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(Stroke 1988;19:340-344)

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Extensive studies have shown that temporary cerebral ischemia results in an initial period of postischemic brain hyperperfusion followed by a sustained and progressive hypoperfusion, the extent of which depends upon the severity and duration of the ischemic period. Recent work has supported the concept that postischemic hypoperfusion can be a primary factor limiting recovery of cerebral energy metabolism. Furthermore, the delayed decrease in cerebral blood flow may, if of sufficient magnitude, produce a secondary ischemic brain insult.

While the mechanism(s) of postischemic hypoperfusion are unknown, some investigators have postulated that oxygen-free-radical–induced microvascular lipid peroxidation (LP) may be a contributing factor. Likewise, progressive microvascular LP has been convincingly implicated in relation to development of posttraumatic central nervous system (CNS) ischemia (i.e., hypoperfusion). Thus, considering the possible involvement of oxygen radicals and LP in delayed hypoperfusion, we have investigated the ability of the nonglucocorticoid 21-aminosteroid U74006F to attenuate cerebral hypoperfusion in cats after a brief episode of near-complete global brain ischemia. U74006F has been shown to be an extremely potent and effective inhibitor of iron-dependent LP in CNS tissue in vitro. Other studies have demonstrated an action of U74006F to retard the development of posttraumatic spinal cord ischemia.

Materials and Methods

General

Adult female specific-pathogen-free cats from Liberty Laboratories (Liberty Corners, New Jersey) weighing 2.5–3.5 kg were anesthetized with 50 mg/kg i.v. α-chloralose in 0.9% saline. A tracheostomy was performed, and femoral venous and arterial cannulas were inserted unilaterally. The cats were then placed in a Kopf stereotactic head frame (Tujunga, California), and positive pressure ventilation was instituted using a Harvard 607B respirator (South Natick, Massachusetts). A 20-mg bolus of gallamine triethiodide was given intravenously to induce neuromuscular paralysis, which was maintained throughout the experiment by slow 5 mg/hr i.v. infusion. \text{Paco}_2, \text{Pao}_2, \text{pH} \text{ were measured with an IL 1301 blood gas analyzer (Instrumentation Laboratories, Dayton, Ohio) and respiration was adjusted to maintain normal postanesthesia, pre-paralysis values. Rectal temperature was monitored and maintained between 37° and 38° C with a heat lamp and Vetco thermal barrier (Harvard). A calvarium evacuation was performed, and a 21-gauge ¼-in. butterfly cannula was inserted into the cisterna magna and sealed in place with cyanoacrylate for the monitoring of intracranial pressure (ICP). Mean arterial blood pressure (MABP) was recorded with a Grass model 7D polygraph (Quincy, Massachusetts).

Two 5 × 5-mm burr holes were drilled through the skull. One burr hole was drilled 2 mm to the right of the sagittal suture and 2 mm anterior to the coronal suture, into which a pair of bipolar electrodes were placed on the dural surface to record somatosensory evoked potentials (SEPs) in response to bipolar stimulation (1.0 V, 0.4 msec, 1 Hz) of the contralateral sciatic nerve. At specified times, 100 successive SEPs were amplified one thousand times with a W-P Instruments DAM-5A AC preamplifier (New Haven, Connecticut) and summed with a Tracor Northern 1550 signal averager (Middleton, Wisconsin).

Cortical blood flow (CBF) was measured using the hydrogen clearance technique. A 125-μm-diameter nail-polish–insulated, platinum–iridium wire was inserted stereotactically through the second burr hole, which was placed 2 mm to the left of the sagittal suture.
and 2 mm posterior to the coronal suture. The electrode was placed 1.5 mm into the cortex.

The blood flow electrode was polarized to +350 mV with respect to a subcutaneous reference electrode, which consisted of a silver-silver chloride pellet resting in a glass syringe plugged at the tip with agar and filled with saturated KCl solution. Hydrogen was administered to the cats by bleeding the gas into the intake line of the respirator at a rate that did not cause significant hypoxia in the cat (approximately 7%). The current generated by the oxidation of hydrogen at the electrode tip was monitored on a Sargent-Welch model 3001 DC polarograph (Skokie, Illinois). Following hydrogen inspiration, the current declined as the tissue concentration of hydrogen fell. CBF was derived from the hydrogen clearance curves using the Fick principle equation

\[ CBF = \frac{(0.693 \div t/2) \times 100}{t} \]

where CBF is expressed in milliliters per 100 grams of tissue per minute, \( t/2 \) is the time in minutes for the current to decay by one half the peak value, and 0.693 is the natural logarithm function constant.

**Global Ischemia**

Following a control period in which at least three stable CBFs and SEPs were obtained, a 5-minute episode of near-total brain ischemia was induced in seven cats according to the tourniquet method of Marcy and Welsh. The beginning of the 5-minute episode was taken as the point at which hydrogen clearance nearly ceased (i.e., CBF < 2 ml/100 g/min), SEPs disappeared, and the pupils dilated fully. At the end of the 5-minute episode, the tourniquet was quickly removed. The cisterna magna ICP cannula was inserted after the ischemic episode. ICP in the initial minutes after ischemia stayed elevated in the initial minutes after ischemia and to approximately 50% by 30 minutes. In ischemia, hypotension was maintained for the duration of the 3-hour postischemic experimental period. ICP increased to >20 mm Hg in the initial minutes after ischemia. However, ICP quickly returned to normal, where it remained throughout the experiment.

**Drug Administration**

U74006F (21-[4-(2,6-di-1-pyrrolidinyl-4-pyrimidinyl)-1-piperazinyl]-16α-methyl-pregna-1,4,9(11)-triene-3,20-dione; monomethane sulfonate) was dissolved in 0.05N HCl in distilled water and injected as a 1.0 ml/kg i.v. dose of 0.05N HC1 alone. The vehicle- and U74006F-treated cats received the injection 15 minutes after the ischemic episode.

**Statistical Analysis**

Differences between vehicle- and U74006F-treated cats were analyzed at individual time points using a one-way analysis of variance (ANOVA). This, instead of a repeated-measures ANOVA of the entire time course, was deemed appropriate since the vehicle- and U74006F-treated groups diverged from the same values.

**Effects of U74006F in Nonischemic Cats**

A 1.0 mg/kg i.v. dose of U74006F was administered to six anesthetized cats not subjected to the 5-minute episode of global brain ischemia. In these six nonischemic cats, U74006F did not significantly affect CBF, MABP, or SEP amplitude (initial positive wave) (Table 1).

**Effects of U74006F After Ischemia**

Induction of near-total global ischemia via tourniquet application resulted in a sustained increase in arterial blood pressure. Accompanying hypertension was a reflex decrease in heart rate, which, however, did not last throughout the ischemic episode. After approximately 1 minute, the heart rate increased above the preischemic level; this may have been due to either tourniquet compression of the vagus nerves or to ischemic depression of brainstem vagal nuclear discharge. Following removal of the tourniquet, there was an immediate and dramatic fall in blood pressure. However, over the next few minutes, blood pressure increased somewhat, but a significant postischemic hypotension was maintained for the duration of the 3-hour postischemic experimental period. ICP increased to >20 mm Hg in the initial minutes after ischemia. However, ICP quickly returned to normal, where it remained throughout the experiment.

**Table 1. Lack of Effect of U74006F on CBF, MABP, and SEP Amplitude in Six Nonischemic Control Cats**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>CBF Before</th>
<th>CBF 5</th>
<th>CBF 30</th>
<th>CBF 60</th>
<th>CBF 90</th>
<th>CBF 120</th>
<th>CBF 150</th>
<th>CBF 180</th>
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</thead>
<tbody>
<tr>
<td>5</td>
<td>39.8±4.2</td>
<td>39.7±5.7</td>
<td>39.6±6.6</td>
<td>37.6±2.8</td>
<td>42.0±5.4</td>
<td>42.2±6.8</td>
<td>38.9±5.7</td>
<td>38.8±6.5</td>
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<tr>
<td>30</td>
<td>146.8±6.9</td>
<td>153.0±6.7</td>
<td>154.7±5.4</td>
<td>154.7±4.2</td>
<td>155.8±4.0</td>
<td>152.2±3.5</td>
<td>148.6±4.0</td>
<td>143.6±3.1</td>
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<tr>
<td>60</td>
<td>33.1±6.6</td>
<td>32.8±6.5</td>
<td>34.3±6.7</td>
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<td>35.8±7.1</td>
<td>35.1±6.7</td>
<td>39.1±7.2</td>
<td>37.1±7.0</td>
</tr>
</tbody>
</table>

CBF, cortical blood flow in ml/100 g/min; MABP, mean arterial blood pressure in mm Hg; SEP, somatosensory evoked potential in μV. Values are mean±SEM.
Figure 1. Recovery of somatosensory evoked potentials (SEPs) after 5-minute episode of near-total global brain ischemia in vehicle- vs. U74006F-treated cats. Values are mean ± SEM for $N$ cats. *Significantly different ($p < 0.05$) from vehicle at same time by one-way analysis of variance. Absolute preischemia values were comparable in both groups.

Figure 2 shows that at 5 minutes after the ischemic episode, CBF in both vehicle- and U74006F-treated cats was elevated by approximately 30% above the preischemic level. This cortical hyperperfusion was shortlived, giving way to hypoperfusion as CBF fell to 20% below control by 30 minutes after ischemia in the vehicle-treated cats; CBF continued to fall to a level 71.7% below the preischemic level at 3 hours.

In absolute terms, this represented a fall from a mean of 51.0 to 14.5 ml/100 g/min, which is below the critical threshold for brain electrical failure and thus may explain the declining SEP (Figure 1).

U74006F treatment, on the other hand, produced a significant maintenance of CBF compared with vehicle treatment from 2–3 hours after ischemia, with CBF falling by only 45.7% ($p < 0.04$, one-way ANOVA compared with vehicle) in relation to its preischemic level. In absolute terms, CBF only declined from a mean of 52.4 to 28.5 ml/100 g/min. At 3 hours after ischemia, CBF in the U74006F-treated cats was nearly twice that observed in the vehicle-treated cats.

Figure 3 indicates that MABP, like CBF, was better maintained in the U74006F-treated cats. In vehicle-treated cats, MABP fell progressively over the experimental time course, with one of the six cats actually dying just before 2.5 hours after ischemia. In contrast, U74006F treatment stabilized MABP, and no cats died.

Finally, Table 2 presents a comparison of the Paco$_2$, PaO$_2$, and pH values between vehicle- and U74006F-treated cats. Immediately after ischemia, Paco$_2$ was elevated, which probably explains the early hyperemia (Figure 2), but gradually returned to the presischemic level. The observation that CBF fell below normal before Paco$_2$ returned to baseline shows a loss of CBF autoregulation. There were no significant differences in Paco$_2$ between groups.

In contrast to the elevated Paco$_2$, PaO$_2$ was depressed in both groups of cats, albeit still within the normal range (i.e., >65 mm Hg). From 30 minutes to 2 hours after ischemia, PaO$_2$ was higher in the U74006F-treated cats. However, this difference was not significant. While the basis for the decrease in PaO$_2$ is unclear, it may be the result of pulmonary edema secondary to the pronounced hypertension during the ischemic episode.

The 5-minute ischemic episode also resulted in a significant lowering of arterial blood pH. From 5 minutes on, there was a gradual recovery from this acidosis. However, by 3 hours after ischemia, pH in the vehicle-treated cats remained significantly below normal. U74006F treatment significantly increased the pH.
Discussion

Our results show that the nonglucocorticoid 21-aminosteroid U74006F can attenuate cerebral hypoperfusion secondary to a brief period of global ischemia. The improved maintenance of CBF after ischemia was also associated with facilitated recovery of brain electrical activity (i.e., SEP).

The mechanism for the improved maintenance in CBF and the associated neurophysiologic recovery in the U74006F-treated cats is probably twofold. First, the fact that U74006F also enhanced the postischemic maintenance of MABP suggests that better support of cerebral perfusion pressure (CPP) may play a role. In respect to improved MABP and CPP, either a peripheral action of U74006F on the heart and resistance vessels or a protection of the brainstem cardiovascular centers and sympathetic outflow could be involved. However, it is clear from the work of others1-4 that delayed cerebral hypoperfusion is not simply the result of a postischemic decrease in CPP. As a second possibility, local cerebral microvascular influences have been postulated to be even more important in the progressive reduction of CBF that follows global brain ischemia. These include excessive microvascular smooth muscle calcium influx, 13 vasoactive prostanoids (e.g., prostaglandin F3, thromboxane A3)15-16 and oxygen-free-radical-generated microvascular LP. 7,3 In the instance of posttraumatic CNS hypoperfusion, recent studies have suggested that these three factors operate in concert within the injured tissue to produce a progressive decrease in microvascular perfusion.9

Thus, the ability of U74006F to antagonize the development of postischemic hypoperfusion may be only in part the result of better maintenance of MABP and CPP. In addition, a direct protective effect on the cerebral microvasculature is probably involved as well. This view is supported by a similar reduction of posttraumatic CNS hypoperfusion by U74006F without an effect on systemic blood pressure.10 The most likely mechanism in this regard concerns the documented ability of U74006F to effectively inhibit iron-dependent LP in CNS tissue.10 As noted above,
microvascular LP has been suggested to play a critical role in posts ischemic cerebral hypoperfusion. Consistent with this view, parallel studies have shown that chronic pretreatment of cats with d-α-tocopherol can also reduce posts ischemic hypoperfusion with no effect on the associated hypotension (E.D. Hall and P.A. Yonkers, unpublished observations). Similarly, intensive dosing with vitamin E and selenium has been found to inhibit the development of posttraumatic spinal cord ischemia but not posttraumatic hypotension.

In summary, the novel 21-aminosteroid U74006F has been shown to retard the development of posts ischemic cerebral hypoperfusion. This effect is probably due to improved maintenance of MABP and CPP with a direct protective action on the cerebral microvasculature, which may involve inhibition of microvascular LP damage. Additional studies are planned to assess in further detail the therapeutic mechanisms and potential of U74006F in CNS ischemia.

References

Key Words • cerebral ischemia • intracranial pressure • lipid peroxides • steroid • subarachnoid hemorrhage
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