Decrease in Cerebral Blood Flow in Rats After Experimental Subarachnoid Hemorrhage: A New Animal Model

ROBERT A. SOLOMON, M.D., J. LOBO ANTUNES, M.D., RICHARD Y.Z. CHEN, M.D., LINDA BLAND, B.S., AND SHU CHIEN, M.D., PH.D.

SUMMARY There continues to be a need for good animal models of experimental subarachnoid hemorrhage (SAH). The rat would be an ideal subject in which to study SAH since it is inexpensive and easier to use than the larger laboratory animals. The present study was undertaken to determine if alterations of cerebral blood flow could be produced in the rat after experimental SAH, and thereby justify using the rat as a model for further study of SAH.

Rats weighing between 450 and 500 grams underwent insertion of a cannula into the cisterna magna at least 5 days prior to physiological testing. One group of rats then received a 0.3 cc injection of fresh autologous arterial blood into the cisterna magna to simulate a SAH. Another group of rats received injection of an equal volume of mock CSF (buffered saline) into the cisterna magna. A third group of rats had no subarachnoid injections. In all three groups, blood flow to the cerebral hemispheres was measured with the labeled microsphere technique.

Rats with experimental SAH showed a 40% decrease of cerebral blood flow, whereas rats with saline injections showed only a 15% decrease. Control rats had no changes of cerebral blood flow. These studies demonstrate that the rat is a potential experimental model for investigations into SAH.

RUPTURE OF AN INTRACRANIAL ANEURYSM or arteriovenous malformation produces a subarachnoid hemorrhage which may have devastating secondary effects on the cerebral circulation. Manifestations of these effects range from mild functional and metabolic disturbances of diencephalic structures to massive cerebral infarction produced by delayed vasospasm. Experimental designs in dogs, cats, and primates have been used to reproduce, in a laboratory setting, the blood flow changes seen in humans. These models, however, tend to be cumbersome and expensive and do not exactly mimic the clinical setting under investigation. Furthermore, these large laboratory animals do not lend themselves easily to studies using the newer techniques of quantitative autoradiography. There continues, therefore, to be a need for improved animal models of subarachnoid hemorrhage. The rat would be an ideal species in which to study this entity since it is relatively inexpensive, easy to use, and is already the preferred model in most current studies of neuroanatomy, neurophysiology, and neuropharmacology. As well, detailed studies of brain me-
tabolism and blood flow using quantitative autoradiography are far easier to conduct in rats than in larger laboratory species. These types of studies will be necessary to improve current research approaches to brain dysfunction after subarachnoid hemorrhage. The usefulness of the rat in this regard has not been adequately addressed.

The present study was undertaken to determine if the rat could be used to produce subarachnoid hemorrhages which are morphologically similar to those observed in humans. Moreover, if these artificial hemorrhages produce quantitative alterations in cerebral blood flow similar to those reported in humans and other animals, then justification would exist to begin development of the rat as a model of human subarachnoid hemorrhage.

Materials and Methods

Cannulation of the Cisterna Magna

Male Sprague-Dawley rats weighing between 450 and 500 grams were used in these studies. All rats underwent insertion of a cannula into the cisterna magna at least 5 days prior to physiological testing. The cannula assembly used was a standard single cannula system made by Plastic Products Company (Roanoke, Va.). It consisted of a 22 gauge stainless steel cannula and a tight-fitting inner stylet to seal the top of the cannula and keep tissue out of the system. The cannula and stylet were ground to a length of 10 mm prior to use.

The rat to be cannulated was anesthetized with intramuscular ketamine hydrochloride (100 mg/kg); the head was shaved and positioned in a stereotaxic head holder (Kopf Instruments, Tujunga, Ca.). Using the operating microscope, the scalp was incised and a midline burr hole was made using an air powered drill just rostral to the interparietal-occipital suture. Another line burr hole was made using an air powered drill just rostral to the interparietal-occipital suture. Another hole was made in the parietal bone for placement of a small anchoring screw. The occipital bone was then cleared of muscular tissue and the atlanto-occipital membrane was identified and cleaned of extraneous connective tissue. Care was taken not to open the cisterna magna while establishing a translucent membrane through which the contents of the cisterna magna could be viewed. The cannula was then mounted on the manipulating arm of the stereotaxic instrument and lowered into the burr hole at an angle of about 60 degrees with the top of the calvarium. This approach allowed the cannula tip to slide along the inner table of the occipital bone. While the cisterna magna was being viewed under high magnification through the atlanto-occipital membrane, the cannula was lowered until the tip was just visible in the cisterna. Methyl methacrylate dental acrylic was then poured over the exposed surface of the skull to fix the cannula and screw assembly. Correct placement was double-checked by observing clear CSF flowing from the free end of the cannula. The cannula was then sealed with the inner stylet and the scalp was sutured. The rat was returned to a separate cage and allowed to recover for several days.

Blood Flow Measurements During Artificial Subarachnoid Hemorrhage

Rats with cannulae implanted in the cisterna magna were anesthetized with ether and immediately tracheotomized. They were then ventilated with 75%/25% N2O/O2 mixture. At least one hour was allowed to elapse before blood flow measurements were made in order to insure complete clearance of the ether. During this time one femoral vein and one femoral artery were cannulated, and a catheter was placed in the left ventricle of the heart via the right common carotid artery. The rats were paralyzed with pancuronium bromide (0.16 mg/kg IV) and ventilation was controlled (rodent ventilator, Harvard Apparatus Corp.) to keep arterial pCO2 close to 32 mm Hg. Arterial blood gases were monitored on a blood gas analyzer (model 213, Instrumentation Laboratory). Continuous recordings of arterial blood pressure, intracranial pressure and EKG were made using Statham transducers and a Grass multichannel recorder. The intracranial pressure was monitored by connecting the free end of the cisterna magna cannula to a pressure transducer located at the level of the right atrium of the heart.

Cerebral blood flow was measured 4 times in each rat using the radioactive microsphere technique.16–18 57Co, 103Ru, 113Sn, and 99mTc labelled 15μ microspheres (New England Nuclear, Boston, Mass.) were employed in these studies. The microspheres were injected into the left ventricle of the heart, while a reference blood sample was withdrawn from the femoral artery by a continuous withdrawal pump (Harvard Apparatus Corp.). Approximately 1.2 cc of blood was withdrawn for each reference sample. This amount of blood was simultaneously replaced by transfusion into the femoral vein of an equal amount of blood from a donor rat. Therefore all blood flow measurements were made under conditions of isovolemia. A total of not more than 100,000 microspheres were used in any one animal, since greater quantities caused significant changes in cardiac output and regional blood flow. With this small number of microspheres, the anatomical resolution of regional cerebral blood flow differences is limited. Preliminary studies have indicated that blood flow is not accurately determined on structures much smaller than the cerebral hemisphere of the rat since too few microspheres are present in small pieces of brain tissue. When approximately 25,000 microspheres are injected into the left ventricle of the heart, about 1200 spheres lodge in each hemisphere of the brain. This number of spheres has been demonstrated to supply accurate information regarding regional blood flow with an error of < 10% at a confidence level of > 95%.16, 17

A control blood flow measurement was first made in each rat. In 6 rats 0.3 cc of fresh autologous arterial blood was then injected into the cisterna magna while the rat was held in a 20° head down position. To insure constancy of blood volume, the removal of 0.3 cc of blood from the femoral artery was accompanied by a simultaneous injection of 0.3 cc of donor rat blood into the femoral vein. Blood flow measurements were re-
peated 15, 30, and 60 minutes after the artificial subarachnoid hemorrhage. In 5 rats, 0.3 cc of mock CSF (normal saline with 25 meq/l of NaHCO₃ equilibrated with 5% CO₂ for pH 7.40) was injected into the cisterna magna and blood flow measurements were repeated at the same intervals. In 7 rats, no cisterna magna injection was made. Blood flow measurements in these rats at 0, 15, 30, and 60 minutes served as control values. The arterial pCO₂ and mean arterial pressure were measured and recorded before each microsphere injection in all rats.

At the completion of each experiment, the rat was sacrificed with intravenous KCl, and the brain was removed and divided into cerebral hemispheres for the determination of blood flow. Blood flow was also measured in the heart and kidneys of each animal. Tissue radioactivities and the energy peaks for the four isotopes were separated and quantitated with the use of a multichannel analyzer (Tracor-Northern, Syosset, New York) connected to a Tri-Carb gamma counter (Packard Instrument Co., Downers Grove, IL). A computer program using the stripping method was used to resolve the radioactivity of each isotope and express it in terms of cpm per 100 gm of tissue sample (Cᵢ), as well as to calculate the flow rate per 100 gm of tissue sample (Qᵢ) and the cardiac output (CO) by the use of the equations given below:

\[
CO = \frac{A_i}{(A_i/Q_B)}
\]

\[
Q_i = \frac{C_i}{(A_i/Q_B)}
\]

where \( A_i \) is the amount of radioactivity injected, \( A_p \) is the total activity of the reference flow sample, and \( Q_p \) is the pump withdrawal rate. Blood flow data for each rat were normalized to the zero time or control value obtained prior to cisternal injection. This normalization was achieved by expressing all blood flow measurements for each individual rat as a percentage of that animal’s zero time or control blood flow value.

**Results**

At the time of necropsy, all rats with experimental subarachnoid hemorrhage were noted to have extensive subarachnoid blood present in the basal cisterns of the brain. No hemorrhage was visualized for the saline treated rats or the control rats (fig. 1).

Mean arterial pressure and arterial pCO₂ remained relatively constant for each rat during the 60 minute

**FIGURE 1.** Examples of the gross appearance of the fresh rat brain at the time of necropsy immediately following physiological experiments. A: Rat given saline injection into the cisterna magna 60 minutes prior to sacrifice. B: Rat given blood injection into the cisterna magna 60 minutes prior to sacrifice. Note extensive subarachnoid hemorrhage in B.
CEREBRAL BLOOD FLOW IN RATS AFTER SAH/Solomon et al

FIGURE 2. A: Mean normalized blood flow to the heart for the three groups of rats over the 60 minute period following the cisterna magna injections. B: Mean normalized blood flow to the kidneys during the same period. C: Mean normalized values of cardiac output for the three groups of rats during the same period. D: Mean normalized mean arterial pressure readings for the three groups of rats. Abbreviations: Saline: rats treated with buffered saline injection into the cisterna magna; SAH: rats treated with blood injection into the cisterna magna; Control: rats that received no cisterna magna injection.

experimental period. The average variation of mean arterial pressure was less than 5% (fig. 2). Two experimental rats, one from the intracisternal saline group and one from the subarachnoid hemorrhage group, demonstrated a change of pCO₂ of greater than 15% during the experimental period. The etiology of these changes was not apparent, and data from these rats were excluded from this report. Cardiac output tended to fall during the course of the experiment in all three groups of rats (fig. 2). It decreased by an average of 6%, 10%, and 15% in the subarachnoid hemorrhage, saline treated, and control rats respectively. This drop may reflect changing depth of anesthesia or other temporal factors in the experimental procedure.

All rats with an artificial subarachnoid hemorrhage showed a dramatic and progressive decrease in hemispheric blood flow over the 60 minutes following the blood injection. The mean decrease for the right and left hemispheres was 48% and 38% respectively, and the values of cerebral blood flow were significantly below control values (p < 0.01). In the intracisternal saline rats a smaller, non-significant decrease of hemispheric blood flow was noted: 12% for the right hemisphere and 15% for the left hemisphere. The seven control rats demonstrated no change in cerebral blood flow over the course of the experiment. The blood flow decreases demonstrated in the subarachnoid hemorrhage group were significantly greater than those observed in the saline treated group (p < 0.02). These data are detailed in table 1 and graphically portrayed in figure 3.

Myocardial blood flow tended to increase slightly during the course of each experiment, but none of these changes achieved statistical significance (fig. 2). Renal blood flow also tended to increase during the course of the experiments, but these changes were also not statistically significant (fig. 2).

No changes in the waveform of the electrocardio-

<table>
<thead>
<tr>
<th>Table 1 Normalized Hemispheric Blood Flow for the Three Groups of Rats at Various Times after Cisterna Magna Injection*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat #</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>SAH 1</td>
</tr>
<tr>
<td>SAH 2</td>
</tr>
<tr>
<td>SAH 3</td>
</tr>
<tr>
<td>SAH 4</td>
</tr>
<tr>
<td>SAH 5</td>
</tr>
<tr>
<td>SAH 6</td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>SD</td>
</tr>
<tr>
<td>SAL 1</td>
</tr>
<tr>
<td>SAL 2</td>
</tr>
<tr>
<td>SAL 3</td>
</tr>
<tr>
<td>SAL 4</td>
</tr>
<tr>
<td>SAL 5</td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>SD</td>
</tr>
<tr>
<td>CTR 1</td>
</tr>
<tr>
<td>CTR 2</td>
</tr>
<tr>
<td>CTR 3</td>
</tr>
<tr>
<td>CTR 4</td>
</tr>
<tr>
<td>CTR 5</td>
</tr>
<tr>
<td>CTR 6</td>
</tr>
<tr>
<td>CTR 7</td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>SD</td>
</tr>
</tbody>
</table>

Abbreviations: RH = right hemisphere; LH = left hemisphere; SAH = rats with artificial subarachnoid hemorrhage; SD = standard deviation; SAL = rats with saline injection into cisterna magna; CTR = rats with no cisternal injection, i.e. controls.

*See materials and methods section for explanation of how normalized blood flow values were obtained.
FIGURE 3. Mean normalized blood flow to the cerebral hemispheres for the three groups of rats over the 60 minute period following the cisterna magna injections. Upper panel shows right hemispheric blood flow and lower panel shows left hemispheric blood flow. Abbreviations: Saline: rats treated with buffered saline injection into the cisterna magna; SAH: rats treated with blood injection into the cisterna magna; Control: rats that received no cisterna magna injections.

gram were noted during injection of blood or saline into the cisterna magna. Transient bradycardia and hypertension were observed during the injection of both agents (fig. 4). These changes correlated with alterations of intracranial pressure that were observed in the experimental animals. Transient increases of intracranial pressure from a baseline of 3–6 mmHg to a peak of 10 to 18 mmHg were noted after the saline or blood injections into the cisterna magna (fig. 4). No differences were observed in the intracranial pressure response to either of these two agents. Intracranial pressure and systemic arterial pressure returned to normal within three to five minutes, and the rats generally became slightly tachycardic thereafter. No changes in the ST or T waves could be demonstrated. However, premature ventricular contractions were observed during the cisternal injections and were seen periodically throughout the following hour. There were no substan-

tial differences in the EKG changes observed between the two experimental groups.

Discussion

The data presented in this study demonstrate a decrease in global cerebral blood flow in the rat after experimental subarachnoid hemorrhage. This decrease of approximately 40% was not reproduced in magnitude by saline injections into the subarachnoid space, indicating the specificity of subarachnoid blood for altering cerebral blood flow. The effects of a sudden increase in intracranial pressure probably account for the slight drop of about 15% observed in the saline treated group. However, it is apparent from the design of this experiment that increased intracranial pressure alone can only account for a small portion of the changes of cerebral blood flow seen after artificial subarachnoid hemorrhage.

Similar blood flow changes following subarachnoid hemorrhage have been documented for humans and other experimental animal models. Nonetheless, controversy still exists regarding the pathophysiological mechanisms which account for these alterations of cerebral blood flow. Acute vasospasm has been postulated to underlie the blood flow changes observed, but other investigators have found little or no correlation between blood flow and angiographically visualized vasospasm.

Recent anatomical and neuropharmacological evidence has demonstrated extensive catecholaminergic and peptidergic innervation of small intraparenchymal blood vessels of the brain. Physiological studies have implicated these neuronal systems in the regulation of regional cerebral blood flow, especially in pathological situations. The cell bodies of origin for neurones innervating the small cerebral blood vessels lie in the locus coeruleus, brain stem reticular formation, and the hypothalamus. These regions of the central nervous system are postulated to be profoundly affected by subarachnoid hemorrhage, as indicated by the disturbances of autonomic function which occur following rupture of an intracranial aneurysm or experimentally induced subarachnoid hemorrhage. The possibility exists, therefore, that alterations of the
CEREBRAL BLOOD FLOW IN RATS AFTER SAH/Solomon et al

microvasculature of the brain, the true resistance vessels of the cerebral circulation, may account for many of the blood flow changes observed after subarachnoid hemorrhage.

As outlined above, several different pathophysiological explanations for cerebral blood flow changes seen after subarachnoid hemorrhage have been invoked by different investigators. It remains for further research to delineate the true mechanisms at work.

There are only three other published reports of studies employing a rat model of subarachnoid hemorrhage. Barry et al used a microfilarium to puncture the basilar artery and thereby produce a subarachnoid hemorrhage. These authors measured basilar artery diameter directly to quantitate the effects of the hemorrhage. These authors measured basilar artery diameter directly to quantify the effects of the hemorrhage. Lacy and Earle used the rat to study electrocardiographic abnormalities observed after the injection of heparinized blood into a catheter placed in the region of the circle of Willis. They concluded that EKG changes were the result of a sudden and sustained increase of intracranial pressure. Svendsgaard et al injected blood into the cisterna magna of rats and used angiography to demonstrate vasospasm. These authors also reported the use of quantitative autoradiography to demonstrate alterations of blood flow and metabolism, although their preliminary report does not give details of their findings or techniques.

The present study represents the first time that the rat has been used to document decreases in hemispheric blood flow after acute experimental subarachnoid hemorrhage. We present a new technique for the creation of hemorrhages which are morphologically similar to those seen in humans and larger experimental animals (fig. 1). Although reduction of cerebral blood flow after subarachnoid hemorrhage has been demonstrated in humans and other animal systems, the pathogenesis of these changes remains obscure. Detailed studies of cerebral blood flow and metabolism using quantitative autoradiography may expand the realm of investigative approaches to these pathophysiological mechanisms. The rat offers many advantages over larger animals in the autoradiographic protocols, and in this regard it seems worthwhile to develop a rat model of subarachnoid hemorrhage. Whether or not the rat will be useful for studies of delayed blood flow changes seen after subarachnoid hemorrhage remain to be investigated.

References

5. Varsos VG, Liszczak TM, Han DH, et al: Delayed cerebral vasospasm is not reversible by aminophylline, nifedipine, or papaverine in a "two-hemorrhage" canine model. J Neurosurg 58: 11-17, 1983
Effect of Small Deep Hemispheric Infarction on the Ipsilateral Cortical Blood Flow in Man

TAKASHI TAKANO, M.D.,* KAZUFUMI KIMURA, M.D.,† MASAICHI NAKAMURA, M.D.,† RYUZO FUKUNAGA, M.D.,† MASAHITO KUSUNOKI, M.D.,† HIDEKI ETANI, M.D.,† MASAYASU MATSUMOTO, M.D.,† SHOTARO YONEDA, M.D.,† and HIROSHI ABE, M.D.,†

SUMMARY The effect of small, deep ischemic lesions on the ipsilateral cortical circulation was investigated in 10 patients with persistent mild or moderate neurological deficits due to infarcts in the internal capsule. rCBF studies by the $^{133}$Xe intracarotid injection method were performed 14–180 days after the onset of the infarction. The rCBF functional image was made up from the data of $^{133}$Xe dynamic images measured by an Anger-type gamma camera and the rCBF values were calculated by the initial slope method. The average value of mean rCBFs (mCBF) in 10 patients was 44.9 ± 7.1 ml/100g/min (average PaCO$_2$: 39.9 ± 4.3 mm Hg). In the rCBF functional images, a focal hypoperfusion area was observed in all cases and localized around the central sulcus, especially in the precentral and central areas. Significant decreases of mCBF and the tendency to decrease of the rCBFs in the hypoperfusion focus were noted in the patients with the larger infarcts in comparison with those with the smaller ones. These results suggest that a small, deep ischemic lesion such as a capsular infarct may have remote effects on the ipsilateral cortical circulation, due probably to the damage of a number of fibers passing through the lesion.

THE PRINCIPLE and method of measuring the cerebral blood flow (CBF) by intracarotid injection of a radioactive inert gas have been established by Lassen and Ingvar. Since then, pathophysiologically valuable information relating to the cerebral circulation and metabolism in cerebrovascular diseases has become available. In the majority of patients with acute stroke, the localization and severity of the reduction of regional CBF (rCBF) correlate well with the anatomical site of the infarct.

It is generally recognized that rCBF is reduced in the ischemic hemisphere and, to a lesser extent, in the nonischemic hemisphere following a unilateral cerebral infarction. This phenomenon suggests that a focal damage may cause a decrease of blood flow at the parts distant from the lesion in the human brain. Several reports have discussed this, but the infarction in these studies has been too large or variable to make a specific analysis on the circulation and metabolism in the distant related area. On the other hand, only a few reports have been presented on the remote effect induced by a minimal lesion in the brain.

The purpose of this study is to investigate the influence of a small, capsular infarct on the ipsilateral cortical blood flow.

Subjects and Methods

Subjects

Ten patients aged 39–78 years old, with a mean ± SD of 56.4 ± 13.5, who suffered from mild or moderate hemiparesis after cerebral infarct were studied. The diagnosis of capsular infarction was made on the basis of clinical symptoms, neurological findings, cerebral angiogram and CT-scan of the brain. Informed consent was obtained from each subject prior to the initiation of the study in accordance with the Helsinki Declaration of 1975. The clinical summary of the subjects studied is shown in table 1. All patients had a clinical history of hypertension. Four patients showed pure motor hemiparesis and 6 manifested both motor and sensory disturbances. Five cases had a mild, and the remainder a moderate severity of neurological deficit. The severity (mild or moderate) of neurological deficit in each case was determined by the activities of daily living. All patients were successfully investigated 14–180 days after the ictus. In each case, CT-scans were carried out 2–3 times before and after the rCBF measurement, and the existence of a small low density area at the internal
Decrease in cerebral blood flow in rats after experimental subarachnoid hemorrhage: a new animal model.
R A Solomon, J L Antunes, R Y Chen, L Bland and S Chien

Stroke. 1985;16:58-64
doi: 10.1161/01.STR.16.1.58
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1985 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/16/1/58

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/