Protective Effect of a 21-Aminosteroid on the Blood–Brain Barrier Following Subarachnoid Hemorrhage in Rats

Mario Zuccarello, MD, and Douglas K. Anderson, PhD

The effects of subarachnoid injection of blood on blood–brain barrier permeability to albumin was assessed in a rat model. Subarachnoid injection of blood caused a significant sixfold increase in Evans blue extravasation, whereas sham operation or NaCl injection had no effect. In addition, subarachnoid injections of arachidonic acid or FeCl₂ increased blood–brain barrier permeability to Evans blue 16- and 10-fold, respectively. The capillary permeability after subarachnoid injection of blood was normalized by pretreatment with a novel 21-aminosteroid, U-74006F, that has antioxidant and antilipolytic activity. Pretreatment with U-74006F also reduced the vascular leakage induced by subarachnoid injection of arachidonic acid or FeCl₂ by 50% and 45%, respectively. We conclude that damage to membrane lipids by peroxidative and/or lipolytic processes is involved in the subarachnoid hemorrhage–induced blood–brain barrier opening and that U-74006F protects the blood–brain barrier against the effects of subarachnoid hemorrhage by preventing or limiting these pathologic membrane lipid changes. (Stroke 1989;20:367-371)

The presence of blood in the subarachnoid space results in alterations in blood–brain barrier (BBB) and blood–arterial wall barrier permeability and vasospasm of the large cerebral vessels. An increase in the permeability of major cerebral vessels and microvessels following subarachnoid hemorrhage (SAH) has been reported in both humans and experimental animals and has been correlated with a poor clinical outcome. Although increased intracranial pressure (ICP), changes in cerebral blood flow (CBF), and loss of CBF autoregulation contribute to the breakdown of the BBB, vasoactive substances originating from the breakdown products of extravasated blood also contribute to the opening of the BBB by directly affecting the brain vessels. Sano et al proposed that blood in the subarachnoid spaces generates lipid peroxides from free radical reactions catalyzed by iron and by hemoglobin degradation products and by the oxidative catabolism of arachidonic acid. It has been hypothesized that this progressive lipid peroxidation also leads to endothelial damage and opening of the BBB. We establish the magnitude of BBB damage following SAH in rats and assess the capacity of a novel 21-aminosteroid with antioxidant properties to prevent BBB breakdown after subarachnoid administration of blood, arachidonic acid, or FeCl₂.

Materials and Methods

We used 51 male albino Sprague-Dawley rats weighing approximately 250–350 g. All rats were anesthetized with 60 mg/kg i.p. pentobarbital sodium, and a femoral vein and artery were catheterized. A burr hole was drilled over the left cerebral convexity, 4 mm caudally from the coronal suture and 3 mm laterally from the sagittal suture. The dura mater was pierced with a 30-gauge needle, and a PE-10 polyethylene catheter was inserted 3–4 mm anteriorly and inferiorly over the cortical surface. Penetration of the subarachnoid space was demonstrated by flow of cerebrospinal fluid (CSF) through the catheter. The burr hole was sealed with bone wax and cyanoacrylate to prevent leakage of CSF. The catheter was connected to a pressure transducer through one outlet of a three-way stopcock. The other outlet was used for the injection of the test materials into the subarachnoid space. Consequently, ICP was not monitored during the injection but was measured immediately before and
after by simply reversing the stopcock. Mean arterial blood pressure (MABP) was continuously monitored for the duration of the experiment.

The rats were divided into nine experimental groups. All subarachnoid injections were over the left cerebral cortex, with an injection duration of 60–80 seconds.

Group I rats were sham-operated controls and received no subarachnoid injections (n=5). Group II rats received 300 μl of 0.9% NaCl injected into the subarachnoid space (n=6). Group III rats received SAH by injection of 300 μl autologous, nonheparinized arterial blood into the subarachnoid space with no treatment (n=6). The rats in Group IV received SAH with U-74006F treatment consisting of an intravenous bolus injection of 1 mg/kg U-74006F 15 minutes before and 2 hours after SAH (n=6). Group V rats received the buffered aqueous vehicle (0.02 M citric acid, 0.0032 M sodium citrate, 0.08 M NaCl) of U-74006F 15 minutes before and 2 hours after SAH (n=5). Group VI rats received subarachnoid injection of 300 μl of an 80 μg/ml solution of arachidonic acid with no treatment (n=6). Group VII rats received an intravenous bolus injection of 1 mg/kg U-74006F 15 minutes before and 2 hours after subarachnoid administration of arachidonic acid (n=5). Group VIII rats had 300 μl of 100 mM FeCl₂ infused into the subarachnoid space without any treatment (n=6). Group IX rats received an intravenous bolus injection of 1 mg/kg U-74006F 15 minutes before and 2 hours after subarachnoid infusion of FeCl₂ (n=7).

BBB permeability to albumin was quantitatively assessed 3 hours after placement of the subarachnoid catheter or subarachnoid injection. We used Evans blue (EB), a diazo dye that quantitatively binds to albumin in vivo,18 to assess BBB breakdown. One hour after operation, 50 mg/kg i.v. EB was administered. Two hours later, EB was cleared from the cerebral vasculature by intra-aortic perfusion of 100 ml 0.9% NaCl/100 g body wt at 90–100 cm H₂O. The brain was then removed; the left and right hemispheres were separated, weighed, and dried overnight in a desiccator. The hemispheres were reweighed and placed in formamide for 72 hours. EB concentration in the formamide extract was determined spectrophotometrically.19

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

**Results**

In Groups III, IV, and V, the blood clot was confined to the left cortical surface; no blood was found extradurally or in the ventricles. In the six other groups, no gross hemorrhage was found over the left cortical surface.

Preceding subarachnoid injection, mean±SEM baseline MABP and ICP were stable at approximately 96.7±0.98 mm Hg and 0.5±0.1 mm Hg, respectively. Group I MABP and ICP remained at
Zuccarello and Anderson  Effect of 21-Aminosteroid on BBB 369

baseline throughout the experiment. Subarachnoid injection of blood, NaCl, arachidonic acid, or FeCl₂ abruptly increased ICP to 7.64±0.36 mm Hg, which then returned to baseline within 8–10 minutes. Subarachnoid injection had no effect on MABP. Additionally, neither MABP nor ICP was affected by bolus injection of U-74006F.

Assessment of Blood–Brain Barrier Integrity

In Groups I and II there was no appreciable increase in BBB permeability (i.e., EB extravasation) in the left cerebral hemisphere compared with that in the right (Figure 1). EB extravasation extended 2–3 mm below the cortical surface under the blood clot.

In rats receiving SAH with (Groups V) or without (Group III) administration of the buffered aqueous vehicle for U-74006F, a significant sixfold increase in EB extravasation was measured in the left hemisphere. In Group IV rats, bolus injection of U-74006F completely prevented the SAH-induced breakdown of the BBB (Figures 1 and 2).

In Group VI, subarachnoid injection of arachidonic acid increased EB extravasation in the left hemisphere 16-fold and in the right hemisphere fourfold (Figure 3). Treatment with U-74006F (Group VII) did not normalize cerebral vascular permeability but significantly reduced EB leakage by 50% (Figure 3).

Subarachnoid injection of FeCl₂ (Group VIII) increased EB leakage in the left and right hemispheres 10-fold and sixfold, respectively (Figure 4). Again, treatment with U-74006F did not completely protect the cerebral vasculature but significantly decreased EB extravasation by 45%.

Discussion

Despite extensive investigation, the identity of the substances in blood responsible for damaging the cerebral vasculature after SAH remains unproven. Sasaki et al.⁹ postulated that free radical reactions and microvascular lipid peroxidation may be the key pathophysiologic factors initiated by blood clot lysis and 15-hydroperoxy arachidonic acid (15-HPAA) release that contribute to posthemorrhagic brain hypoperfusion. Furthermore, Travis and Hall¹¹ reported that chronic twofold dietary supplementation with α-tocopherol antagonizes acute cerebral hypoperfusion following experimental SAH, which supports the view that lipid peroxidative mechanisms play an important role in the development of this hypoperfusion. Recently, it was reported that lipid peroxidation coupled with insufficient antioxidant defense mechanisms in the arterial wall and in the CSF might be responsible for the occurrence of cerebral vasospasm and brain edema following SAH.²² The clinical significance of BBB breakdown after SAH has been stressed, and a correlation between BBB opening, poor neurologic grade, and outcome has been reported.⁷ In an attempt to elucidate the pathochemical mechanism of BBB dysfunction after SAH, we tested the hypothesis that BBB opening is induced by the action of lipid hydroperoxides gener- ated from free radical reactions catalyzed by iron and by hemoglobin degradation products and by the oxidative catabolism of arachidonic acid. Lipid hydrolysis with subsequent eicosanoid production may also be a pathochemical event occurring early after SAH.⁹-¹⁴ Lipid peroxidation and eicosanoids can damage cerebral vascular endothelial cells with subsequent opening of the BBB (Figure 5).

A new 21-aminosteroid antioxidant, U-74006F, was used to protect the BBB against the effects of subarachnoid injections of blood, arachidonic acid, or FeCl₂. This compound is an effective inhibitor of in vitro iron-dependent lipid peroxidation and was specifically developed to have antioxidant activity.¹⁶ U-74006F inhibits in vitro iron-dependent lipid peroxidation with a potency that equals that of the antioxidant α-tocopherol and surpasses that of the iron chelator deferoxamine.¹⁶ In a number of in vitro assays, U-74006F scavenges both the superoxi-
ide radical (J.M. Braughler, personal communication) and, like α-tocopherol, lipid peroxyl radicals. Also, U-74006F blocks the release of arachidonic acid from cultured pituitary cells in response to hypoxia or lipid peroxidation, which indicates some antilipolytic activity for this agent. Thus, it is not possible now to separate the antioxidant actions of U-74006F from its antilipolytic functions. U-74006F inhibits acute progressive SAH-induced cerebral hypoperfusion in cats. U-74006F also attenuates posttraumatic spinal cord hypoperfusion and postischemic cortical hypoperfusion. In each instance (i.e., SAH, trauma, ischemia), intensive pretreatment with α-tocopherol also antagonizes central nervous system (CNS) tissue hypoperfusion. The similar effects of both α-tocopherol and U-74006F on CNS microvascular perfusion suggests that the protection afforded by U-74006F is mediated, at least in part, by its antioxidant activity.

Travis and Hall reported that in cats, both α-tocopherol and U-74006F reduce the SAH-induced rise in ICP. They proposed that this reduction in ICP was mediated by a decrease in vasogenic edema. Our results demonstrating an inhibition of post-SAH EB extravasation are consistent with this speculation.

Our studies demonstrate that U-74006F can completely prevent the SAH-induced breakdown of the BBB in rats. U-74006F pretreatment also significantly reduced the arachidonic acid- or FeCl₂-induced vascular leakage of albumin by 50% and 45%, respectively. It is likely that concentrations of both arachidonic acid and FeCl₂ were too high and/or that the dose of U-74006F was too low to completely protect the BBB. Further studies are necessary to elucidate whether or not a dose dependency exists.

Our findings support the hypothesis that microvascular lipid peroxidation along with eicosanoid production are involved in the SAH-induced opening of the BBB. This interpretation may have implications for the clinical treatment of SAH, that is, it emphasizes the necessity for more effective attempts to reduce the leakage that occurs from cerebral capillaries during the early stage of SAH. Furthermore, if lipid peroxidation is implicated in post-SAH damage to the BBB, one can reasonably speculate that the same pathochemical mechanism may play a role in the development of cerebral vasospasm. Further studies will investigate this possible association and the potentially beneficial effects of U-74006F.

Acknowledgments

We are grateful to John T. Povlishock, PhD, Department of Anatomy, Medical College of Virginia, Richmond, Virginia, and to Edward D. Hall, Central Nervous System Diseases Research Unit, The Upjohn Company, for their critical review of the manuscript and to Mrs. Saundra K. Eversole for manuscript preparation. We are indebted to The Upjohn Company, Kalamazoo, Michigan, for supplying the U-74006F.

References


**Key Words** • blood–brain barrier • lipid peroxides • subarachnoid hemorrhage • rats
Protective effect of a 21-aminosteroid on the blood-brain barrier following subarachnoid hemorrhage in rats.
M Zuccarello and D K Anderson

doi: 10.1161/01.STR.20.3.367

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1989 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/20/3/367

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Stroke* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Stroke* is online at:
http://stroke.ahajournals.org/subscriptions/