Attenuation of Postischemic Cerebral Hypoperfusion by the 21-Aminosteroid U74006F

Edward D. Hall, PhD, and Patricia A. Yonkers, BS

The ability of the nonglucocorticoid 21-aminosteroid U74006F, a potent inhibitor of iron-dependent lipid peroxidation, to antagonize progressive brain hypoperfusion after a 5-minute episode of global brain ischemia was examined in α-chloralose-anesthetized cats. Immediately after a 5-minute episode of near-total tourniquet-induced brain ischemia, cortical blood flow returned to normal or above normal. Thereafter, cortical blood flow fell progressively to a level 71.7% below normal by 3 hours after ischemia. In contrast, in cats that received 1 mg/kg i.v. U74006F 15 minutes after the ischemic episode, cortical blood flow remained significantly greater than that seen in vehicle-treated cats. At 3 hours, cortical blood flow had declined by only 45.7% (p < 0.04 compared with vehicle). In addition, U74006F treatment significantly improved postischemic maintenance of blood pressure and recovery of somatosensory evoked potentials and reduced postischemic arterial blood acidosis. U74006F had no effect on cortical blood flow, somatosensory evoked potentials, or blood pressure in nonischemic cats. Our results suggest that U74006F may be useful in the early treatment of global cerebral ischemia.

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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

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

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 was <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.

**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. bolus (1.0 mg/ml) followed by a 1.0 ml saline flush. Vehicle-treated cats received a 1.0 ml/kg i.v. bolus of 0.05N HC1 alone. The vehicle- and U74006F-treated groups diverged from the same values.

**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.

There was total suppression of SEPs during the ischemic episode. The time course of postischemic SEP recovery is shown in Figure 1. In both vehicle- and U74006F-treated cats, the SEP (initial positive wave) recovered by approximately 8% by 5 minutes after ischemia and to approximately 50% by 30 minutes. In vehicle-treated cats, SEP recovery plateaued from 30 to 90 minutes before falling off gradually thereafter to a mean level of 71.5% below the preischemia level at 3 hours. In contrast, U74006F-treated cats showed a progressive increase in SEP amplitude throughout the experiment. At 2.5 and 3 hours, mean SEP amplitude was significantly greater than in the vehicle-treated group.

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

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>5</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
<th>150</th>
<th>180</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBF</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>
</tr>
<tr>
<td>MABP</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>
</tr>
<tr>
<td>SEP</td>
<td>33.1±6.6</td>
<td>32.8±6.5</td>
<td>34.3±6.7</td>
<td>35.7±7.1</td>
<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 3 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 failure13 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 Paco₂, Pao₂, and pH values between vehicle- and U74006F-treated cats. Immediately after ischemia, Paco₂ was elevated, which probably explains the early hyperemia (Figure 2), but gradually returned to the preischemic level. The observation that CBF fell below normal before Paco₂ returned to baseline shows a loss of CBF autoregulation. There were no significant differences in Paco₂ between groups.

In contrast to the elevated Paco₂, Pao₂ 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, Pao₂ was higher in the U74006F-treated cats. However, this difference was not significant. While the basis for the decrease in Pao₂ 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.
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 others 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, vasoactive prostanoids (e.g., prostaglandin F_2, thromboxane A_2), and oxygen-free-radical-generated microvascular LP. 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.

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. The most likely mechanism in this regard concerns the documented ability of U74006F to effectively inhibit iron-dependent LP in CNS tissue.

### TABLE 2. PacO_2, PacO_2, and pH in Vehicle- and U74006F-Treated Cats Before and for 3 Hours After 5 Minutes of Near-Complete Global Brain Ischemia

<table>
<thead>
<tr>
<th></th>
<th>Pre-Ischemia (min)</th>
<th>Postischemia (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td><strong>PacO_2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>25.1 ± 0.5</td>
<td>34.9 ± 3.3</td>
</tr>
<tr>
<td>U74006F</td>
<td>25.3 ± 0.9</td>
<td>34.9 ± 2.3</td>
</tr>
<tr>
<td><strong>PacO_2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>97.6 ± 2.2</td>
<td>70.8 ± 9.0</td>
</tr>
<tr>
<td>U74006F</td>
<td>92.9 ± 2.2</td>
<td>77.9 ± 3.5</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>7.30 ± 0.03</td>
<td>7.16 ± 0.03</td>
</tr>
<tr>
<td>U74006F</td>
<td>7.31 ± 0.01</td>
<td>7.12 ± 0.01</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. Vehicle or U74006F was injected 15 minutes after ischemic episode. *p<0.05 by paired t test compared with preischemic (only 3 hour time was analyzed). †p<0.05 by one-way analysis of variance vs. vehicle at same time.
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-alpha 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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