Cerebrovascular CO₂ Reactivity During Delayed Vasospasm in a Canine Model of Subarachnoid Hemorrhage

Michael N. Diringer, MD; Dan S. Heffez, MD; Lee Monsein, MD; Jeffrey R. Kirsch, MD; Daniel F. Hanley, MD; and Richard J. Traystman, PhD

While the in vitro reactivity of cerebral conducting vessels following subarachnoid hemorrhage has been extensively studied, in vivo cerebrovascular CO₂ reactivity has not been systematically investigated. We tested the hypothesis that, in the canine model of subarachnoid hemorrhage, the rise in cerebral blood flow normally seen with hypercapnia is blunted during delayed vasospasm. Four groups of animals were studied: one received two 4-ml subarachnoid injections of nonheparinized arterial blood into the cisterna magna (n=8), one received three subarachnoid injections of 5 ml blood (n=5), one received two subarachnoid injections of 4 ml saline (n=5), and a control group (n=5) had no subarachnoid injections or angiography. Basilar artery diameter was measured from baseline and follow-up angiography. We determined CO₂ reactivity by randomly varying the concentration of inspired CO₂ and measuring regional cerebral blood flow with radiolabeled microspheres. Basilar artery diameter was not affected by saline injection and was reduced by 26±2.9% in the two-hemorrhage group and 55±1.9% in the three-hemorrhage group. Baseline cerebral blood flow and CO₂ reactivity were similar in all four groups. We conclude that, in this model of delayed vasospasm, regional cerebral vascular CO₂ reactivity is intact and extrapolation of in vitro data regarding basilar artery diameter and reactivity to cerebral blood flow must be done cautiously. (Stroke 1991;22:367-372)

Delayed cerebral ischemia is a major cause of morbidity and mortality following subarachnoid hemorrhage (SAH). This ischemia is often attributed to the vasospasm syndrome, which is manifest angiographically by the narrowing of large conducting blood vessels and clinically by delayed neurologic deficits. Angiographic evidence of vasospasm can be seen in a majority of patients following SAH, while the clinical syndrome develops in only 20–30%. Thus, the physiologic significance of the angiographic changes is not clear.

The effects of SAH on cerebral vascular reactivity have been investigated using a canine model. In this model there is transient vasospasm immediately following hemorrhage, and it recurs, in a delayed form, 4–8 days later. Enhanced vasoconstriction, impaired vasodilation, altered mechanical properties, and ultrastructural changes have been demonstrated coincident with angiographic vasospasm. However, these experiments have studied isolated segments of large conducting vessels in vitro, without correlation with in vivo vascular reactivity or cerebral blood flow (CBF). While large conducting vessels contribute significantly to cerebrovascular resistance, evidence suggests that further regulation of CBF occurs at the level of small pial vessels. Thus, the relevance of in vitro investigations to our understanding of the mechanisms and treatment of delayed cerebral ischemia is unclear.

To improve our understanding of CBF and cerebrovascular regulation during delayed vasospasm, we studied in vivo cerebrovascular CO₂ reactivity in a canine model of delayed vasospasm following SAH. We tested the hypothesis that the normal rise in CBF in response to increased arterial partial pressure of CO₂ (PaCO₂) is blunted in this model of delayed basilar artery vasospasm. Delayed vasospasm was produced by cisternal injection of nonheparinized arterial blood...
autologous arterial blood and was demonstrated by angiography. Cerebrovascular reactivity to changes in PaCO₂ was determined by measuring regional cerebral blood flow (rCBF) using the radiolabeled microsphere technique.

**Materials and Methods**

Experiments were performed on 23 adult male beagle-type dogs weighing 10−12 kg. Four groups of animals were studied (Table 1). In the two-hemorrhage group (n=8) CO₂ reactivity was measured following two subarachnoid injections of blood and angiography. On day 1, following baseline angiography, 4 ml fresh, autologous, nonheparinized arterial blood was injected into the cisterna magna. On day 3 the injection of blood was repeated. On day 6 angiography was repeated followed by the determination of CO₂ reactivity. The three-hemorrhage group (n=5) received three subarachnoid injections of blood to produce more severe vasospasm. On day 1 baseline angiography was followed by the injection of 5 ml blood; 5 hours later another 5 ml arterial blood was injected. On day 3 5 ml blood was again injected. Follow-up angiography was performed on day 9 followed by determination of CO₂ reactivity. The saline group (n=5) received cisternal injections of 4 ml sterile, preservative-free saline on days 1 and 3. On day 6 follow-up angiography was performed followed by determination of CO₂ reactivity. In the control group (n=5) CO₂ reactivity was determined without subarachnoid injection or angiography.

During the performance of angiography, the dogs were anesthetized with 20−25 mg/kg i.v. thiamylal, intubated, and mechanically ventilated (Model 607, Harvard Apparatus, Millis, Mass.) to maintain PacO₂ at 35−40 torr. Due to the larger number of vertebral artery branches in dogs, injection of contrast material into the vertebral artery does not allow for consistent opacification of the basilar artery, especially when vasospasm increases the resistance and shunts contrast into more proximal collaterals. Therefore, a 2.7F microcatheter (Traker-18, Target Therapeutics, San Jose, Calif.) was passed, through an angiographic catheter positioned in one vertebral artery, into the ventral spinal artery via the third cervical branch of the vertebral artery. This allowed for the injection of contrast material into the ventral spinal artery, which is the major supply of the basilar artery. Blood pressure was recorded, and arterial blood was obtained for analysis. Five milliliters of the iodinated contrast material meglumine diatrizoate was injected at a rate of 3 ml/sec, and films were recorded at 2/sec. A radiopaque ruler was filmed after each injection to document the degree of magnification. The film that best demonstrated the basilar artery was enlarged under standard magnification (×3.3) and printed on high-contrast film (Grade 5 Kodachrome, Kodak, Rochester, N.Y.). Measurements were made at three points along the basilar artery by two independent observers who were blinded to the groups and the relation of angiography to subarachnoid injection. The six measurements were corrected for magnification and averaged to obtain the final vessel diameter.

For the injection of subarachnoid blood, the dogs were anesthetized with 20−25 mg/kg i.v. thiamylal, intubated, and allowed to breathe spontaneously. Blood was hand-injected over 2 minutes into the cisterna magna via a percutaneous puncture. The animal was immediately placed in the head-down position at an angle of 30° for at least 20 minutes. For the determination of CO₂ reactivity, the dogs were anesthetized with barbiturate (20−30 ml/kg thiaramyl or 10 mg/kg pentobarbital) and 50 μg/kg fentanyl and maintained on an infusion of 3 mg/kg/hr pentobarbital for the remainder of the study. Core temperature was maintained at 38±0.5°C, end-tidal CO₂ was continuously monitored (Model 78356A Hewlett-Packard, Boeblingen, FRG, or Model LB-2, Beckman, Fullerton, Calif.), and arterial blood gases were frequently determined (ABL-3 Blood Gas Analyzer, Radiometer, Copenhagen, Denmark). A catheter was inserted into the sagittal sinus approximately 0.5 cm caudal to the coronal suture to obtain cerebrovenous blood for calculation of cerebral oxygen consumption (CMRO₂). Intracranial pressure and blood pressure were continuously monitored. During preparation for the CBF measurements, ventilation was adjusted to lower PacO₂ to 20−22 torr. The inspired concentration of CO₂ was then increased to achieve a baseline PacO₂ of 38−40 torr. Following preparation and a 30-minute stabilization period, baseline blood gases, pH, blood pressure, intracranial pressure, and core temperature were measured, and microspheres were injected. The concentration of inspired CO₂ was then adjusted, in random order, to alter PacO₂ to 20±3, 30±3, 50±3, 60±3, and 75±3 torr. Following 10 minutes of stabilization at each PacO₂ level, all measurements were repeated. Cerebral perfusion pressure was calculated by subtracting the intracranial pressure from the mean arterial blood pressure, and CMRO₂ was calculated by dividing the difference between the arterial and cerebrovenous oxygen contents (Model IL-

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**Table 1. Experimental Groups of Dogs**

<table>
<thead>
<tr>
<th>Group</th>
<th>Subarachnoid injection into cisterna magna</th>
<th>Angiography</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two-hemorrhage</td>
<td>4 ml fresh nonheparinized arterial blood on days 1 and 3</td>
<td>Days 1 and 6</td>
</tr>
<tr>
<td>Three-hemorrhage</td>
<td>5 ml fresh nonheparinized arterial blood twice on day 1 and once on day 3</td>
<td>Days 1 and 9</td>
</tr>
<tr>
<td>Saline</td>
<td>4 ml saline on days 1 and 3</td>
<td>Days 1 and 6</td>
</tr>
<tr>
<td>Control</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

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TABLE 2. Blood Pressure, Blood Gases, and pH During Angiography in Dogs

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Mean blood pressure (mm Hg)</th>
<th>pH</th>
<th>PaCO2 (torr)</th>
<th>PaO2 (torr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Baseline</td>
<td>Follow-up</td>
<td>Baseline</td>
<td>Follow-up</td>
</tr>
<tr>
<td>Saline</td>
<td>5</td>
<td>137±6</td>
<td>135±3</td>
<td>7.32±0.01</td>
<td>7.32±0.02</td>
</tr>
<tr>
<td>Two-hemorrhage</td>
<td>8</td>
<td>127±6</td>
<td>132±6</td>
<td>7.34±0.01</td>
<td>7.38±0.05</td>
</tr>
<tr>
<td>Three-hemorrhage</td>
<td>5</td>
<td>147±6</td>
<td>136±4</td>
<td>7.32±0.03</td>
<td>7.34±0.02</td>
</tr>
</tbody>
</table>

Values are mean±SEM. PaCO2, arterial partial pressure of CO2; PaO2, arterial partial pressure of O2.

282, CO-Oximeter, Instrumentation Laboratories, Lexington, Mass.) by the hemispheric CBF.

The rCBF was measured with radiolabeled microspheres (16±0.5 μm diameter, Du Pont–New England Nuclear, Boston, Mass.) using the reference sample method.13 Microspheres were injected via a catheter in the left cardiac ventricle. The brain was dissected into discrete areas including the brain stem, cerebellum, caudate nucleus, medial occipital lobe (posterior cerebral artery territory cortex), corpus callosum and centrum semiovale (white matter), frontal and parietal cortex, and deep nuclei (carotid artery territory). At the three highest levels of PacO2, rCBF was significantly different from the values at baseline PacO2; however, there were no differences within groups in PaCO2, hemoglobin concentration, cerebral perfusion pressure, or CMRO2. There were also no differences among groups in PaCO2, PaO2, hemoglobin concentration, cerebral perfusion pressure, or CMRO2.

At baseline PaCO2 rCBF was similar in all four groups. There was a significant effect of PaCO2 on rCBF in regions supplied by the basilar artery (Figure 1) and the carotid arteries. In all groups post hoc comparisons indicated that rCBF at the two highest levels of PaCO2 was significantly different from that at baseline PaCO2 in the brain stem, cerebellum, posterior cerebral artery cortex, and carotid artery territory. At the three highest levels of PaCO2, rCBF was different from that at baseline PaCO2 in the white matter and caudate nucleus (data not shown). There was no difference among groups for the change in cerebrovascular resistance over the range of PaCO2 tested. In all groups, PaCO2 had a significant effect on cerebrovascular resistance in the brain stem and carotid territory (Figure 2), with cerebrovascular...
Discussion

We demonstrate that baseline rCBF and cerebrovascular CO₂ reactivity in brain regions supplied by the basilar artery were unchanged in a canine model of delayed cerebral vasospasm following experimental SAH. Inclusion of the saline group demonstrates that prior anesthesia, cisterna magna injection, and angiography did not alter CO₂ reactivity. Two SAH groups were included to investigate CO₂ reactivity in mild and severe delayed vasospasm. These data indicate that in this model of severe delayed vasospasm of the basilar artery the cerebrovascular response to changes in Paco₂ is not altered.

The canine model of SAH has been used extensively to produce delayed angiographic vasospasm. The degree of basilar artery narrowing we achieved is similar to that reported by others using two15 and three16 subarachnoid injections of arterial blood. However, it should be noted that the selective angiographic technique we used enhances opacification of the basilar artery, especially when there is increased resistance, and therefore tends to underestimate the degree of vasospasm compared with conventional techniques. Thus, the degree of vasospasm achieved in our study may be greater than that in previous reports. The canine model has been employed to demonstrate ultrastructural changes9,10 and altered mechanical properties8 of the basilar artery and has been used to test the activity of a variety of drugs to overcome vasospasm.5-7 While studies have demonstrated altered in vitro basilar artery reactivity, the responsibility of the intact system in vivo had not been previously studied. Our findings suggest that extrapolation of altered in vitro reactivity of the basilar artery to alterations in CBF and correlation of basilar...
artery diameter with ischemia and therapy for vasospasm must be done with caution. Measurements in patients following SAH have demonstrated a progressive decline in CBF that appears to be inversely proportional to the clinical grade.17-19 Reductions in rCBF have been demonstrated in patients with focal neurologic deficits due to vasospasm.18,20,21 CO2 reactivity is reported to be disturbed in patients following SAH.22-25 However, investigation of CO2 reactivity in patients following SAH requires the study of acutely ill patients, often under varying conditions; therefore, it is not surprising that these studies are frequently limited to measurements of global CBF, performed under poorly controlled conditions and at only two levels of Paco2. Measurement of CBF in primates following SAH suggests impaired CO2 reactivity.26-28 However, these investigations are limited to the study of acute CBF changes after SAH25-27 or to changes over a limited range of Paco2.28

We measured rCBF in different vascular territories and investigated CO2 reactivity over a wide range of Paco2 under strictly controlled conditions. We found preserved CO2 reactivity in all brain regions. There are several possible explanations for these results. First, the degree of vasospasm may not have been severe enough with this model to alter CO2 reactivity. Although mild SAH in humans is associated with preserved CO2 reactivity,18 inadequate vasospasm is unlikely to explain our findings because a 55% reduction in basilar artery diameter was found in the three-hemorrhage group. Although the change in vessel size is usually not calculated in humans, the changes seen in the three-hemorrhage group would be considered moderate to severe vasospasm in humans. In addition, although no dog in the three-hemorrhage group developed a focal deficit, global neurologic dysfunction was evident by their being much less alert, ataxic, and uninterested in their environment. Second, the vascular anatomy of dogs differs from that of humans in that the circle of Willis is complete,12 with large posterior communicating arteries. It may be that in this study the luxuriant collateral flow provided via the anterior circulation overcame any restriction in blood flow due to basilar artery vasospasm. Finally, it is possible that vasospasm of large pial vessels, those demonstrated angiographically, does not alter CO2 reactivity because the ability of smaller vessels to dilate remains intact. This possibility is suggested by the finding of increased cerebral blood volume in humans with severe vasospasm,18 and it has been shown that small pial vessels (<100 µm diameter) have a much greater response to hypercarbia than do large pial vessels.29

In summary, we were able to achieve a dose-dependent delayed vasospasm of the basilar artery by injecting blood into the cisternal magna of dogs. In this model baseline rCBF is unchanged and cerebrovascular CO2 reactivity is preserved. We speculate that the mechanism for preserved reactivity involves preservation of blood flow via collaterals and/or vasodilation of small vessels distal to the narrowed segments.

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References


**KEY WORDS** • angiography • cerebral blood flow • hypercapnia • dogs
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