Increased Lipid Peroxidation in Vulnerable Brain Regions After Transient Forebrain Ischemia in Rats

Christine Bromont, MS, Christine Marie, PhD, and Jean Bralet, PhD

We examined cerebral lipid peroxidation, estimated by a thiobarbituric acid test, in rat brain regions after 30 minutes of severe forebrain ischemia and at recirculation periods of up to 72 hours. The lipid peroxide levels remained unaltered in all brain regions during ischemia and during the first hour of recirculation but were selectively increased between 8 and 72 hours of recirculation in the ischemia-sensitive regions of the hippocampus, striatum, and cortex. The most pronounced increases (30–37%) were seen at 48 hours of recirculation. In contrast, lipid peroxide levels were unchanged in infarcted brain regions 24 hours after intracarotid injection of microspheres, indicating that reoxygenation of the ischemic brain is a prerequisite for lipid peroxidation. We assessed the lipid peroxidation capacity of cerebral homogenates obtained from rats subjected to ischemia and recirculation by measuring the production of lipid peroxides after aerobic incubation. The homogenates from rats exposed to 30 minutes of ischemia or to 1 hour of recirculation were not more susceptible to peroxidation. However, the production of lipid peroxides was selectively increased in the hippocampus, striatum, and cortex at 8–48 hours of recirculation, suggesting a loss of efficacy of the antioxidant systems. These results, showing a delayed and long-lasting increase in lipid peroxidation that occurs in ischemia-sensitive brain regions and parallels the development of neuronal necrosis, support the hypothesis that free radical processes participate in postischemic neuronal damage. (Stroke 1989;20:918–924)
Weighed, and their radioactivity was measured.

was dissolved in isotonic saline, and 5 μCi were

anesthetized with ether and tracheotomized. One group of rats was used for the measurement of regional blood flow and another group for the measurement of lipid peroxides.

This test estimates the level of malondialdehyde (MDA) precursors, including hydroperoxides and endoperoxides.21,22 Rats subjected to four-vessel occlusion or embolization were decapitated. The brain was removed and dissected at 0°C. Cerebral structures were homogenized in 10 vol ice-cold 0.05 M phosphate buffer (pH 7) containing 0.015 M Na and 0.145 M K.23 The buffer solution had previously been equilibrated with 100% nitrogen for 1 hour before use. To 0.2 ml homogenate were added 1 ml buffer solution and 1.5 ml trichloroacetic (TCA)-thiobarbituric acid (TBA)-HCl reagent prepared in 0.85N HCl with 13.5% wt/vol TCA and 0.33% wt/vol TBA and deoxygenated by bubbling with nitrogen. Samples were then placed in sealed tubes containing nitrogen and were heated for 15 minutes in a boiling water bath. After cooling, 1 ml TCA 70% wt/vol was added, and the precipitate was removed by centrifugation at 2,500g for 10 minutes. The fluorescence of the supernatant was measured on an Aminco-Bowman spectrofluorometer at 553 nm with an excitation wavelength of 515 nm.24 The amounts of TBARS were quantified using a standard curve of MDA prepared with malondialdehyde-bis-dimethylacetal (Aldrich-Chemie, France) and expressed as nanomoles MDA per gram wet weight.

Table 1 gives the physiologic parameters of rats subjected to measurement of cerebral blood flow. There were no consistent differences in the parameters between the experimental groups except for an increase in mean arterial blood pressure after 30 minutes of vascular occlusion (158 vs. 128 mm Hg in control rats, p<0.001).

The evolution of regional blood flow after ischemia and recirculation is shown in Table 2. During ischemia, blood flow was reduced to approximately 32% of the control values in the brainstem, to 10%
in the hypothalamus, to 7% in the thalamus and hippocampus, and to 3% in the striatum and parietotemporal cortex. After 1 hour of recirculation, a significant hypoperfusion was observed in all brain regions. Flow rates were reduced to approximately 60–70% of control values in the brainstem and hypothalamus, to 45% in the thalamus and hippocampus, and to 30% in the striatum and parietotemporal cortex. At 24 hours of recirculation, blood flow had returned to control values in the striatum but remained reduced (60–80% of control values) in the other brain regions.

The time course of changes in lipid peroxide levels, quantified as nanomoles MDA per gram wet weight, is shown in Figures 1 and 2. Before ischemia, the peroxide levels were similar in all sampled brain regions, with the highest level in the hypothalamus (16.9±0.7 nmol/g) and the lowest in the brainstem (14.6±0.7 nmol/g). In all brain regions, a 30-minute ischemic exposure did not alter significantly the peroxide levels. During recirculation, the pattern of changes differed according to brain region. In the brainstem, hypothalamus, and thalamus (Figure 1), peroxide concentrations did not differ significantly from preischemic values throughout the entire recirculation period. More important changes were found in the hippocampus, striatum, and cortex (Figure 2). After 1 hour of recirculation, the peroxide level was unchanged in the cortex and slightly reduced in the hippocampus (−9%, p=NS) and striatum (−12%, p=NS). These reductions might be related to dilution secondary to the slight increase (about 1%) in brain water content, which was reported in this model during the first hour of recirculation. During the 8–72-hour interval, significant increases in peroxide levels occurred in all three regions. The most pronounced change was found after 48 hours of recirculation and reached 30% in the hippocampus (p<0.01), 34% in the parietotemporal cortex (p<0.01), and 37% in the striatum (p<0.01). Afterward, levels declined but remained significantly increased in the cortex (24%) at 72 hours of recirculation.

In our embolic stroke model, intracarotid microsphere injection produces multiple infarcts located ipsilaterally in the striatum, thalamus, hippocampus, and cortex.27 Lipid peroxides were measured 24 hours after embolization in these regions and in the brainstem, which was not affected by the intracarotid injection of microspheres. As shown in Table 3, peroxide levels did not differ significantly between embolized and control rats. The infarcted regions showed a trend toward reduction of peroxide levels (−10 to −15%) that can be explained by
FIGURE 2. Graph of lipid peroxide levels in hippocampus, striatum, and parietotemporal cortex during ischemia and recirculation. There were 22 rats in control group, six rats subjected to 30 minutes of four-vessel occlusion, and eight, 10, 14, seven and eight subjected to 1, 8, 24, 48, and 72 hours of recirculation after 30 minutes of ischemic exposure, respectively. Values are mean±SEM. (*p<0.05; **p<0.01 vs. control group.)

Table 3. Effect of Embolization on Cerebral Lipid Peroxide Levels

<table>
<thead>
<tr>
<th>Structure</th>
<th>Control (n=8)</th>
<th>Embolized (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hippocampus</td>
<td>16.8±0.7</td>
<td>15.2±0.9</td>
</tr>
<tr>
<td>Striatum</td>
<td>17.9±0.8</td>
<td>15.5±1.0</td>
</tr>
<tr>
<td>Parietotemporal cortex</td>
<td>18.9±0.9</td>
<td>15.8±1.4</td>
</tr>
</tbody>
</table>

Values are mean±SEM; n, number of rats. MDA, malondialdehyde. Embolized rats were killed 24 hours after injection of microspheres into left internal carotid artery, and lipid peroxides were measured in brainstem and in left hemispheric structures.

Discussion

The characteristics of the cerebral ischemia induced by four-vessel occlusion in rats vary according to the rat strain.13,16,17 Changes in regional cerebral blood flow reported in our study are comparable with those described by Pulsinelli et al.28 Transient four-vessel occlusion in rats produces delayed neuronal necrosis in specific brain regions, the localization and the degree of damage being related to the duration of ischemia.14 After 30 minutes of ischemia, neuronal damage was found mainly in the hippocampus, striatum, and neocortex, and to a lesser extent, in the thalamus; the brainstem and hypothalamus were not affected.

Our study results show increases in TBARS levels limited to vulnerable brain regions during the postischemic reperfusion period. Changes were not observed during the ischemic period or after 1 hour of recirculation but became manifest between 8 and 72 hours of recirculation. The TBA reaction has frequently been used for measuring lipid peroxidation in biologic samples but has limitations because a part of the TBA-positive material does not originate from lipid peroxides.29 Nevertheless, change in TBARS level remains a reliable index of lipid peroxidation, especially in comparative studies made on the same biologic material. In brain homogenates incubated under aerobic conditions, Rehncrona et al.23 have shown that increases in TBARS level were correlated with the loss of polyunsaturated fatty acids.

Postischemic lipid peroxidation has previously been demonstrated during the first hours of recirculation in various experimental models of brain ischemia.3-5,8 Using the four-vessel occlusion model, MacMillan12 failed to observe a significant increase in cortical peroxide level for up to 72 hours of recirculation. These data disagree with our findings. The discrepancy might originate from differences in the severity of ischemia or in the nature of the tissue samples. Our study was performed on the parieto-
An overproduction of free radicals may also account for the increased lipid peroxidation seen after reperfusion of the ischemic brain, with several potential sources for the oxygen radicals. Ischemia is accompanied by a marked accumulation of free fatty acids, mainly arachidonic acid, that triggers production of prostaglandins and leukotrienes and generates free radicals. However, this process is of short duration because of rapid normalization of arachidonic acid level.

Normally, a major source of oxygen radicals is the electron transport chain located on the inner mitochondrial membrane. Single electrons may leak at the sites of transfer, permitting the reduction of oxygen to superoxide anion. Decrease in the state 3 respiration has been reported to occur in mitochondria from animals exposed to brain ischemia and recirculation. Although the reduction in oxygen uptake rate may result from a variety of mitochondrial perturbations, an overproduction of oxygen radicals may occur if accumulated electrons cannot flow into cytochrome aa₃ despite a resupply of oxygen. Such a mechanism may become critical during the recirculation period as metabolic demand is increased and may lead to a progressive deterioration of mitochondrial function. Brain mitochondria exposed to free radical–generating systems in vitro showed an inhibition of respiratory activity similar to that observed after brain ischemia.

Considering the delayed increase in lipid peroxidation that parallels the development of neuronal damage, it cannot be ruled out that the increase represents a late phenomenon in an already irreversibly injured tissue rather than the primary cause of the damage. However, if lipid peroxidation were associated with neuronal necrosis, it would be seen after brain infarction. Our results do not support this hypothesis since lipid peroxide levels were not increased in infarcted brain regions, indicating that reoxygenation of the ischemic brain is a prerequisite for lipid peroxidation.
Regardless of the mechanisms initiating lipid peroxidation after reperfusion of the ischemic brain, the pathogenic importance of peroxidative processes in vivo is not well established. What remains to be determined is whether reduction of postischemic lipid peroxidation by administration of antioxidants or free radical–trapping agents can prevent postischemic brain damage and improve the clinical outcome.

Acknowledgments

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