Effects of the 21-aminosteroid U74006F on posttraumatic spinal cord ischemia in cats

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The ability of a single intravenous dose of the 21-aminosteroid U74006F to affect the development of posttraumatic spinal cord ischemia was examined in pentobarbital-anesthetized cats. After surgical preparation, each animal received a 300 gm-cm contusion injury to the exposed L-3 vertebral segment, followed by a single bolus injection of vehicle or U74006F (3 or 10 mg/kg) at 30 minutes postinjury. Spinal cord white matter blood flow (SCBF) was measured by hydrogen clearance in the dorsolateral funiculus in the center of the injured segment before and at various times up to 4 hours after injury. In vehicle-treated cats, there was a progressive decline in SCBF over the course of the experiment. By 4 hours postinjury, SCBF had decreased from a preinjury value of 15.9 ± 2.4 ml/100 gm/min (mean ± standard error of the mean) to 5.8 ± 0.8 ml/100 gm/min, representing a decline of 63.5%. In contrast, the SCBF measured 4 hours postinjury in cats that were treated with a single 10-mg/kg dose of U74006F was 13.6 ± 1.7 ml/100 gm/min (p < 0.001 vs. vehicle). Animals that received a 3-mg/kg intravenous dose of U74006F displayed a drop in SCBF equal to that of vehicle-treated cats. However, when a 3-mg/kg dose of U74006F was given to four vehicle-treated cats at the end of the experiment, a partial reversal of ischemia was recorded. Blood flow increased within 30 minutes from a mean of 4.5 ± 0.8 to 7.4 ± 1.0 ml/100 gm/min or an increase of 64.4% (p < 0.05). This rather surprising effect of U74006F in reversing posttraumatic ischemia once it has developed significantly is not shared by a 30-mg/kg intravenous dose of methylprednisolone sodium succinate (MP), although MP has previously been shown to attenuate the posttraumatic drop in SCBF when given before the SCBF drop occurs. The mechanism of action of U74006F in antagonizing posttraumatic ischemia development is believed to involve the ability of the compound to inhibit iron-dependent lipid peroxidation in central nervous system tissue.

KEY WORDS  •  spinal cord injury  •  posttraumatic ischemia  •  21-aminosteroid  •  U74006F  •  cat

SEVERE blunt injury to the spinal cord is known to result in a progressive decline in blood flow in the injured spinal segment. This may play an important role in secondary posttraumatic spinal cord degeneration.17 This posttraumatic ischemic phenomenon, which has been studied most extensively in the white matter of the injured spinal cord, is independent of alterations in systemic blood pressure or blood gases (such as arterial pCO2).11 Instead, the progressive decline in spinal cord white matter blood flow (SCBF) is primarily a local event within the injured spinal cord segment probably caused by an injury-initiated molecular cascade involving massive intracellular calcium accumulation,16,19 liberation of vasoactive prostanoids (principally prostaglandin F2α (PGF2α),15 and thromboxane A2 (TXA2),6,14) and microvascular lipid peroxidative reactions.5,8 Consistent with this scheme is the finding that treatment of cats prior to spinal cord contusion with certain calcium antagonists (such as diltiazem or nifedipine), cyclo-oxygenase inhibitors (such as ibuprofen or meclofenamate), or antioxidants (such as α-tocopherol plus selenium) can significantly attenuate development of posttraumatic ischemia.10

In a more clinically relevant protocol involving postinjury drug administration in cats, it has been shown that injecting a large 30-mg/kg intravenous bolus of the glucocorticoid methylprednisolone sodium succinate (MP) 30 minutes after injury can also inhibit the predictable decrease in SCBF, while lesser doses are relatively ineffective.12,18 Moreover, a repeated dosing protocol with MP over 48 hours, beginning with a 30-mg/kg intravenous dose, has been shown to improve chronic neurological recovery of spinal cord-injured cats.13 If the above pathogenetic scheme is correct, the
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prevention of posttraumatic ischemia by MP is most likely based upon the ability of the same 30-mg/kg dose to reduce the accumulation of PGF₂α and TXA₂ and the occurrence of lipid peroxidation in injured spinal cord tissue.

In view of the large dose of MP required to antagonize posttraumatic prostanoïd formation, lipid peroxidation, and progressive ischemia development, it has been hypothesized that these cerebroprotective actions of MP are unrelated to its glucocorticoid receptor activity. Other studies have in fact demonstrated that the ability of MP to inhibit lipid peroxidation in vitro in rat central nervous system (CNS) tissue and to enhance the early recovery of mice after a severe concussive head injury can be duplicated by U72099E, a non-glucocorticoid analog of MP. Very recently, it has been shown that another compound, U74006F (21-[4-(2,6-di-1-pyrrolidinyl-4-pyrimidinyl)-1-piperazinyl]-16α-methyl-pregna-1,4,9(11)-triene-3,20-dione, mono-methane sulfonate), a non-glucocorticoid 21-aminosteroid, is extremely potent and effective as an inhibitor of lipid peroxidation and a promoter of the recovery and survival of severely head-injured mice. In the presently reported investigation, the ability of U74006F to prevent and reverse posttraumatic ischemia has been examined within the context of the severely contused cat spinal cord.

Materials and Methods

Adult female specific pathogen-free (SPF) cats, each weighing approximately 2.4 to 3.2 kg, were used in this study. All experiments were performed under intravenous sodium pentobarbital anesthesia (30 mg/kg initial dose plus 5-mg/kg supplements as required to maintain nearly complete obtundation of the corneal reflex). Details of the surgical technique, blood flow measurement (hydrogen clearance), cardiovascular monitoring, and injury methods have been given elsewhere. The only difference in the present studies was the use of a lesser injury force (300 gm-cm: 50-gm weight dropped 6 cm) than that employed in the past (500 gm-cm). Upon switching from mongrel to SPF cats, it was discovered that the same injury force in the SPF animals produced a much more rapid and severe decline in SCBF than in the mongrel cats. While the basis for this difference is not fully known, it seems to relate to a greater susceptibility of the SPF cats to posttraumatic tissue hemorrhage, since a more prolonged clotting time has been observed in these animals. This may explain their difference from mongrel cats. In any case, a 300 gm-cm injury caused an equal or slightly greater drop in SCBF in the SPF cats over the 4-hour postinjury time course than that seen with a 500 gm-cm force in mongrel animals.

The U74006F was dissolved in 0.05 N HCl (pH 3.0) and given as a 0.5-ml/kg bolus. A 1.0-ml saline flush was administered just prior to the 30-minute postinjury SCBF measurement. The effects of U74006F in doses of 3 and 10 mg/kg were studied.

Results

Posttraumatic Ischemia Development

Figure 1 displays the postinjury changes in SCBF in vehicle-treated versus U74006F-treated animals over the 4-hour study period. The 300 gm-cm injury in the vehicle-treated animals resulted in a progressive decline in SCBF from a preinjury mean ± standard error of the mean of 15.9 ± 2.4 ml/100 gm/min to a severely ischemic mean of 5.8 ± 0.8 ml/100 gm/min by 4 hours. This represents a decrease of 63.5%. The lower boundary for normal SCBF is approximately 10.0 ml/100 gm/min, although some authors give a higher minimum normal value. Therefore, by 2 hours after injury, the SCBF entered a subnormal range (< 10.0 ml/100 gm/min).

In comparison, the administration of a 10-mg/kg intravenous dose of U74006F at 30 minutes after injury resulted in nearly complete support of the SCBF over the experimental period. The 4-hour postinjury mean was only 9.9% less than the paired preinjury value. Comparing the means at 3 and 4 hours with the vehicle SCBF's at the same time points gave p values (one-way analysis of variance) of 0.004 and 0.001, respectively. The decrease in SCBF after the 3-mg/kg dose of U74006F was essentially identical to that seen in the vehicle-treated animals. The decrease seen at 4 hours...
TABLE 1
Mean arterial blood pressure in vehicle- and U74006F-treated spinal cord-injured cats*

<table>
<thead>
<tr>
<th>Injectate</th>
<th>No. of Cats</th>
<th>Preinjury</th>
<th>10 Min Postinjury</th>
<th>30 Min Postinjury</th>
<th>1 Hr Postinjury</th>
<th>2 Hrs Postinjury</th>
<th>3 Hrs Postinjury</th>
<th>4 Hrs Postinjury</th>
</tr>
</thead>
<tbody>
<tr>
<td>vehicle</td>
<td>6</td>
<td>119.7 ± 7.8</td>
<td>103.7 ± 7.6</td>
<td>106.2 ± 10.2</td>
<td>112.8 ± 9.7</td>
<td>114.7 ± 8.0</td>
<td>108.3 ± 8.1</td>
<td>97.0 ± 6.6</td>
</tr>
<tr>
<td>U74006F (10 mg/kg)</td>
<td>6</td>
<td>119.7 ± 6.9</td>
<td>102.7 ± 5.6</td>
<td>102.8 ± 5.1</td>
<td>113.8 ± 3.9</td>
<td>117.2 ± 2.5</td>
<td>110.5 ± 2.6</td>
<td>100.2 ± 2.6</td>
</tr>
</tbody>
</table>

* Values are means ± standard error of the means, expressed in mm Hg.

TABLE 2
Arterial blood gases and pH in vehicle- and U74006F-treated spinal cord-injured cats*

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of Cats</th>
<th>Preinjury</th>
<th>4 Hrs Postinjury</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6</td>
<td>7.24 ± 0.04</td>
<td>7.32 ± 0.03</td>
</tr>
<tr>
<td>pCO₂ (mm Hg)</td>
<td>6</td>
<td>27.2 ± 1.7</td>
<td>24.7 ± 2.8</td>
</tr>
<tr>
<td>pO₂ (mm Hg)</td>
<td>6</td>
<td>119.6 ± 5.2</td>
<td>104.0 ± 6.1</td>
</tr>
<tr>
<td>U74006F (10 mg/kg)</td>
<td>6</td>
<td>7.38 ± 0.02</td>
<td>7.35 ± 0.03</td>
</tr>
<tr>
<td>pCO₂ (mm Hg)</td>
<td>6</td>
<td>25.5 ± 1.0</td>
<td>23.1 ± 2.3</td>
</tr>
<tr>
<td>pO₂ (mm Hg)</td>
<td>6</td>
<td>103.4 ± 2.7</td>
<td>114.0 ± 12.9</td>
</tr>
</tbody>
</table>

* Values are means ± standard error of the means.

Postinjury compared with the preinjury levels was 59.9% in the 3 mg/kg experiments.

Posttraumatic Hypotension

The 300 gm-cm lumbar spinal contusion caused an initial hypertensive episode, with an increase in mean arterial blood pressure (MABP) of 50 to 100 mm Hg. This increase receded within a few minutes, and significant postraumatic hypotension followed which was sustained for the remainder of the experiment (Table 1). A 10-mg/kg intravenous dose of U74006F, given just prior to 30 minutes after contusion injury, had no effect on the MABP at any subsequent measurement time in comparison to vehicle-treated cats. The 3-mg/kg intravenous dose (data not shown) was also without effect.

Table 2 shows that arterial blood gases and pH were essentially stable in both the vehicle- and the U74006F-treated cats. It was of perhaps the greatest importance to CNS blood flow that pCO₂ was not significantly different between groups either before trauma or at 4 hours after injury. The slight drop in pCO₂ in each group was not statistically significant.

Partial Reversal of Posttraumatic Ischemia

Table 3 shows the ability of a 3-mg/kg intravenous dose of U74006F to partially reverse posttraumatic ischemia once it has developed. While the magnitude of this effect was variable, it was observed in all of the four experiments in which this was tested. The SCBF at 4 hours postinjury was increased from a mean of 4.5 ml/100 gm/min to 7.4 ml/100 gm/min after the administration of U74006F (p < 0.05, an increase of 64.4%).

On the other hand, the MABP was not significantly altered.

Discussion

The non-glucocorticoid 21-aminosteroid U74006F was shown to completely prevent the development of posttraumatic spinal white matter ischemia when administered in a 10-mg/kg intravenous bolus 30 minutes after a severe spinal cord contusion injury. While the mechanism of this action cannot be definitely assigned, U74006F and related compounds are known to be potent inhibitors of iron-dependent lipid peroxidation. Specifically, U74006F is thought to inhibit lipid peroxidation by multiple molecular mechanisms including a vitamin E-like membrane antioxidant action, scavenging of superoxide anion, and perhaps a membrane-localized iron-binding effect. Thus, considering the purported role of microvascular lipid peroxidation in the progressive decline in SCBF that follows severe cord injury, it is conceivable that the anti-ischemic effect of U74006F is based upon an inhibition of posttraumatic lipid peroxidation reactions.

Earlier work has documented similar action of a 30-
mg/kg intravenous dose of the glucocorticoid steroid MP to inhibit posttraumatic ischemia development. This effect has been largely attributed to the ability of MP to inhibit posttraumatic spinal tissue lipid peroxidation. However, several advantages of U74006F over MP have been documented. First, U74006F lacks glucocorticoid receptor-mediated activities. This reasonably predicts that prolonged dosing with U74006F should be devoid of typical "steroid" side effects such as immunosuppression, diabetic-like disturbances, or impaired wound healing. Second, U74006F is enormously more potent and effective than MP as an inhibitor of iron-dependent lipid peroxidation in CNS tissue, which leads to the prediction that U74006F should have much greater cerebroprotective efficacy. This is apparent in the present study in regard to the compound's greater anti-ischemic potency and its unexpected action to partially reverse ischemia after it has developed to a significant degree. In contrast, MP is almost totally ineffective in improving SCBF after a significant decline has occurred.

A third advantage of U74006F over MP concerns the possible exacerbation of the posttraumatic hypotension that follows severe experimental and clinical CNS trauma. While this phenomenon does not seem to play a significant role in the progressive decline of SCBF in the present model, it could, if intensified, compromise spinal cord (or brain) perfusion pressure. A large (30 mg/kg) intravenous dose of MP has peripheral vasodilator activity and increases the magnitude of posttraumatic hypotension. This may, in some instances, work against the direct beneficial effect on the spinal cord microvasculature. On the other hand, U74006F did not exacerbate the usual posttraumatic depression in MABP.

Additional studies are in progress to assess the ability of repeated dosing with U74006F over a 48-hour period to improve the chronic neurological recovery of spinal cord-injured cats. In other completed studies, U74006F has been shown to promote early recovery and long-term survival of severely head-injured mice. Based upon the present results, it is conceivable that the effects of the compound in experimental head injury are related to an antagonism of posttraumatic brain ischemia. Further work is needed to explore this possibility.

References

Manuscript received March 27, 1987. Accepted in final form August 4, 1987.
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