A Model for Experimental Cerebral Arterial Spasm

BY AKIO KUWAYAMA, M.D.,* NICHOLAS T. ZERVAS, M.D.,† ROGER BELSON,‡ AKIRA SHINTANI, M.D.,§ AND KENNETH PICKREN†

Abstract:
Vasospasm of the basilar artery of the dog was induced by subarachnoid injection of arterial blood through the cisterna magna. Cerebral angiography was employed to evaluate quantitative assessment of the spasm. Chronic vasospasm was successfully induced in 100% of surviving dogs.

Biphasic vasoconstriction was observed. The acute phase occurred within 30 minutes after the blood injection and tended to abate. Chronic spasm was demonstrated on the second day's angiograms and persisted to the seventh day in some cases. Etiology of the chronic spasm using this model is now under investigation.

Vasospasm was not due to alterations in blood gas concentration or blood pressure, to increased CSF pressure or to injury from contrast medium or direct trauma. The primacy of blood as the offending agent is strongly suggested.

ADDITIONAL KEY WORDS aneurysm subarachnoid hemorrhage

Despite the intensive work of many investigators the pathogenesis of cerebral arterial spasm following ruptured aneurysm has not been elucidated and satisfactory management of spasm has not been established. In 1965, Echlin† suggested that spasm was due primarily to some irritant property of blood in the subarachnoid space. He based this theory on his observation that the basilar artery of the dog would constrict following the application of blood to the arterial wall. Others‡–12 have confirmed this in dog, cat and monkey, and a number of experimental models have been developed to permit the study of arterial spasm. This report describes a reproducible method for inducing both acute and chronic vasospasm in the basilar artery of dogs that can be quantitated by vertebral angiography.

Methods
A total of 48 beagle mongrels were studied. Dogs of either sex weighing between 7 and 15 kg were premedicated with Ketamine* and atropine sulfate IM and were anesthetized with sodium pentobarbital given intravenously. Ketamine (15 mg/kg) failed to cause any side effects except for slight increase in secretion of saliva, which was well controlled by atropine. The dogs were intubated when needed, but were permitted to breathe spontaneously. Intra-arterial blood pressure was monitored with a strain gauge transducer (Statham P-23-Ac) via a femoral catheter. Rectal temperature was monitored and an electric blanket was used to maintain the body temperature.

The right vertebral artery was cannulated approximately 3 cm proximal to its entrance into the foramen transversarium for subsequent angiographical procedures. The dog was then placed in a stereotaxic frame in the prone position. At least 30 minutes were allowed to elapse prior to control angiography to avoid the influence of vertebral cannulation on intracranial vessel dynamics. All angiograms were obtained with 2 ml of Renografin-76.† After a control angiogram the dura

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Ketamine Hydrochloride, Bristol Laboratories, Syracuse, New York.

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at the cisterna magna was exposed, and a 25-gauge spinal needle held by the stereotaxic holder was advanced into the cisterna; 2.0 ml of autogenous fresh arterial blood was injected slowly at a rate of 0.4 ml/min with an exchange of the same amount of cerebrospinal fluid. In order to facilitate the contact of blood with the basilar artery the dog was tilted tail-up at approximately 30° for ten minutes following injection. Follow-up angiograms were obtained.

The cannula was then removed and the site of cannulation was sutured under the operating microscope. The vertebral artery was recannulated for repeat angiograms on the second, fourth, seventh and fourteenth days, and arterial blood gas analysis was obtained before each injection.

Angiograms were taken with fixed magnification, and the same amount of (2.0 ml) contrast material was instilled using an automatic injector. The diameter of the basilar artery was measured under the dissection microscope or by a light microdensitometer (fig. 1). All measurements were performed independently by several observers. The error of measurement was estimated as ±0.1 mm.

Results

Controls

Angiograms were carried out in 11 normal dogs. In two dogs angiograms were carried out at 15-minute intervals, but blood vessel caliber did not change appreciably and blood gases and blood pressure were unaffected. Two normal dogs were hyperventilated for five to ten minutes. $P_{O_2}$ was increased (52% and 69% respectively) and vasoconstriction occurred (4% and 18% of original vessel diameter). $CO_2$ mixed with air inhalation in three dogs caused marked elevation of $P_{CO_2}$ and slight hypotension with minimal dilatation of the vessels (table 1). It was concluded that vessel caliber was not affected by moderate changes in respiration, and only slightly by major changes in respiration.

Several dogs were subjected to acute severe hemorrhage hypotension, but change in vessel caliber was minimal. Both constriction and dilatation occurred but of less than 13% magnitude.

To evaluate the effect of contrast material on vessel size, a second angiogram was performed.

Table 1

<table>
<thead>
<tr>
<th>Response of Normal Basilar Artery to Respiratory Change</th>
<th>% change of vessel</th>
<th>Blood pressure, %</th>
<th>$P_{O_2}, %$</th>
<th>$P_{CO_2}, %$</th>
<th>pH, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperventilation</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Case 1</td>
<td>-4</td>
<td>-15</td>
<td>+53</td>
<td>-38</td>
<td>+2.6</td>
</tr>
<tr>
<td>Case 2</td>
<td>-18</td>
<td>0</td>
<td>+69</td>
<td>-54</td>
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<tr>
<td>$CO_2$ inhalation</td>
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<tr>
<td>Case 1</td>
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<td>-13</td>
<td>+19</td>
<td>+176</td>
<td>-5</td>
</tr>
<tr>
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<td>+4</td>
<td>-4</td>
<td>+200</td>
<td>-6</td>
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<tr>
<td>Case 3</td>
<td>+4</td>
<td>-20</td>
<td>+13</td>
<td>+46</td>
<td>-4</td>
</tr>
</tbody>
</table>
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FIGURE 2

performed within one to three minutes of the first contrast study in four normal dogs. Slight vasodilatation (10%) occurred. This was also carried out in three dogs with chronic hemorrhagic vasospasm, and average vasodilatation was 17%. However, dilatation was not present on angiograms five to ten minutes later. The injection of normal saline also had no effect on angiograms taken at 1 and 15, 30 and 150 minutes after injection.

VASOSPASM FOLLOWING CISTERNAL BLOOD INJECTION

Acute
Nine dogs were studied at intervals of 15, 30, 60 and 120 minutes following the injection of blood into the cisterna magna (fig. 2). Constriction varied from 29% to 59% of the original diameter. The arteries recovered to some extent by 120 minutes, but none reexpanded to control size or greater (fig. 3). Changes in arterial blood pressure, $P_{O_2}$, $P_{CO_2}$ and pH are shown in figure 4, but no clear relationship could be found between the acute vasospasm and these factors. A tenth dog failed to develop vasospasm, but autopsy revealed that the thrombus was present solely in the spinal subarachnoid space.

Chronic
Seven of the above dogs and another six were studied on the second and later days. All showed constriction of from 25% to 59% with an average of 37% of the control (fig. 5). The diameter of the arteries on the second day was

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very stable and independent of changes in blood gases or blood pressure. At this time the standard deviation of arterial blood pressure, \( P_{O_2}, P_{CO_2} \) and \( pH \) from the mean was \( \pm 9.8, \pm 8.7, \pm 5.1 \) and \( \pm 0.22\% \), respectively. On later angiograms spasm was variable but persisted as long as the seventh day in two. Once spasm subsided it did not recur at a later date (table 2).

**Biphasic Phenomenon**

Six of these animals were studied in the acute and chronic stage. Maximal vasoconstriction in these dogs occurred within 30 minutes. Partial recovery then occurred in three within two hours but on the second day all six had severe vasoconstriction (table 2 and figure 3).

**Meningitis**

One dog developed meningeal infection traced to diarrhea and fecal contamination. The angiogram revealed 75% constriction of the basilar artery, which appeared as a fine thread. A brown-stained clot covered the opaque arachnoid membrane at autopsy. The cerebrospinal fluid grew numerous gram-negative rod bacilli. This dog had by far the greatest narrowing of the basilar artery seen in this study (fig. 6).
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Hemorrhagic vasospasm expressed as the average percent change from the control size. The number shows the total cases for each point.

TABLE 2

Vasospasm Following Subarachnoid Blood Transfusion and Necropsy Findings

<table>
<thead>
<tr>
<th>Acute stage</th>
<th>Chronic stage</th>
<th>Necropsy findings</th>
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<tbody>
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<td>Bas. art.</td>
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<tr>
<td>Deg no.</td>
<td>Time (min)</td>
<td>1 day, %</td>
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<tr>
<td>20</td>
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<td>23</td>
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<td>30</td>
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<tr>
<td>66</td>
<td>33</td>
<td>30</td>
</tr>
</tbody>
</table>

*Meningeal infection.
Vasoconstriction is expressed as percent decrease from control size of the basilar artery.

Cerebrospinal Fluid
In five cases cisternal pressure was measured (four on the second day and one on the seventh day). The pressure never exceeded more than 150 mm H2O, and the cerebrospinal fluid in all was not infected. Cerebrospinal fluid was taken within four days following subarachnoid blood injection and was detectably

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xanthochromic. Within two weeks xanthochromia was absent.

**Mortality**

Two dogs died following angiography on the first day. Vasoconstriction was 38% and 58% and both had an extensive clot at the brain base. Necropsy was performed within four days in nine dogs and blood clot was found in each at the basilar artery or the circle of Willis, although the amount of thrombus was not uniform (fig. 7). Two dogs were sacrificed on the seventh day. No thrombus was found in one although severe vasospasm was present throughout the full course, and the CSF was clear and aseptic. In the other dog thrombus was found around the posterior communicating arteries bilaterally. This dog had early but not late spasm; however, the posterior communicating arteries failed to visualize on the seventh-day angiogram. Two dogs were sacrificed on the fourteenth day but thrombus was not found. In summary, clot was always found in animals with spasm when necropsy was performed within the first four days. Thereafter it was not possible to detect thrombus, possibly because of clot lysis (table 2).

**Discussion**

Wilkins and Levitt and others have studied chronic arterial spasm of the vessels of the circle of Willis and the middle cerebral artery in dogs by carotid angiography. Vertebroangiography, in contrast to carotid angiography in the dog, gives a consistent view of the vertebrobasilar system as well as the circle of Willis. A clear, well-defined image of the basilar artery was obtained using axial projection, thus facilitating quantitative assessment of arterial diameter. Careful vertebral cannulation with the aid of the microscope and repair of the cut-down site permits cannulation days later and minimizes blood flow alterations that might obscure the results of chronic study. Vertebral angiography by this method failed to influence vital signs or size of the basilar artery.

The effects of contrast media on vessel tone are not well understood. Huber and Handa observed vasodilatation of small arteries of humans. This persisted less than one minute, but was not seen with larger vessels. This was also seen in normal dogs in our experiments but subsided within ten minutes. Transient vasodilatation was also observed when the arteries were in spasm, and this was slightly more pronounced than in the normal vessels. The prior injection of normal saline did not cause this phenomenon. This suggests that...
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FIGURE 7

Base of the brain of a dog following injection of 2 ml of fresh autogenous arterial whole blood into the cisterna magna two days before. Note clot in entire basal subarachnoid cistern.

Dilatation was not due to transient ischemia caused by the passage of unoxygenated fluid but rather by the direct action of the contrast material on the smooth muscle of the arteries as Huber mentioned. Because of this, a 15-minute interval was employed between angiographies in this project.

Tearing or direct puncture of the artery around the circle of Willis was employed to induce vasospasm by many authors and was reported to be very reliable. Echlin experienced high morbidity and mortality rate (uncontrollable bleeding, seizure, respiratory arrest, death) with this method. He also pointed out that intracranial hypertension and brain swelling, followed by uncontrollable hemorrhage with puncture method, exerted influence to the spasm other than blood itself. Additionally, release of some vasoactive substances from the damaged brain was suggested by Wilkins and Odom, and Osterholm and Meyer. In our studies blood was injected into the cisterna magna by exchange with an equal volume of cerebrospinal fluid, and the cisterna was punctured under direct microscopic examination so as to avoid a traumatic puncture. In this way increase in CSF pressure was minimized as a variable; and in fact on subsequent measurement was found to be normal despite the presence of spasm. Thus increased intracranial pressure and mechanical destruction of the vessels do not appear to be major factors in the production of spasm in our animals.

Kapp reported that extreme changes in $P_{O_2}$ or $P_{O_2}$ did influence the size of vertebral vessels but to a small degree. This was noted in several of the stressed controls mentioned above. However, changes in blood gas concentration at the time of angiography were minimal and can be dismissed as a significant factor in the production of spasm in our animals. Systemic arterial pressure is another variable. Kapp noted that the basilar artery of a cat constricted 13% when the mean arterial pressure was reduced by 50%. We also noted only minimal dilatation with hemorrhagic or drug-induced hypotension.

In examining the collated data, average arterial constriction appeared to be maximal within 30 minutes of cisternal blood injection and recovered slowly (fig. 3). On the second day vasospasm was again present. Since studies were not performed between 120 minutes and the second day, it is not known whether further fluctuations occurred in that interval.

This "biphasic" phenomenon was demonstrated by Brawley et al. in 1968, applying a strain-gauge device to the internal carotid artery to detect that constriction was greatest at five minutes. This subsided in one hour but recurred on the third day. This was termed "biphasic." A similar observation was reported by Kågström et al. and Echlin in monkeys by angiogram, who named this "recurrent" and "delayed" spasm, respectively.

The persistence of spasm into the first or second week in our dogs is in agreement with observations in monkeys that vasoconstriction induced by blood injection into the subarachnoid space recurred in most animals within 24 hours, was greater in two to four days and frequently persisted for at least seven days. 

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The substance in blood inducing spasm in the acute stage is thought by many authors to be serotonin, but others are convinced of the presence of other vasoactive compounds in whole blood.

The etiological factor in the chronic stage is also controversial. Wilkins and Levitt incubated purple-brown serum taken from blood at 37°C for two to eight days. This produced spasm, whereas fresh serum had no such effect. Buckell demonstrated unknown substances derived from the hematoma fluid of ruptured cerebral aneurysm capable of contracting smooth muscle. FDP (fibrinogen degradation products) was suggested by Kowalski and Osbahr et al.

References
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