SOD is much higher than its reactivity with tissue components.

Another important implication of the experimental studies reported above is that the therapeutic window for any intervention directed against radicals is likely to be short, on the order of 1 to 2 hours. It is therefore likely that the most productive approach to maximize the effectiveness of any radical directed therapies is to administer the agents at the time of reperfusion.

Finally, it is important to keep in mind that the considerations above have dealt with the early phase of reperfusion following ischemia. There are important subsequent changes,23 such as inflammation and gene activation, that may afford important targets for therapies that may be effective in protecting cerebral tissue in ischemia.

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