Effect of the Aminosteroid U74006F After Cardiopulmonary Arrest in Dogs

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The oxygen free radical–induced lipid peroxidative reactions that occur during resuscitation from normothermic cardiac arrest may contribute to the degree of neurologic dysfunction sustained. A blinded, randomized experimental trial was performed to determine whether U74006F, a potent inhibitor of lipid peroxidation, reduces morbidity and 24-hour mortality after 10 minutes of normothermic cardiopulmonary arrest; ventricular fibrillation was induced by electrical stimulation in 24 open-chest, halothane-anesthetized dogs, and circulation was reestablished by direct cardiac compressions, administration of a standardized drug regime, and internal countershocks. When spontaneous circulation was restored, a bolus injection of 1.5 mg/kg U74006F (n = 12) or 25 mM citrate vehicle (n = 12) was infused intravenously in 15 minutes and an infusion was continued at 0.125 mg/kg/hr for the next 12 hours. In the drug-treated group, plasma U74006F concentration averaged 0.13 μg/mL between 3 and 12 hours after cardiac arrest. By 24 hours after arrest, 10 of 12 (83%) vehicle-treated dogs had died but only four of 12 (33%) U74006F-treated dogs had died (p = 0.017). U74006F-treated dogs survived significantly longer (mean ± SEM 22 ± 1 hr) than vehicle-treated dogs (18 ± 1 hr), with significantly better neurologic function 1, 2, and 24 hours after arrest. Plasma fatty acid hydroperoxide concentrations 12 hours after arrest were 88 ± 81 pmol/mL in U74006F-treated and 241 ± 49 pmol/mL in vehicle-treated dogs (p < 0.05). Vitamin E concentrations were significantly higher in the plasma of U74006F-treated dogs 2, 3, and 6 hours after arrest compared with vehicle-treated dogs. We conclude that the nonglucocorticoid 21-aminosteroid U74006F reduces mortality and sensorimotor deficit after transient global cerebral ischemia, perhaps by inhibiting lipid hydroperoxide formation. (Stroke 1988;19:1371–1378)
Materials and Methods

Twenty-four adult male mongrel dogs weighing 14.2–22.2 kg were fasted for 24 hours. Thirty minutes after they were premedicated with 1.5 mg/kg i.m. morphine sulfate, anesthesia was induced in the dogs with 5% halothane in oxygen via face mask (Foregger, Compact-75, Puritan-Bennett Corp., Westmont, Illinois). The dogs were intubated and mechanically ventilated with 1–2% halothane in oxygen to maintain surgical analgesia and suppression of corneal reflexes. Paralyzing agents were not used. Expired CO₂ tension was monitored and maintained between 3% and 5% (Beckman LB-2, Sensor Medics Co., Anaheim, California). Ventilation and bicarbonate administration were manipulated to maintain arterial blood pH between 7.38 and 7.41 (Model 113, Instrumentation Laboratories, Lexington, Massachusetts). Blood glucose concentration was determined spectrophotometrically (Seralyzer reflectance photometer, Miles Laboratories, Inc., Elkhart, Indiana). Deep esophageal temperature was monitored and maintained at 39.0±1.0° C before arrest with a heating pad and proportional controller. A urethral catheter was inserted. Two venous catheters were inserted; one passed through the left external jugular vein to the right atrium for drug administration, and the other passed into a muscular branch of the femoral vein for fluid administration. Arterial blood pressures were measured through a catheter placed in a muscular branch of the femoral artery (Statham P23XL transducer, Gould Inc., Oxnard, California). Subcutaneous disk electrodes were placed to monitor lead II electrocardiogram (ECG).

The left chest was opened at the fifth intercostal space, and the pericardial sac was opened to facilitate direct cardiac compression. All catheters and electrical leads were passed subcutaneously to exit the skin in the dorsal midscapular region for later attachment to the dog jacket and hydraulic/electric swivel. The arterial blood pressure, heart rate, and expired CO₂ were continuously recorded on a six-channel oscillograph (Model 200, Gould-Brush, Cleveland, Ohio). Each dog received 500 ml physiological saline via the saphenous vein to assure adequate hydration before arrest.

Halothane was discontinued and ventilation was continued (Model 607, Harvard, Millis, Massachusetts) with room air to reduce and standardize the level of anesthesia at which ventricular fibrillation was induced. As soon as corneal reflexes returned (stage 3, plane 1 of surgical anesthesia), the heart was fibrillated by delivering a 10–15-second, 60-Hz, 2-msec square-wave stimulus to the left ventricular epicardium; circulatory arrest was confirmed by the ECG, arterial blood pressure, and direct observation of the heart.

After 10 minutes of normothermic ventricular fibrillation, direct cardiac massage maintained mean arterial blood pressure (MABP) above 75 mm Hg. Vasopressor support was initiated by an injection of 40 µg/kg epinephrine and either 6 µg/kg/min epinephrine in 13 dogs (six U74006F-treated and seven vehicle-treated) or 10 µg/kg/min dopamine in 11 dogs (six U74006F-treated and five vehicle-treated). This injection was followed in rapid succession by central intravenous administration of 1) 1 mg/kg lidocaine, 2) 4 meq/kg sodium bicarbonate, and 3) 25 mg/kg calcium chloride. Defibrillation was accomplished by delivering a charge of 40–80 J (LifePak 3 defibrillator/monitor, Physio-Control, Redmond, Washington), with 31-cm² paddles placed on the right and left ventricular surfaces.

When MABP reached 75 mm Hg without cardiac compression, U74006F or vehicle (each prepared by The Upjohn Company, Kalamazoo, Michigan, in coded vials) was infused through a catheter in the left saphenous vein. Dogs received either U74006F (1.5 mg/ml in citrate vehicle [0.45% NaCl, 25 mM citrate buffer, pH 2.9], pH 2.9) as a 1.5-mg/kg bolus over 12–15 minutes followed by a 0.125 mg/kg/hr infusion for 12 hours (n = 12) or an equivalent volume of citrate vehicle infused in the same manner (n = 12). The investigators were unaware of the treatment of each dog.

Postresuscitation epinephrine or dopamine infusion maintained MABP between 75 and 100 mm Hg as long as necessary but no longer than 6 hours. After closing the chest, each dog was ventilated for up to 10 hours or until spontaneous ventilation ensued and was extubated on return of the gag reflex.

Spectinomycin (10 mg/kg i.m.) was administered, and morphine sulfate provided for analgesia if attention to wound sites or aggressive behavior suggested the presence of pain. Each dog was placed in a jacket and swivel (Alice King Chatham Medical Arts, Los Angeles, California) permitting three electrical and three hydraulic connections while allowing free movement about the cage. Dogs surviving 24 hours after arrest were killed with 120 mg/kg i.v. sodium pentobarbital following final neurologic deficit scoring and blood sampling. The heart, lungs, and wound sites were examined postmortem in all dogs to identify iatrogeny. This experimental procedure conformed to guidelines established by the

![Chemical structure of 21-aminosteroid U74006F, potent inhibitor of lipid peroxidation.](image_url)

**FIGURE 1.** Chemical structure of 21-aminosteroid U74006F, potent inhibitor of lipid peroxidation.
**TABLE 1. Neurologic Deficit Score for 24 Dogs After 10 Minutes of Normothermic Cardiopulmonary Arrest**

<table>
<thead>
<tr>
<th>Neurologic Function</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Consciousness (range 0–18)</strong></td>
<td></td>
</tr>
<tr>
<td>Normal, consistently alert</td>
<td>0</td>
</tr>
<tr>
<td>Continually conscious but clouded</td>
<td>3</td>
</tr>
<tr>
<td>Intermittently conscious, aroused with minimum effort</td>
<td>6</td>
</tr>
<tr>
<td>Stuporous, aroused with persistent effort</td>
<td>12</td>
</tr>
<tr>
<td>Light coma, reflex movement only</td>
<td>15</td>
</tr>
<tr>
<td>Deep coma, no movement</td>
<td>18</td>
</tr>
<tr>
<td><strong>Respiration (range 0–18)</strong></td>
<td></td>
</tr>
<tr>
<td>Normal, extubated and normal</td>
<td>0</td>
</tr>
<tr>
<td>Extubated/abnormal, extubated but abnormal</td>
<td>6</td>
</tr>
<tr>
<td>Intubated/spontaneous, intubated but off ventilator</td>
<td>12</td>
</tr>
<tr>
<td>On ventilator, intubated and on ventilator</td>
<td>18</td>
</tr>
<tr>
<td><strong>Cranial nerves (range 0–16)</strong></td>
<td></td>
</tr>
<tr>
<td>Corneal reflex</td>
<td></td>
</tr>
<tr>
<td>Strong, consistently blinks in response to touch or saline in eye area</td>
<td>0</td>
</tr>
<tr>
<td>Weak, inconsistently blinks in response to touch or saline in eye area</td>
<td>1</td>
</tr>
<tr>
<td>Absent, does not respond to touch or saline in eye area</td>
<td>2</td>
</tr>
<tr>
<td>Pupillary light reflex</td>
<td></td>
</tr>
<tr>
<td>Strong, constricts pupil to light quickly and completely</td>
<td>0</td>
</tr>
<tr>
<td>Weak, constricts pupil to light slowly and/or incompletely</td>
<td>1</td>
</tr>
<tr>
<td>Absent, does not constrict pupil to light or pupil fixed and constricted</td>
<td>2</td>
</tr>
<tr>
<td>Facial sensation</td>
<td></td>
</tr>
<tr>
<td>Strong, reacts consistently to touch in any area of face</td>
<td>0</td>
</tr>
<tr>
<td>Weak, reacts to touch only in certain areas or inconsistently</td>
<td>1</td>
</tr>
<tr>
<td>Absent, does not react to touch in any facial area</td>
<td>2</td>
</tr>
<tr>
<td>Gag reflex</td>
<td></td>
</tr>
<tr>
<td>Strong, rapid and strong reaction to endotracheal tube or forceps in throat</td>
<td>0</td>
</tr>
<tr>
<td>Weak, slow, weak, or inconsistent reaction</td>
<td>1</td>
</tr>
<tr>
<td>Absent, no gag response on stimulation of throat</td>
<td>2</td>
</tr>
<tr>
<td>Jaw reflex</td>
<td></td>
</tr>
<tr>
<td>Strong, strongly resists rapid opening of jaw</td>
<td>0</td>
</tr>
<tr>
<td>Weak, weakly resists rapid opening of jaw</td>
<td>1</td>
</tr>
<tr>
<td>Absent, jaw flaccid</td>
<td>2</td>
</tr>
<tr>
<td>Pinna reflex</td>
<td></td>
</tr>
<tr>
<td>Strong, twitches ear in response to touch on outer/inner hairs</td>
<td>0</td>
</tr>
<tr>
<td>Weak, twitches ear in response to touch on deep inner hairs only</td>
<td>1</td>
</tr>
<tr>
<td>Absent, does not move ear in response to touch</td>
<td>2</td>
</tr>
<tr>
<td>Olfactory reflex</td>
<td></td>
</tr>
<tr>
<td>Strong, strong reaction to acetic acid near nostril</td>
<td>0</td>
</tr>
<tr>
<td>Weak, weak reaction to acetic acid near nostril</td>
<td>1</td>
</tr>
<tr>
<td>Absent, no reaction</td>
<td>2</td>
</tr>
<tr>
<td>Swallowing reflex</td>
<td></td>
</tr>
<tr>
<td>Strong, consistently swallows water when injected into mouth</td>
<td>0</td>
</tr>
<tr>
<td>Weak, inconsistent swallowing of water</td>
<td>1</td>
</tr>
<tr>
<td>Absent, does not swallow</td>
<td>2</td>
</tr>
</tbody>
</table>

**TABLE 1. (Continued)**

<table>
<thead>
<tr>
<th>Neurologic Function</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Spinal nerves (range 0–20)</strong></td>
<td></td>
</tr>
<tr>
<td>Limb tone (fore/hind)</td>
<td></td>
</tr>
<tr>
<td>Normal, limb has tone without stiffness</td>
<td>0/0</td>
</tr>
<tr>
<td>Spastic, stiff; resists movement</td>
<td>2/2</td>
</tr>
<tr>
<td>Flaccid, no tone</td>
<td>4/4</td>
</tr>
<tr>
<td>Pain reflex (fore/hind)</td>
<td></td>
</tr>
<tr>
<td>Strong, quick, complete withdrawal from toe pinch</td>
<td>0/0</td>
</tr>
<tr>
<td>Weak, slow, incomplete, or inconsistent withdrawal from toe pinch</td>
<td>2/2</td>
</tr>
<tr>
<td>Absent, no withdrawal from toe pinch</td>
<td>4/4</td>
</tr>
<tr>
<td>Knee jerk</td>
<td></td>
</tr>
<tr>
<td>Strong, normal slow response</td>
<td>0</td>
</tr>
<tr>
<td>Weak, incomplete response</td>
<td>2</td>
</tr>
<tr>
<td>Absent, no response or hyperreflexive</td>
<td>4</td>
</tr>
<tr>
<td><strong>Motor function (range 0–28)</strong></td>
<td></td>
</tr>
<tr>
<td>Normal, walks normally</td>
<td>0</td>
</tr>
<tr>
<td>Minimal ataxia, walks with some impairment of gait</td>
<td>2</td>
</tr>
<tr>
<td>Ataxia, just able to walk</td>
<td>4</td>
</tr>
<tr>
<td>Stands spontaneously, falls with a few steps</td>
<td>6</td>
</tr>
<tr>
<td>Stands if posed, falls with any movement</td>
<td>8</td>
</tr>
<tr>
<td>Sits spontaneously, without falling</td>
<td>10</td>
</tr>
<tr>
<td>Sits if posed, falls with any movement</td>
<td>12</td>
</tr>
<tr>
<td>Spontaneous dorsal recumbancy</td>
<td>14</td>
</tr>
<tr>
<td>Posed dorsal recumbancy</td>
<td>16</td>
</tr>
<tr>
<td>Spontaneous purposeful movement, unprovoked, nonconvulsive movement</td>
<td>18</td>
</tr>
<tr>
<td>Provoked purposeful movement, provoked, nonconvulsive movement</td>
<td>20</td>
</tr>
<tr>
<td>Reflex, spastic, or convulsive movement only</td>
<td>24</td>
</tr>
<tr>
<td>No movement</td>
<td>28</td>
</tr>
</tbody>
</table>

Dogs that died are included because their patterns of death suggest primary cerebral event.

American Physiological Society and the "Guide for the Care and Use of Laboratory Animals," NIH Publication No. 85-23, 1985, and was approved by The University of Michigan Unit for Laboratory Animal Medicine's Vertebrate Animal Use Committee (approval #D001068D).

To assess neurologic deficit a well-standardized score (Table 1) was assigned 1, 2, 6, 12, and 24 hours after arrest. Interobserver variability was resolved through consultation of the detailed description of each neurologic functional level. Of the 100 points possible, 18 are assigned to consciousness. 18 to respiratory function, 16 to cranial nerve function, 20 to spinal nerve function, and 28 to motor function.

Plasma U74006F and vitamin E concentrations were determined using high-performance liquid chromatography (HPLC) with electrochemical detection (460, Waters, Milford, Massachusetts). For analysis of vitamin E concentration, 50 µl plasma was added to 100 µl methanol, vortexed, then extracted with 500 µl hexane. The samples were centrifuged, and 300 µl of the hexane layer was dried under a stream...
of argon. The dried extract was dissolved in 1 ml methanol, and 20 μl was injected onto a Waters C-18 Bondapak column (0.46×25 cm) and eluted with methanol:pyridine (99.9:0.1) containing 6.1 mg/ml NaClO₄ at a flow rate of 1 ml/min. Extraction and analysis of U74006F was similar to that for vitamin E except that 1.5 ml plasma was added to 3 ml methanol; 15 ml hexane was then added, and 9 ml hexane extract was dried. Standards for vitamin E (Sigma Chemical Co., St. Louis, Missouri) and U74006F (synthesized by The Upjohn Company) were prepared, and standard curves were produced to quantify these compounds. Recoveries assessed from spiked plasma samples were >98% for vitamin E and 80% for U74006F.

Plasma fatty acid hydroperoxides were extracted from 1 ml plasma and assayed using HPLC and a Farrand Model A4 fluorometer as described by Yamamoto et al. ¹³

Multiple linear regression analysis was used to determine if the prearrest variables (body weight, operative time, MABP, heart rate, arterial pH, arterial plasma glucose concentration, end-expiratory CO₂, and esophageal temperature) were associated with neurologic outcome and survival. Prearrest and resuscitation variables (resuscitation time, number of shocks, ventilation time, extubation time, epinephrine dose, pressor agent infusion time, lidocaine dose, bicarbonate dose, and calcium chloride dose) were compared between U74006F- and vehicle-treated groups using Student’s t test. Neurologic deficit scores were compared parametrically using Student’s t test and nonparametrically using the Mann-Whitney U test, and we report probability values for both tests. Fisher’s exact test and Breslow survival analysis (BMDP statistical program) provided significance values for survival data. ¹⁴ We report the group mean±SEM. Statistical analysis was performed using the Michigan Interactive Data Analysis System (MIDAS) and BMDP Statistical Software on an IBM 3090-400 computer.

Results

The prearrest (Table 2) and resuscitation (Table 3) variables were found to not differ (p>0.05, Student’s t test) between groups. Likewise, no association was detected between prearrest variables and neurologic deficit scores or survival by multiple linear regression analyses. Neither MABP nor heart rate differed between groups at any time before or after arrest. No differences in survival time or 24-hour neurologic deficit scores (p>0.20) were detected using Student’s t test among the subgroups of dogs given epinephrine or dopamine as pressor agents.

All 24 dogs were successfully resuscitated as indicated by the return of spontaneous ventilation and MABP of >75 mm Hg. While 10 of 12 (83.3%) vehicle-treated dogs died by 24 hours, only four of 12 (33.3%) U74006F-treated dogs died by 24 hours after arrest (Figure 2, p = 0.017, Fisher’s exact test).

### Table 2. Comparison of Prearrest Physiologic Variables for 24 Dogs After 10 Minutes of Normothermic Cardiopulmonary Arrest

<table>
<thead>
<tr>
<th>Prearrest variable</th>
<th>Treatment</th>
<th>Vehicle (n = 12)</th>
<th>U74006F (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial plasma glucose concentration (mg/dl)</td>
<td>150 ± 10</td>
<td>132 ± 7</td>
<td></td>
</tr>
<tr>
<td>End-expiratory CO₂ (%)</td>
<td>3.4 ± 0.3</td>
<td>13.6 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Esophageal temperature (°C)</td>
<td>38.5 ± 0.2</td>
<td>38.2 ± 0.3</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SEM. No differences between treatment groups were significant at p = 0.05 by Student’s t test.

U74006F-treated dogs survived significantly longer than vehicle-treated dogs (p = 0.033, Breslow survival analysis; 21.9 ± 1.1, range 14.3–24 hours vs. 18.0 ± 1.2, range 10.4–24 hours, p = 0.03, Student’s t test). In those dogs dying within 24 hours, there was a developing and persistent pattern of fixed and dilated pupils, sustained forced limb extension with subsequent apnea, and collapse of arterial blood pressure. This preagonal sequence is consistent with, albeit not definitive of, ischemic damage to the central nervous system (CNS). No dog died of ventricular fibrillation, hemorrhage, or iatrogeny after arrest.

Neurologic deficit score indicated significantly less impairment in the U74006F-treated than in the

### Table 3. Comparison of Resuscitation Variables for 24 Dogs After 10 Minutes of Normothermic Cardiopulmonary Arrest

<table>
<thead>
<tr>
<th>Resuscitation variable</th>
<th>Treatment</th>
<th>Vehicle (n = 12)</th>
<th>U74006F (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resuscitation time (min)</td>
<td>3.4 ± 0.6</td>
<td>2.6 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Number of shocks</td>
<td>2.2 ± 0.5</td>
<td>1.7 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Ventilation time (min)</td>
<td>19.0 ± 1.8</td>
<td>17.6 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Exubation time (min)</td>
<td>315 ± 17</td>
<td>369 ± 92</td>
<td></td>
</tr>
<tr>
<td>Total epinephrine bolus dose (μg/kg)</td>
<td>62.4 ± 10.0</td>
<td>52.5 ± 6.0</td>
<td></td>
</tr>
<tr>
<td>Total pressor agent infusion time (min)</td>
<td>41.9 ± 14.0</td>
<td>53.0 ± 20.0</td>
<td></td>
</tr>
<tr>
<td>Total lidocaine dose (mg/kg)</td>
<td>2.7 ± 0.5</td>
<td>2.5 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Total NaHCO₃ dose (meq/kg)</td>
<td>5.0 ± 0.2</td>
<td>5.4 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Total CaCl₂ dose (mg/kg)</td>
<td>33.2 ± 4.6</td>
<td>25.0 ± 0.0</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SEM. No differences between treatment groups were significant at p = 0.05 by Student’s t test.
vehicle-treated group 24 hours after arrest (Student's t test, \( p = 0.008 \); Mann-Whitney U test, \( p = 0.020 \)) (Figure 3). At 1 and 2 hours after arrest the U74006F-treated group exhibited more normal sensorimotor function than the vehicle-treated group (\( p < 0.05 \), Student's t and Mann-Whitney U tests).

Figure 4 indicates the time course of plasma U74006F concentrations, which fell rapidly following the bolus injection. By the third hour of infusion, mean plasma U74006F concentration was approximately 0.13 \( \mu g/ml \) and remained essentially stable for the rest of the 12-hour infusion. By 24 hours after arrest, U74006F was no longer detected in the plasma.

Significantly higher plasma vitamin E concentrations were detected in the U74006F-treated than in the vehicle-treated group 2, 3, and 6 hours after arrest (Figure 5). While vitamin E concentration fell during cardiopulmonary arrest, the reduction did not reach significance in either group. However, vitamin E concentration in the vehicle-treated group tended to remain low following resuscitation, whereas in the U74006F-treated group it returned to prearrest values.

Twelve hours after arrest the mean ± SEM concentration of fatty acid hydroperoxides in plasma was 241 ± 49 pmol/ml for the vehicle-treated group but significantly lower (88 ± 81 pmol/ml) for the U74006F-treated group (\( n = 11 \) for each group; \( p < 0.01 \), Student's t test).
Although vitamin E concentrations declined during cardiopulmonary arrest in both U74006F-treated (filled boxes, n=12) and vehicle-treated (open boxes, n=11) groups, they rose toward control in former but fell in latter group. *p=0.05, analysis of variance.

Plasma glucose concentrations before and after cardiopulmonary arrest are illustrated in Figure 6. The groups did not differ except at 24 hours; at that time, however, there were only two dogs in the vehicle-treated group.

Discussion

The greatest change in neurologic deficit has been reported to occur in the first 24 hours after cardiac arrest as demonstrated by long-term recovery studies in which some minimal additional deficit resolved by 7 days in a group of treated dogs. Assuming that our 10 minutes of normothermic cardiac arrest produced a similar pattern of recovery, we chose a 24-hour study period. Continuation of the study for 7 instead of 1 day would provide only limited additional information concerning neurologic recovery or mortality while exposing the dogs to unnecessary discomfort as a result of sepsis, pneumonia, etc.

Assignment of a neurologic deficit score of 100 to all dogs that die after resuscitation is justified because all the evidence we have suggests that these dogs die of CNS insult, not ventricular fibrillation, hemorrhage, pneumothorax, or other postischemic non-CNS organ dysfunction. While removal of these dead dogs from the analysis eliminates the statistical separation of the groups by neurologic deficit score, the exclusion of dogs with maximum scores would leave a few (two of 12) of only the least impaired dogs in the vehicle-treated group to compare with the full range of neurologic deficit scores in the live dogs (eight of 12) of the U74006F-treated group. The fact that all dogs survived at least 10 hours despite termination of vasopressor support after 42 (vehicle-treated group) and 53 (U74006F-treated group) minutes suggests that cardiac function was fully restored following resuscitation. Likewise, ventilatory support was stopped in all dogs by 31.5 minutes because spontaneous ventilation produced end-expiratory CO₂ of <5%. Although our neurologic deficit score has been used successfully in several studies, it does not detect any and all neurologic dysfunction, and furthermore, it focuses only on discrete time points. Continuous rather than intermittent monitoring of neurologic function would assist in more clearly defining the precise time course of the development of neurologic deficit, but no such continuously recording functional monitor is currently available.

The 21-aminosteroid U74006F reduced morbidity and 24-hour mortality concurrent with lower concentrations of plasma fatty acid hydroperoxides and elevated plasma vitamin E concentrations after resuscitation following 10 minutes of global cerebral ischemia. The factors responsible for this protection cannot be provided by our data; however, in vitro studies have shown that U74006F inhibits iron-dependent lipid peroxidation. The precise mechanism of its antioxidant activity is unclear, U74006F may act in a manner similar to that of vitamin E by scavenging the lipid peroxyl radical and inhibiting the lipid radical chain reactions (J.M. Braughler, unpublished observations). In that regard, U74006F has been shown to protect vitamin E from oxidation during lipid peroxidation (J.M. Braughler, unpublished observations). The reduced concentrations of plasma lipid peroxidation products and the maintained concentration of vitamin E in U74006F-treated dogs suggest an antioxidant activity that could account for the potential tissue-protective effects of U74006F. However, verification of brain tissue sparing of vitamin E is necessary to support this hypothesized protective mechanism.

Transient cerebral ischemia results in initial postischemic brain hyperperfusion followed by progres-
sive hypoperfusion. Postischemic formation of vasoactive compounds and occlusion of the cerebral microvasculature with platelets contribute to a secondary ischemic brain insult and impair cerebral metabolic recovery. Microvascular lipid peroxidation may contribute to the progression of postischemic hypoperfusion. Although we did not measure cerebral blood flow in this study, in cats U74006F attenuated decreases in cerebral blood flow, reduced postischemic arterial acidosis, improved postischemic blood pressure, and enhanced recovery of somatosensory evoked potentials (SEPs) following experimental global cerebral ischemia.

The normothermic cardiac arrest model has been useful in testing interventions designed to improve or degrade neurologic function and/or survival. In a similar but less severe ischemic insult (6 minutes of cardiac arrest), we demonstrated that ibuprofen, an agent that limits granulocyte aggregation and lysosomal enzyme release, decreased 24-hour mortality and morbidity. Only two other compounds have improved neurologic outcome and survival in this model, ketamine and U74006F. The proposed neuroprotective actions of these compounds are dissimilar; ketamine reportedly antagonizes hippocampal N-methyl-D-aspartate receptors and reduces the ischemic excitotoxic action of glutamate, whereas U74006F inhibits systemic lipid peroxidation. The literature contains few other reports of functional, rather than biochemical or morphologic, cerebral ischemic protective agents.

One recent relevant investigation described the protective effects of combined superoxide dismutase (SOD) and deferoxamine on recovery of SEPs and cerebral blood flow after 7 minutes of asphyxial cardiac arrest. Interestingly, the protection provided by those two drugs and our current study with U74006F both implicate oxygen free radical mechanisms of reperfusion injury. However, three important issues should be addressed when comparing these two studies. First, the combination might well have substantially greater efficacy than either SOD or deferoxamine alone or than the sum of their individual effects; therefore, comparing the relative efficacies of U74006F and either SOD or deferoxamine is not appropriate. Second, neurologic function and the presence or absence of SEPs must be associated cautiously. Brunko and Zegers de Beyl investigated the prognostic value of SEPs after resuscitation from cardiac arrest in patients and found a relation between the absence of cortical SEPs and poor prognosis, but the presence of SEPs did not predict neurologic function and recovery. Finally, elevated blood glucose concentrations in several animal models, including cardiac arrest models, augment ischemic CNS injury. Although prearrest or postarrest blood glucose concentrations were not reported by Cerchiar et al., both SOD/deferoxamine-treated and control groups received 4 ml/kg/hr 5% dextrose in 0.45% NaCl before arrest, but only the control group was maintained on this intravenous fluid support after arrest. Because the quantity and timing of dextrose administration was not standardized between groups, a differential effect of exogenous dextrose on neurologic outcome cannot be excluded. We have recently demonstrated worsened neurologic function and survival in dogs receiving 5% dextrose following 6 minutes of cardiac arrest and resuscitation.

Our study confirms the lack of an effect of U74006F on blood glucose concentration, supporting previous studies that demonstrated the absence of any glucocorticoid-like hormonal activity of this compound. An important characteristic of any postischemic intervention must be that it not exacerbate CNS ischemic tissue injury by elevating blood glucose concentrations.

In summary, the 21-aminosteroid U74006F reduced morbidity and 24-hour mortality after resuscitation following 10 minutes of normothermic cardiopulmonary arrest in dogs. This protective effect may be attributed to the demonstrated inhibition of lipid peroxidation by oxygen free radicals during reperfusion and the sparing of vitamin E in the plasma of U74006F-treated dogs. Additional studies are necessary to further elucidate additional components of the neuroprotective action of U74006F, such as amelioration of postischemic hypoperfusion. The clinical significance of our findings are speculative; however, our study suggests that U74006F is a promising therapeutic candidate for restoration of postischemic CNS function.

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References


KEY WORDS • cerebral ischemia • lipid peroxidation • heart arrest • mortality • dogs
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J E Natale, R J Schott, E D Hall, J M Braughler and L G D'Alecy

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