Effect of the 21-Aminosteroid Tirilazad on Cerebral pH and Somatosensory Evoked Potentials After Incomplete Ischemia

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Background and Purpose: Postischemic evoked potential recovery correlates with acidosis during ischemia and early reperfusion. Acidosis promotes lipid peroxidation in vitro. We tested the hypothesis that the 21-aminosteroid tirilazad mesylate (U74006F), an inhibitor of lipid peroxidation in vitro, ameliorates somatosensory evoked potential recovery and acidosis during reperfusion after severe incomplete cerebral ischemia.

Methods: Cerebral perfusion pressure was reduced to 11±1 mm Hg (±SEM) for 30 minutes by cerebral ventricular fluid infusion in anesthetized dogs. Cerebral intracellular pH and high-energy phosphates were measured by magnetic resonance spectroscopy. Dogs were randomized to receive vehicle (citrate buffer; n=8) or tirilazad (1 mg/kg; n=8) before ischemia in a blinded study.

Results: Cerebral blood flow was reduced to 6±1 mL/min per 100 g during ischemia, resulting in nearly complete loss of high-energy phosphates and an intracellular pH of 6.0–6.1 in both groups. Initial postischemic hyperemia was similar between groups but lasted longer in the vehicle group. Tirilazad accelerated mean recovery time of intracellular pH from 31±5 to 15±3 minutes and of inorganic phosphate from 13±2 to 6±1 minutes. Recovery of somatosensory evoked potential amplitude was greater with tirilazad (49±3%) than vehicle (33±6%). Fractional cortical water content was less with tirilazad (0.819±0.003) than vehicle (0.831±0.002).

Conclusions: Tirilazad attenuates cerebral edema and improves somatosensory evoked potential recovery after incomplete ischemia associated with severe acidosis. Accelerated pH and inorganic phosphate recovery indicates that this antioxidant acts during the early minutes of reperfusion. (Stroke 1993;24:724–730)

Key Words • acidosis • cerebral blood flow • cerebral ischemia • evoked potentials, somatosensory • spectroscopy, nuclear magnetic resonance

Oxygen radicals are thought to make a significant contribution to ischemic/reperfusion injury in several major organs, but the magnitude of their role in brain injury is less clear.1 Where positive evidence based on markers of oxygen radical production and lipid peroxidation has been described,2–5 the changes are in some cases relatively small and spatially heterogeneous,6 gender dependent,7 and delayed by 24 hours.8 Administration of oxygen radical scavengers such as superoxide dismutase can limit the extent of infarction during focal ischemia,9,10 which may be attributed in part to a vascular effect of this large protein.11,12 With global ischemia significant protection has been reported with superoxide dismutase administration,13 although others have reported only modest effects14 possibly caused by poor penetration across the blood–brain barrier.15

See Editorial Comment, page 730

The 21-aminosteroids are a family of lipophilic antioxidants that are potent inhibitors of lipid peroxidation in vitro,16,17 including brain.7 The most frequently studied drug in this family for ischemic protection is tirilazad mesylate (U74006F; 21-[4-2(2,6-di-1-pyrrolidinyl-4-pyrimidinyl)-1-piperazinyl]-16α-methyl-pregn-1,4,9(11)-triene-3,20-dione; monomethane sulfonate). Tirilazad has been reported to ameliorate electrophysiological deficit,18 metabolic deficit,19 edema,20 and neurological deficit and histological injury21–24 in many but not all25–27 experimental models of cerebral ischemia.

Acidosis augments lipid peroxidation,28 particularly when bicarbonate levels are reduced.29 We have previously found that recovery of somatosensory evoked potentials (SEP) varies with the severity and duration of cerebral acidosis during both the ischemic and reperfusion periods,30–32 particularly when end-ischemic bicarbonate levels are reduced.33 We postulated that an effect of tirilazad on SEP recovery would be evident when acidosis was severe and accompanied by low bicarbonate levels. Accordingly, we used a model of reversible incomplete cerebral ischemia that was severe and prolonged. Phosphorus magnetic resonance spectroscopy (MRS) was used.
to follow changes in intracellular pH (pHi) and high-energy phosphates. We tested the hypothesis that pretreatment with tirilazad mesylate improves SEP recovery and that improved SEP recovery correlates with accelerated pH and metabolic recovery during early reperfusion. A preliminary report has been presented.34

Materials and Methods

Male dogs weighing approximately 10 kg were anesthetized with fentanyl (50 μg/kg i.v.) and pentobarbital (10 mg/kg i.v.). Additional pentobarbital was infused continuously (3 mg/kg per hour) throughout the experiment. Lungs were mechanically ventilated with 25−30% O2, and end-tidal CO2 concentration was controlled. Femoral and axillary arteries were catheterized for cerebral blood flow (CBF) measurements with radiolabeled microspheres.30,31 Muscles were paralyzed with pancuronium bromide (0.3 mg/kg), and temporalis muscles were fully retracted. Through burr holes in the skull, catheters were inserted into the sagittal sinus and left lateral ventricle, and a thermistor was inserted into the epidural space. An electrode was secured in a burr hole in the skull over the right somatosensory cortex. Amplitude of the primary cortical wave complex of SEP with left foreleg stimulation was measured.30,31

Phosphorus MRS was performed with a 3.5-cm surface coil tuned to 32.5 MHz in a 1.89-T superconducting magnet (25-cm-bore diameter; Oxford Instruments, Oxford, UK) as previously described.27,30,31 Areas under the peaks corresponding to inorganic phosphate (P), phosphocreatine, and the β-phosphorus of adenosine triphosphate (ATP) were measured by planimetry and normalized by the corresponding area of the preischemic spectra.30 Intracellular pH was calculated from the chemical shift of P,30,35 Intracellular bicarbonate ion concentration ([HCO3−]) was estimated from the Henderson-Hasselbalch equation using the MRS-derived pH i and sagittal sinus partial pressure of CO2 (Pco2) as an estimate of mean tissue Pco2.30,31,33

To maintain normothermia while in the magnet, the body of the dog was wrapped in plastic and placed on a blanket perfused with warm water. A blanket of fiberglass insulation was placed above the skull and surface coil to reduce radiant heat loss. Global incomplete ischemia was produced by infusion of artificial cerebrospinal fluid from a 38°C pressurized reservoir through a line in a 38°C water jacket and into the lateral ventricular catheter. Intracranial pressure was kept to 10 to 15 mm Hg below mean arterial pressure while arterial pressure spontaneously changed. This procedure maintains CBF relatively constant throughout the ischemic period.31,33 After 30 minutes of ischemia, the fluid reservoir was disconnected, and intracranial pressure rapidly decreased. After 3 hours of reperfusion, the anesthetized dogs were killed by intravenous potassium chloride injection. A 200-mg sample of cortical gray matter was dried at 100°C for 48 hours to determine the fractional water content.

Phosphorus MRS spectra were analyzed in 15-minute epochs (225 free-induction decays) in duplicate before ischemia, in a 6-minute epoch followed by three 8-minute epochs during ischemia, in four 5-minute epochs during the first 20 minutes of reperfusion, and in 15-minute epochs for the remainder of reperfusion. Sagittal sinus Pco2 and SEP amplitude were measured at the midpoint of each MRS spectra. Arterial and sagittal sinus blood gases (ABL3, Radiometer, Copenhagen, Denmark), O2 content (CO-Oximeter 282, Instrumentation Laboratories, Lexington, Mass.), and glucose (23A, Yellow Springs Instrument Co., Yellow Springs, Ohio) were measured before ischemia, at 17 minutes of ischemia, and at 7.5, 30, 90, and 180 minutes of reperfusion. Radiolabeled microspheres (15±0.5-μm diameter; Du Pont-NEN Products, Boston, Mass.) were injected also at these time points, and CBF was calculated as previously described.30 Cerebral O2 consumption was calculated from the arterial−sagittal sinus O2 content difference and blood flow to the entire cerebrum.

Dogs were randomized into two groups. One group of eight dogs received 1 mg/kg of tirilazad mesylate (1.5 mg/mL) intravenously over a 10-minute period starting 20 minutes before ischemia. During 3 hours of reperfusion, tirilazad mesylate was continuously infused at a rate of 0.2 mg/kg per hour. A second group of eight dogs received the vehicle (0.02 M citric acid monohydrate, 0.0032 M sodium citrate dihydrate, 0.077 M NaCl, pH 3.0). Dogs with CBF values of less than 1 or greater than 14 mL/min per 100 g during ischemia were excluded. Investigators were blinded to the treatment and remained blinded until all data were analyzed and excluded dogs were replaced (two dogs in the vehicle group and one dog in the drug group).

Data were analyzed by two-way analysis of variance (ANOVA) in which group drug treatment was a between-subject factor and time was a within-subject factor. If there was a significant group effect or group×time interaction, mean values were compared between groups at individual time points using orthogonal contrasts. In addition, to obtain a measure of recovery rate based on all of the data of the recovery-time profile, mean recovery time of MRS-derived measurements was calculated by the stochastic approach of area/height, where area is the time integral of the difference between the recovery values and the preischemic value, and height is the difference between the end-ischemic and preischemic values. Mean recovery times were compared between groups by t test. Values of p<0.05 were considered significant in all tests. Values are presented as mean±SEM.

Results

Arterial blood gases were controlled during ischemia and reperfusion, and there were no differences between groups in arterial oxygen or glucose contents (Table 1). Arterial pH was slightly lower in the tirilazad group during reperfusion.

During ischemia, cerebral perfusion pressure was reduced to 11±1 mm Hg, CBF was reduced to 6±1 mL/min per 100 g, and cerebral O2 consumption was reduced to 0.6±0.1 mL O2 per minute per 100 g in both groups (Table 2). During reperfusion, cerebral perfusion pressure was rapidly restored to normal levels, and postischemic hyperemia at 7.5 minutes was equivalent in both groups. At 30 minutes of reperfusion, CBF remained elevated in the vehicle group but not in the tirilazad group. Delayed hypoperfusion was significant in both groups at 90 minutes. Cerebral O2 consumption recovered to preischemic levels, and there were no differences between groups. Epidural temperature was maintained at normothermic levels during ischemia and...
reperfusion. Intracranial pressure was significantly lower in the tirilazad group at 30 minutes of reperfusion. Fractional water content of cortical gray matter measured after 180 minutes of reperfusion was less in the tirilazad group (0.819±0.003) than in the vehicle group (0.831±0.002).

During incomplete ischemia, ATP and phosphocreatine were reduced nearly to undetectable levels (Figure 1). Recovery of ATP was rapid in both groups. However, two-way ANOVA indicated a significant treatment×time interaction for recovery of phosphocreatine and \( \text{Pi} \).

End-ischemic pH\(_i\) was 6.09±0.07 in the vehicle group and 5.98±0.08 in the tirilazad group (Figure 2). Estimated \([\text{HCO}_3^-]_i\) decreased to 1.7±0.3 mM in the vehicle group and to 1.5±0.2 mM in the tirilazad group. During reperfusion pH\(_i\) and \([\text{HCO}_3^-]_i\) eventually recovered to preischemic levels. However, two-way ANOVA indicated a significant effect of tirilazad on the pH\(_i\) and \([\text{HCO}_3^-]_i\) recovery profiles.

Recovery rates were analyzed by calculating mean recovery times, which integrate all data in the recovery profile. Mean recovery times of pH\(_i\), \([\text{HCO}_3^-]_i\), and \( \text{Pi} \) were reduced by half with tirilazad treatment (Figure

### Table 1. Arterial Blood Analysis Before, During, and After 30 Minutes of Incomplete Ischemia

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Ischemia</th>
<th>7.5</th>
<th>30</th>
<th>90</th>
<th>180</th>
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<td>pH</td>
<td></td>
<td></td>
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<td>Vehicle</td>
<td>7.39±0.01</td>
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<td>7.30±0.01</td>
<td>7.31±0.01</td>
<td>7.33±0.01</td>
<td>7.33±0.02</td>
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<td>Tirilazad</td>
<td>7.39±0.01</td>
<td>7.33±0.02</td>
<td>7.30±0.01</td>
<td>7.28±0.02</td>
<td>7.26±0.02*</td>
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<td>PCO(_2) (mm Hg)</td>
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<td>Vehicle</td>
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<td>39±1</td>
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<td>36±1</td>
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<td>Tirilazad</td>
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<td>42±2</td>
<td>38±1</td>
<td>38±1</td>
<td>39±1</td>
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<td>PO(_2) (mm Hg)</td>
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<tr>
<td>Vehicle</td>
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<td>112±6</td>
<td>113±5</td>
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<td>105±4</td>
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<td>113±6</td>
<td>109±5</td>
<td>106±6</td>
<td>102±5</td>
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<td>O(_2) content (mL/dL)</td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>Vehicle</td>
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<td>21.4±1.1</td>
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<tr>
<td>Tirilazad</td>
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<td>22.3±2.0</td>
<td>24.2±1.1</td>
<td>24.5±1.3</td>
<td>24.0±1.4</td>
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<tr>
<td>Glucose (mg/dL)</td>
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<tr>
<td>Vehicle</td>
<td>64±2</td>
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<tr>
<td>Tirilazad</td>
<td>72±12</td>
<td>72±11</td>
<td>57±5</td>
<td>60±7</td>
<td>60±12</td>
<td>63±14</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

*p<0.05 different from vehicle group.

### Table 2. Cerebral Hemodynamics Before, During, and After 30 Minutes of Incomplete Ischemia

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Ischemia</th>
<th>7.5</th>
<th>30</th>
<th>90</th>
<th>180</th>
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</thead>
<tbody>
<tr>
<td>Intracranial pressure (mm Hg)</td>
<td>Vehicle</td>
<td>17±1</td>
<td>123±18</td>
<td>27±3</td>
<td>17±2</td>
<td>16±2</td>
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<td></td>
<td>Tirilazad</td>
<td>14±2</td>
<td>143±19</td>
<td>23±4</td>
<td>11±2*</td>
<td>12±2</td>
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<tr>
<td>Cerebral perfusion pressure (mm Hg)</td>
<td>Vehicle</td>
<td>105±6</td>
<td>11±1</td>
<td>106±10</td>
<td>116±5</td>
<td>108±5</td>
</tr>
<tr>
<td></td>
<td>Tirilazad</td>
<td>105±7</td>
<td>11±1</td>
<td>128±13</td>
<td>126±7</td>
<td>104±8</td>
</tr>
<tr>
<td>Cerebral blood flow (mL/min per 100 g)</td>
<td>Vehicle</td>
<td>28±3</td>
<td>6±1</td>
<td>108±18</td>
<td>51±6</td>
<td>23±2</td>
</tr>
<tr>
<td></td>
<td>Tirilazad</td>
<td>30±3</td>
<td>6±1</td>
<td>112±23</td>
<td>36±5*</td>
<td>23±1</td>
</tr>
<tr>
<td>Arteriovenous O(_2) content difference (mL/dL)</td>
<td>Vehicle</td>
<td>12.0±0.7</td>
<td>8.6±1.4</td>
<td>5.0±1.2</td>
<td>6.3±1.0</td>
<td>13.2±0.8</td>
</tr>
<tr>
<td></td>
<td>Tirilazad</td>
<td>11.2±0.6</td>
<td>9.9±1.2</td>
<td>3.6±0.5</td>
<td>9.9±1.3*</td>
<td>15.1±1.4</td>
</tr>
<tr>
<td>Cerebral O(_2) consumption (mL/min per 100 g)</td>
<td>Vehicle</td>
<td>3.27±0.27</td>
<td>0.63±0.13</td>
<td>4.06±0.44</td>
<td>2.93±0.27</td>
<td>3.02±0.29</td>
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<td>Tirilazad</td>
<td>3.31±0.30</td>
<td>0.65±0.15</td>
<td>3.66±0.67</td>
<td>3.33±0.43</td>
<td>3.43±0.29</td>
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<tr>
<td>Epidural temperature (°C)</td>
<td>Vehicle</td>
<td>36.7±0.2</td>
<td>37.4±0.3</td>
<td>...</td>
<td>37.6±0.3</td>
<td>37.7±0.4</td>
</tr>
<tr>
<td></td>
<td>Tirilazad</td>
<td>37.0±0.5</td>
<td>38.0±0.3</td>
<td>...</td>
<td>37.6±0.3</td>
<td>37.8±0.3</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

*p<0.05 different from vehicle group.
3). Mean recovery times of ATP and phosphocreatine were not significantly reduced.

Baseline SEP amplitude was similar in the vehicle (86±19 μV) and tirilazad (98±12 μV) groups. In all dogs, SEP was isoelectric from 3 minutes through 30 minutes of ischemia. By 2.5 minutes of reperfusion, SEP waveforms were detectable in two of eight vehicle-treated dogs and five of eight tirilazad-treated dogs. Percent recovery of SEP amplitude was significantly greater in the tirilazad group at 90 minutes of reperfusion and thereafter (Figure 4). In individual data pooled from both groups, percent recovery of SEP amplitude at 180 minutes of reperfusion was correlated with mean recovery time of P, (r=0.69), pH, (r=0.53), and [HCO₃⁻], (r=0.60), but not with that of ATP (r=0.11) or phosphocreatine (r=0.31).

Discussion

The principal findings of this randomized, blinded study on anesthetized dogs are that treatment with the 21-aminosteroid tirilazad before 30 minutes of severe incomplete global cerebral ischemia 1) doubles the recovery rate of pH, [HCO₃⁻], and P, during early reperfusion, 2) improves recovery of SEP amplitude, and 3) reduces cerebral edema. The drug does not appear to act by reducing the severity of the ischemic insult because the reductions in CBF, cerebral O₂ consumption, high-energy phosphates, pH, and SEP were equivalent in the vehicle and drug groups during ischemia. In addition, the effect of the drug was not attributable to hypothermia because epidural temperature was maintained in both groups.

Studies of the drug's properties indicate that tirilazad is a potent inhibitor of lipid peroxidation in vitro. In brain tissue homogenates tirilazad is a more potent inhibitor than vitamin E. The importance of lipid peroxidation as a mediator of ischemic/reperfusion injury in brain is unclear. Postischemic increases in conjugated diene formation have been reported, but the magnitude of these changes is often relatively small and spatially heterogeneous. On the other hand, posts ischemic depletion of vitamin E in gerbil is attenuated by tirilazad treatment. Indirect measurements of lipid peroxidation were not obtained in the present study.
lower cortical water content at 3 hours of reperfusion support the hypothesis of improved electrolyte recovery with tirilazad, consistent with observations during focal ischemia. Second, tirilazad may improve recovery of mitochondrial function and thereby reduce postischemic lactic acid production. Rapid recovery of ATP and cerebral O₂ consumption in both groups argues against major mitochondrial dysfunction in this model of normoglycemic ischemia. Nevertheless, dysfunction in a small portion of mitochondria undetected by global ATP and O₂ consumption measurements could give rise to substantial lactate accumulation in a subpopulation of cells. Because such a subpopulation would likely have elevated P₁ and because MRS measurements of pHᵢ are heavily weighted by compartments containing most of the Pᵢ, the more rapid recovery of pHᵢ in our study may reflect rapid recovery in a subpopulation of selectively vulnerable neurons or selectively acidic astrocytes.

Third, faster pHᵢ recovery also might be attributable to more effective reperfusion. The lack of effect of tirilazad on global CBF during the early hyperemia or the delayed hypoperfusion argues against this possibility. However, tirilazad is concentrated in plasma membranes of brain endothelial cells in culture. In vivo, the drug’s hydrophobicity may limit diffusion to neurons beyond the endothelial membrane. Moreover, ischemia/reperfusion can cause superoxide dismutase–inhibitable changes in endothelial membrane fluidity. Superoxide anion is detectable in the extracellular space surrounding cerebral vessels and production is most prominent during early reperfusion. Thus, based on the site of oxygen radical production and on the hydrophobicity of tirilazad, our findings do not exclude the possibilities that 1) tirilazad acts by improving endothelial transport processes or control of capillary microflow undetected by microspheres, or 2) that release of toxic oxygen species from leukocytes and monocytes is inhibited, as reported for related 21-aminosteroids, which in turn could affect endothelial function or microflow.

After 10 minutes of carotid occlusion plus arterial hypotension in rats, Haraldseth et al observed faster ATP and phosphocreatine but not pHᵢ recovery with tirilazad treatment. The lack of effect on pHᵢ recovery may be related to the less severe level of acidosis during ischemia in their model (pHᵢ, 6.55) than in our model (pHᵢ, 6.09). On the other hand, the lack of effect of tirilazad on ATP recovery rate in our study may be related to less temporal resolution associated with the lower magnetic field strength in our study (1.89 versus 4.7 T). Consistent with their data, we did find greater phosphocreatine during early reperfusion as indicated by the drug × time interaction with ANOVA. However, in our study much of this difference could be attributed to the effect of pHᵢ on creatine kinase equilibrium. Despite some of these differences, our results are in general agreement with Haraldseth et al that tirilazad exerts an effect during the early minutes of reperfusion. In addition, their observation that tirilazad was equally effective when administered either before or at the end of ischemia further supports the premise that the drug acts during reperfusion.

We also observed that recovery of SEP amplitude was improved by tirilazad and that this improvement correlated with mean recovery time of pHᵢ. A similar correlation was observed after complete ischemia of different

**Figure 3.** Bar graph shows mean recovery time of adenosine triphosphate (ATP), phosphocreatine (PCr), inorganic phosphate (Pᵢ), intracellular pH (pHᵢ), and intracellular bicarbonate ion concentration ([HCO₃⁻]) after 30 minutes of incomplete ischemia in dogs pretreated with vehicle or tirilazad. Bars represent SEM. *p<0.05 different from vehicle group.

However, our data demonstrating a doubling of pHᵢ and Pᵢ recovery rates during the first half hour of reperfusion indicate that the effect of the drug is expressed during early reperfusion. If the drug’s mechanism of action in vivo is inhibition of lipid peroxidation, then our data indicate that lipid peroxidation can occur during early reperfusion after severe incomplete ischemia and that the magnitude of peroxidation is sufficient to exert a physiological effect on restoration of brain pH and on electrophysiological deficit. Moreover, the observation that pHᵢ and [HCO₃⁻], recovery can be accelerated by drug treatment implies that postischemic normalization of buffering capacity is not merely a passive process.

There are several potential explanations for more rapid pHᵢ recovery. First, plasma membrane ionic conductance may be more rapidly restored by tirilazad, which would improve antipporter-dependent pHᵢ recovery. The lower intracranial pressure at 30 minutes and
durations.\textsuperscript{30} Moreover, delaying pH\textsubscript{i} recovery with acetazolamide or hypercapnic reperfusion resulted in depressed SEP recovery.\textsuperscript{32} These data taken together with the present results of accelerated pH\textsubscript{i} recovery associated with improved SEP recovery are consistent with the hypothesis that part of the postischemic electrophysiological deficit is attributable to the magnitude and duration of acidosis during the reperfusion period. Acidosis promotes lipid peroxidation in vitro.\textsuperscript{28} By inhibiting initial lipid peroxidation and limiting the duration of severe acidosis, tirilazad may act to limit further lipid peroxidation and improve SEP conduction. Furthermore, resolution of postischemic hyperemia corresponds temporally to resolution of acidosis.\textsuperscript{30,32} In the present study, more rapid pH\textsubscript{i} recovery with tirilazad was also associated with quicker return of CBF, as indicated by the 30-minute measurements. Thus, by limiting the duration of hyperemia and consequent overoxygenation of tissue, tirilazad may also act to limit further peroxidation. Therefore, efficacy of the drug in vivo may be amplified by the integration of several interdependent mechanisms.

It should be appreciated that we did not address whether the shortened duration of postischemic acidosis and the modest improvement in SEP amplitude with tirilazad lead to improved histological or neurological outcome. Moreover, tirilazad did not fully restore SEP amplitude to preischemic levels, thereby indicating that additional mechanisms of cell injury may be operational. These may include excitotoxic mechanisms or white matter edema unaffected by tirilazad treatment. In neuronal culture, tirilazad ameliorates cell death from N-methyl-D-aspartate administration and from oxygen and glucose deprivation, but the protection is only partial.\textsuperscript{42} Thus, one component of the excitotoxic injury process may not depend on lipid peroxidation, and this component may prevent complete SEP recovery. A second component that depends on lipid peroxidation may be pH sensitive. This hypothesis may explain why tirilazad does not offer full neuroprotection\textsuperscript{25,26} or offers protection in only selected regions\textsuperscript{23} in models involving carotid occlusion plus hypotension in which excitotoxic mechanisms presumably predominate. In this type of incomplete ischemia model, the level of acidosis may be less severe, particularly when ischemic duration is limited to 10 minutes,\textsuperscript{19} compared with our study, in which CBF was reduced by 80\% for 30 minutes.

In contrast to the present results with 30 minutes of incomplete ischemia, we previously observed no effect of tirilazad on early metabolic recovery or SEP recovery after 12 minutes of complete ischemia despite similar reductions in end-ischemic pH\textsubscript{i}.\textsuperscript{47} Lack of effect of tirilazad with complete ischemia was attributed either to use of a suboptimal drug dose for the degree of lipid peroxidation that might occur with complete ischemia, possible traumatic injury associated with much greater intracranial hypertension used to achieve complete ischemia, or to mechanisms other than lipid peroxidation limiting recovery when ischemia is complete. An additional difference between complete and incomplete ischemia is that at equivalent reductions in pH\textsubscript{i}, [HCO\textsubscript{3}⁻] is greater during complete ischemia secondary to carboxidic acidosis. For example, end-ischemic [HCO\textsubscript{3}⁻] was estimated at 1–2 mM in the present study but 2–6 mM during complete ischemia.\textsuperscript{27,30,33} We previously found that SEP recovery corresponded better with end-ischemic [HCO\textsubscript{3}⁻] than with end-ischemic pH\textsubscript{i}.\textsuperscript{32} Rehncrona et al\textsuperscript{32} proposed that iron mobilization sufficient to augment lipid peroxidation occurs only when low pH is accompanied by low [HCO\textsubscript{3}⁻]. Thus, the impact of tirilazad on early metabolic and electrophysiological recovery may be more evident when the severity of metabolic acidosis is sufficient to reduce [HCO\textsubscript{3}⁻] to less than 2 mM. This situation is prominent when incomplete ischemia is severe and prolonged.

Therefore, our results indicate that tirilazad exerts an effect on physiological recovery during early reperfusion as evidenced by accelerated pH\textsubscript{i} recovery, greater SEP recovery, and attenuated edema. We suggest that this early effect is most prominent with severe acidosis accompanied by low [HCO\textsubscript{3}⁻]. Our focus was on early reperfusion kinetics, and our results do not address the therapeutic impact of early improvement of SEP and pH\textsubscript{i} recovery. Furthermore, these results do not exclude that tirilazad may also act later during reperfusion because improvement in neurological outcome after complete ischemia has been reported in some laboratories.\textsuperscript{22,24}

Acknowledgments

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References

In a carefully conducted, randomized, and blinded study, Maruki et al have shown that low doses of tirilazad mesylate (one of the 21-aminosteroid family) improve early metabolic and functional recovery after severe incomplete cerebral ischemia in the dog. Previously, they found no protection with tirilazad mesylate in total cerebral ischemia in dogs. The aminosteroids are hydrophobic and potent inhibitors of lipid peroxidation. They do not readily cross the blood–brain barrier, suggesting that the site of action involves the microvasculature. Similar conclusions have been reached with the superoxide scavenger polyethylene glycol–conjugated superoxide dismutase, which also does not readily cross the blood–brain barrier and yet can reduce injury in cerebral ischemia. This growing literature strongly supports a role of oxygen radicals in microvascular injury to the brain. The low doses of tirilazad mesylate used in this study (1 mg/kg) are far too low to directly scavenge hydroxyl radical. The hydrophobicity of tirilazad suggests that the most likely mechanism involves lipid and fatty acid peroxidation products or possibly a direct attack on membrane proteins. The exciting challenge remains to identify how tirilazad mesylate protects in partial but not total ischemia in dogs.

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