Effect of the 21-aminosteroid U-74006F on cerebral vasospasm following subarachnoid hemorrhage

MARIO ZUCCARELLO, M.D., JEFFERY T. MARSH, M.D., GERALD SCHMITT, B.S.B.A., JAMES WOODWARD, M.S., AND DOUGLAS K. ANDERSON, PH.D.

Cincinnati Veterans Administration Medical Center and Departments of Neurology and Neurosurgery, University of Cincinnati College of Medicine, Cincinnati, Ohio

The purpose of this study was to use a new 21-aminosteroid (U-74006F) with in vitro antioxidant and antilipolytic properties as a pharmacological probe to assess the role of lipid hydrolysis and peroxidation in a rabbit model of subarachnoid hemorrhage (SAH)-induced vasospasm. Cerebral angiograms were performed on 15 rabbits. Eighteen hours later, 1 cc/kg of autologous blood was infused into the cisterna magna of all 15 animals. Six rabbits received no treatment, six received U-74006F starting 30 minutes after SAH, and three rabbits received the vehicle for U-74006F starting 30 minutes after SAH. At 72 hours post-SAH, a second angiogram was obtained. Digital subtraction angiographic techniques were used to measure the diameter of and contrast material flow through the basilar artery. At 72 hours post-SAH, vasospasm was evident in all untreated and vehicle-treated rabbits. The diameter of and the flow through the basilar artery were significantly reduced 42.3% ± 6.6% and 46.8% ± 5.8%, respectively, below pre-SAH levels (means ± standard error of the means). Treatment with U-74006F eliminated the SAH-induced vasospasm; in treated animals, both the flow through and the diameter of the basilar arteries were at pre-SAH levels. These findings indicate that: 1) membrane lipid changes (that is, hydrolysis with eicosanoid production and/or peroxidation) contribute to the chronic vasospasm resulting from SAH, and 2) U-74006F prevents the SAH-induced chronic vasospasm in this model by limiting these pathological membrane events.

KEY WORDS: subarachnoid hemorrhage • cerebral vasospasm • U-74006F • 21-aminosteroid • digital subtraction angiography • rabbit

CEREBRAL vasospasm after subarachnoid hemorrhage (SAH) is a major clinical problem because its occurrence evokes severe impairment of local cerebral blood flow with subsequent brain dysfunction and poor outcome of patients. While the pathological mechanism responsible for cerebral vasospasm has not been identified, it appears to involve a multifactorial process. The phenomenon occurs most commonly with aneurysmal hemorrhage, and the volume of blood at the site of hemorrhage has been closely correlated with the occurrence of severe vasospasm. Several investigators have proposed that substances produced during hemolysis of the subarachnoid blood clot play a fundamental role in the genesis of cerebral vasospasm. Hemoglobin, especially oxyhemoglobin (oxyHb), produces a prolonged constriction of vascular smooth muscle. Temporally, oxyHb release following SAH in humans seems to correspond with the development of vasospasm. Oxyhemoglobin can participate in the generation of oxygen radicals which can initiate peroxidative reactions in membrane polyunsaturated fatty acids (PUFA). In addition, oxygen radicals can stimulate membrane phospholipases resulting in the deacylation of PUFA (including arachidonic acid) from glycerophospholipids. The unesterified arachidonic acid is then available for conversion to the eicosanoids by cyclo-oxygenase and lipoxygenase.

Recently, it was demonstrated that elevated concentrations of prostaglandins in the cerebrospinal fluid (CSF) also correlate with the occurrence of SAH-induced vasospasm. Thus, a role for eicosanoids and oxygen free radicals in the induction of cerebral vasospasm has been postulated. Additionally, Sasaki, et al., and Sakaki, et al., have reported a close correlation between the occurrence of vasospasm and an increase in the content of lipid peroxides in the CSF of patients with SAH. On the basis of these clinical and experimental data, an attempt has been made to verify the hypothesis that lipid peroxidation and hydrolysis resulting from lysis of the subarachnoid clot are involved in the genesis of cerebral vasospasm. A novel
Effect of U-74006F on post-SAH cerebral vasospasm

21-aminosteroid, U-74006F, with demonstrated in vitro antioxidant and antilipolytic properties was used as a pharmacological probe to assess the role of these membrane lipid changes in a rabbit model of SAH-induced vasospasm. In the process, the capacity of U-74006F to prevent cerebral vasospasm was tested.

Materials and Methods

Fifteen male New Zealand White rabbits, each weighing 2.7 to 4 kg, were used in this study. Each animal included two transfemoral cerebral angiograms performed 3½ days apart. The animals were anesthetized with ketamine hydrochloride (30 mg/kg, intramuscularly) and xylazine (6 mg/kg, intramuscularly) and were placed on the angiography table in a supine position. A No. 3 French catheter was positioned in the aortic arch via a femoral arteriotomy. Prior to each angiogram, arterial blood was collected for analysis of blood gases.

Angiovist 282 was injected at a constant rate (4 cc/sec for 5 seconds) and serial digital subtraction angiography (DSA) images were obtained to document the diameter of and flow through the vertebrobasilar system. A rapid sequential angiographic technique was used to take images at two per second for 14 seconds. The DSA was performed with the small focal spot, at 60 kV and 0.8 mA. All films were taken in the 3° left anterior oblique projection. The catheter was then removed and the animals were allowed to recover from anesthesia. At 18 hours after the first angiogram, the rabbits were reanesthetized with ketamine hydrochloride and xylazine and intubated with a Portex 3-mm endotracheal tube. A femoral vein and artery were catheterized and anesthesia was maintained by intravenous sodium pentobarbital (20 mg/kg). The animals were positioned in a stereotaxic frame and the cisterna magna was entered percutaneously with a No. 21 butterfly needle; 1 cc/kg of autologous blood was then infused into the subarachnoid space over 2 minutes.

Arterial blood pressure and intracranial pressure (ICP) were continuously monitored and recorded. Some of the rabbits had to be artificially ventilated immediately following SAH; otherwise they were allowed to breathe spontaneously. Immediately after SAH, the animals were tilted at 30° in a head-down position for 15 minutes to ensure the pooling of blood into the basal cistern. When the rabbits were breathing unaided, they were transferred to a small-animal intensive care unit* and were maintained there until the second angiogram was performed.

The animals were separated into three groups: Group I included six rabbits with SAH but no treatment, serving as a control group; Group II comprised six rabbits that received U-74006F infusion following SAH; and Group III comprised three rabbits that received the citrate-buffered saline vehicle of U-74006F after SAH.

In Group II, the treatment regimen for U-74006F infusion required a 1.5-mg/kg bolus intravenous injection of U-74006F to be given 30 minutes after SAH followed 2 hours later by another intravenous bolus injection of 0.75 mg/kg. Immediately after the second injection, an intravenous infusion was started that delivered a total of 2 mg/kg U-74006F over the next 14 hours. After termination of the infusion, U-74006F was given as 1-mg/kg intravenous injections four times daily for the duration of the study (56 hours). The same schedule was followed in Group III rabbits with the exception that only the vehicle of U-74006F was administered.

At 72 hours following SAH, a second or "post-SAH" angiogram was obtained, after which the rabbits were killed by intracardiac perfusion fixation with 10% formalin. Each angiogram was recorded on tape and digital images were acquired to 512 x 512 x 8-byte matrix resolution. From the subtracted images, the diameter of the basilar artery was measured at three locations using a DSA computer program. The three points measured were designated as the top (below the basilar-posterior cerebral artery junction), the middle, and the bottom (above the basilar-vertebral artery junction). For every group, the mean diameters (± standard error of the means (SEM)) were calculated for each of the three locations on the basilar artery. In addition, the mean of the three individual basilar artery diameter measurements were calculated for every animal and these were averaged for each group as an index of whole basilar artery constriction. The results are reported as mean percent constriction (± SEM) of the post-SAH basilar artery relative to the diameter of the pre-SAH basilar artery.

Images from each study were also transferred to an analytical processing unit system (256 x 256 x 8-byte matrix resolution). From the subtracted images, three regions of interest (ROIs) on each basilar artery that corresponded with the three points used for vessel diameter measurements were analyzed for the rate of contrast material flow. This flow determination was based on the degree of x-ray attenuation that was proportional to the amount of Angiovist passing through each ROI at each second during the study. The area for each ROI was 1 x 1 mm (20 x 20 pixels). In addition, a background ROI was selected within the radiographic image for quantification of radiation scatter and image-intensifier glare. During the performance of each DSA series, the initial arterial phase was followed by a parenchymal blush and subsequent venous phase. The data were extrapolated and plotted to show the iodine concentration in the three basilar artery ROI's at 2-second intervals for 14 seconds, corrected for radiation scatter. Image-intensifier glare was eliminated by linearly subtracting the background ROI intensity from that for each basilar artery ROI at each point on the iodine concentration/time curve. The area under each curve was calculated as an index of blood flow and these values were averaged (± SEM) for each of the three ROI's on the basilar artery. The mean of

* Small-animal intensive care unit, Model 107B, manufactured by Harvard Bioscience, South Natick, Massachusetts.
the three individual ROI values was calculated for every rabbit, and these were averaged for each group as an index of blood flow in the whole basilar artery. The results are reported as the percent change in blood flow ($\pm$ SEM) in the post-SAH basilar artery relative to the blood flow in the pre-SAH basilar artery.

Differences among the groups were assessed by analysis of variance. Differences between individual groups were determined by Duncan’s multiple range test. The minimum level of significance was set at 0.05.

**Results**

Subarachnoid hemorrhage (with or without treatment with U-74006F) had no effect on mean arterial blood pressure, whereas ICP was transiently elevated 67- to 85-fold (Table 1). Regardless of the group, there was no significant difference between levels of blood PO$_2$, pCO$_2$, and pH that were measured before the first artery, the mean percent constrictions were: 32.5% (Fig. 2). For the three individual regions of the basilar animals (Fig. 1). In Group I rabbits, the percent constriction with U-74006F) had no effect on mean arterial blood pressure, and on the basal surface of the brain. By 72 hours after SAH, substantial vasospasm was apparent in all untreated (Group I) and vehicle-treated (Group III) animals (Fig. 1). In Group I rabbits, the percent constriction of the whole basilar artery averaged 42.3% $\pm$ 6.6% (Fig. 2). For the three individual regions of the basilar artery, the mean percent constrictions were: 32.5% $\pm$ 11.5% (upper), 46.6% $\pm$ 9.4% (middle), and 45.9% $\pm$ 4.1% (lower, Fig. 2). These changes were not significantly different from those for the rabbits receiving SAH and treated with vehicle (Group III, data not shown).

The SAH-initiated vasospasm of the basilar artery was essentially eliminated by U-74006F treatment (Fig. 3). The mean percent constriction of the whole basilar artery at 72 hours post-SAH was 4.9% $\pm$ 3.0% (Fig. 2). The mean percent constriction for the three individual areas on the basilar artery were: 2.7% $\pm$ 5.6% (upper), 7.2% $\pm$ 3.2% (middle), and 4.1% $\pm$ 5.5% (lower, Fig. 2). These values were not significantly different from pre-SAH values but were significantly less than those for the untreated and vehicle-treated rabbits.

Blood flows (as measured by the amount of contrast material passing through each ROI per second) for each animal at 72 hours after SAH are presented as the percent change from the pre-SAH values (Fig. 4). In Group I (SAH, untreated rabbits), the mean calculated flow for the whole basilar artery was 46.8% $\pm$ 5.8% below pre-SAH levels. The mean calculated flow for the three individual ROI's on the basilar artery were: 47.8% $\pm$ 7.0% (upper), 41.9% $\pm$ 9.1% (middle), and 47.2% $\pm$ 5.8% (lower) below pre-SAH flows. These changes were not significantly different from those for Group III (SAH, vehicle-treated rabbits, data not shown). In Group II (U-74006F-treated rabbits), the mean calculated post-SAH flow for the whole artery was 1.0% $\pm$ 4.6% below pre-SAH values (Fig. 4). The mean regional flows in this group were: 2.3% $\pm$ 3.7% above pre-SAH flow (upper), 1.6% $\pm$ 6.1% below pre-SAH flow (middle), and 3.3% $\pm$ 5.2% below pre-SAH flow (lower, Fig. 4). These changes were significantly less than those seen in the untreated or vehicle-treated rabbits, but not significantly different from the pre-SAH flows.

**Discussion**

Serial DSA images were used to measure the diameter of the basilar artery and to quantify blood flow through it. Two recent studies in dogs and one in rabbits have used this DSA technique to assess flow in the coronary artery, in the myocardium, and in the middle cerebral artery.

Cerebral vasospasm after SAH has been directly related to the amount of blood in the subarachnoid space. How blood in the subarachnoid space leads to vasospasm is still unknown. It is generally agreed that the etiology of vasospasm is related to the release of substances from the subarachnoid blood clot which causes prolonged and abnormal contraction (and impairment of vasodilatation) of the vessels and results in cytoarchitectural changes in the vessel wall. Erythrocytes appear to be essential for generating the prolonged contraction and the pathological changes associated with cerebral vasospasm. Numerous studies link oxyHb and cerebral vasospasm with cerebral vasospasm. Hemo-globin, particularly when oxygenated, possesses vaso-constrictive properties. In addition, the transformation of oxyHb to methemoglobin generates activated species of oxygen such as the superoxide anion, hydro-

---

**TABLE 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>MABP (mm Hg)</th>
<th>ICP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (SAH only)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre-SAH</td>
<td>90.0 $\pm$ 3.4</td>
<td>1.8 $\pm$ 0.3</td>
</tr>
<tr>
<td>post-SAH</td>
<td>82.0 $\pm$ 6.6</td>
<td>120.0 $\pm$ 7.1</td>
</tr>
<tr>
<td>Group II (SAH + U-74006F)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre-SAH</td>
<td>84.0 $\pm$ 3.7</td>
<td>1.6 $\pm$ 0.2</td>
</tr>
<tr>
<td>post-SAH</td>
<td>89.0 $\pm$ 8.2</td>
<td>136.0 $\pm$ 13.4</td>
</tr>
</tbody>
</table>

* All values are means $\pm$ standard error of the means. SAH = subarachnoid hemorrhage; MABP = mean arterial blood pressure; ICP = intracranial pressure.

---

**TABLE 2**

<table>
<thead>
<tr>
<th>Angiogram</th>
<th>pO$_2$ (mm Hg)</th>
<th>pCO$_2$ (mm Hg)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>92.7 $\pm$ 0.9</td>
<td>36.4 $\pm$ 0.6</td>
<td>7.404 $\pm$ 0.007</td>
</tr>
<tr>
<td>2nd</td>
<td>92.4 $\pm$ 0.9</td>
<td>35.5 $\pm$ 0.7</td>
<td>7.411 $\pm$ 0.009</td>
</tr>
</tbody>
</table>

* Values are the means $\pm$ standard error of the means of all the blood gas and pH values from all three groups.
Effect of U-74006F on post-SAH cerebral vasospasm

FIG. 1. Digital subtraction angiographic images of a Group I rabbit’s vertebrobasilar system. The appearance was similar in Group III rabbits. Left: Vertebral and basilar arteries before subarachnoid hemorrhage (SAH) showing a normal appearance. Right: The vertebrobasilar system in the same rabbit 72 hours after SAH (infusion of 1 ml/kg of autologous blood into the cisterna magna) with no treatment.

gen peroxide, and singlet oxygen.\textsuperscript{38,69} Oxygen radicals can initiate peroxidative reactions in membrane PUFA producing lipid peroxides.\textsuperscript{10,48,58} Other degradation products of hemoglobin such as hematin can also catalyze the autoxidation of lipids.\textsuperscript{49,59} Lipid peroxides produced in the subarachnoid space can initiate free-radical reactions in the arterial wall.\textsuperscript{50}

Lipid peroxides act as very potent vasoconstrictors.\textsuperscript{1,2} Sasaki, \textit{et al.},\textsuperscript{21} reported that 5-hydroxyeicosatetraenoic acid (5-HETE) was the primary lipid peroxide measured in the CSF after SAH and was found in significantly higher concentrations in patients with vasospasm. In addition, these investigators induced prolonged vasospasm of the basilar artery in dogs by the intracisternal injection of 15-hydroperoxy-arachidonic acid.\textsuperscript{24} Sasaki, \textit{et al.},\textsuperscript{47} concluded that increased lipid peroxidation resulting from blood in the subarachnoid space may overwhelm normal cellular defense mechanisms, thereby contributing to the genesis of prolonged vasospasm.

Lipid peroxides contribute to the chronic cerebral vasospasm that is a consequence of SAH, antioxidant therapy should be effective in its prevention. Asano, \textit{et al.},\textsuperscript{1} tested the effect of the antioxidant 1,2-bis(nicotinamide) propane in a dog model of SAH and reported that this treatment reduced the magnitude of vasospasm. More recently, Travis and Hall\textsuperscript{62} demonstrated that intensive antioxidant treatment with vitamin E antagonized post-hemorrhagic cerebral hypoperfusion in a cat model of SAH.

In the present experiment, a novel 21-aminosteroid (U-74006F) was used as a pharmacological probe to assess the role of membrane lipid changes in SAH-
induced cerebral vasospasm. This compound is an effective inhibitor of in vitro iron-dependent lipid peroxidation and was specifically developed to have antioxidant activity. With 50% inhibiting concentrations ranging from 25 to 60 μM, U-74006F inhibits in vitro iron-dependent lipid peroxidation with a potency that equals that of the antioxidant vitamin E, and surpasses that of the iron chelator desferrioxamine. In a number of in vitro assays, U-74006F scavenges both the superoxide radical and (like alpha-tocopherol) lipid peroxyl radicals. In addition, U-74006F may have some antilipolytic activity. In preliminary studies, U-74006F blocked the release of arachidonic acid from cultured pituitary cells in response to hypoxia or lipid peroxidation. Thus, while U-74006F appears to have substantial "membrane stabilizing" capacity, it is not possible at this time to separate its antioxidant actions from its antilipolytic functions.

It has been shown that U-74006F reduced the loss of cortical and hippocampal CA1 neurons in a gerbil model of cerebral ischemia, and prevented the development of white-matter ischemia following a severe contusion of the spinal cord. Moreover, U-74006F can partially restore posttraumatic spinal cord blood flow (SCBF) even after it has declined significantly. Hall and Wolf have recently shown that the injury-initiated decline in SCBF involves lipid peroxidation of the spinal cord microvasculature. Thus, the protection afforded posttraumatic SCBF by U-74006F is consistent with its antioxidant properties.

In the present experiment, rabbits were treated with U-74006F starting 30 minutes after the onset of experimental SAH and treatment was continued for 72 hours. This treatment completely prevented both the chronic vasospasm and the reduction of flow in the basilar artery that occurred in the untreated and vehicle-treated rabbits 3 days after SAH. These results support the hypothesis that vascular lipid peroxidation, possibly along with lipid hydrolysis and eicosanoid production, are involved in the genesis of cerebral vasospasm. Previously, we demonstrated that pretreatment with U-74006F completely prevented the SAH-induced break-
down of the blood-brain barrier in rats. These data along with the present findings implicate lipid peroxidation and/or lipid hydrolysis in the genesis of SAH-initiated microvascular damage and prolonged vasospasm of the large cerebral vessels.

Acknowledgments

The authors thank the Upjohn Company, Kalamazoo, Michigan, for providing the U-74006F and Mrs. Saundra K. Eversole for typing the manuscript.

References

30. Ishii S, Nonaka T: [Cerebral vasospasm in subarachnoid hemorrhage with reference to its mechanism.] No To Shinkei 29:829–840, 1977 (Jpn)


Manuscript received September 16, 1988.

This work was supported by the Veterans Administration.

Address reprint requests to: Douglas K. Anderson, Ph.D., Medical Research Service (151), VA Medical Center, 3200 Vine Street, Cincinnati, Ohio 45220.