Experimental Model of Symptomatic Vasospasm in Rabbits

Shunro Endo, MD, Patricia J. Branson, MA, and John F. Alksne, MD

The common carotid arteries were ligated bilaterally 2 weeks before induction of subarachnoid hemorrhage in rabbits. The rabbits were observed closely for clinical symptoms, and angiographic and pathologic investigations were performed. Thirteen experimental rabbits showed a progressing neurologic deficit that was worst on the fourth or fifth day after the subarachnoid hemorrhage. This symptomatic change did not occur in five rabbits without previous carotid ligation. Presumably, the rabbits with carotid ligation became symptomatic because they no longer had a collateral blood flow to compensate for the reduced blood flow in the basilar artery after subarachnoid hemorrhage. Our model of symptomatic vasospasm after subarachnoid hemorrhage may be beneficial for future studies. (Stroke 1988;19:1420-1425)

The pathophysiology of vasospasm after subarachnoid hemorrhage (SAH) and its influence on cerebral perfusion and function remain poorly understood. One obstacle to the laboratory study of this important problem is that the common animal models of vasospasm produce arterial narrowing but not the development of a neurologic deficit. A recently reported primate model produces neurologic deficit, but primates are expensive and cumbersome for studies requiring many animals.

We report our attempt to make a rabbit model of symptomatic vasospasm. SAH was induced in rabbits previously subjected to bilateral carotid artery ligation. Based on pilot studies that showed that the basilar artery dilates during the first 2 weeks after carotid ligation, SAH was delayed until at least 2 weeks after ligation.

Materials and Methods

We entered 27 female rabbits weighing 3.0–3.5 kg into this study. In 21 rabbits, both common carotid arteries were ligated 2 weeks before the planned SAH. All surgical and angiographic procedures were performed under anesthesia with 50 mg/kg i.m. ketamine hydrochloride and 10 mg/kg i.m. xylazine. Each rabbit was placed in the supine position with its head fixed such that the orbitomeatal line was horizontal. Using sterile technique a midline cervical incision was made, the carotid arteries were isolated bilaterally, and the common carotid arteries were ligated at the carotid bifurcation. An 18-gauge polyethylene catheter was inserted retrograde into one common carotid artery just below the bifurcation and advanced until the tip of the catheter was positioned at the ascending aorta. The catheter was connected to a three-way stopcock and used for angiography, arterial blood pressure measurement, and blood gas analysis. Subsequent angiograms were performed in a similar manner by reopening the neck and using the other carotid artery.

We discarded six of these 21 rabbits from the study; two died shortly after carotid ligation and four had severe neurologic symptoms, presumably due to lack of collateral blood flow. The remaining 15 asymptomatic or minimally symptomatic rabbits with carotid ligation and six rabbits without carotid ligation were subjected to experimental SAH. Each rabbit received two subarachnoid blood injections 48 hours apart, the first injection on Day 0. Each rabbit was placed in the prone position with its head down. Using sterile technique, a 2-cm vertical suboccipital incision was made to expose the craniocaudal junction. A 23-gauge needle inserted into the cisterna magna (as evidenced by obtaining a free flow of spinal fluid) was used for manual injection over 2 minutes of 2.5 ml autologous arterial blood for the first SAH and 1.5 ml for the second. The needle was then removed, and the point of insertion was covered by a small piece of muscle and sealed with adhesive. During this procedure, respiration was maintained spontaneously without intubation. $P_{aco_2}$ was monitored and remained at 35–45 mm Hg. Eighteen rabbits, 13 with previous carotid ligation (Group 1) and five without (Group 2), recovered from anesthesia within 3 hours.
All rabbits were maintained on standard pellet feed and tap water in day/night regulated quarters at 23° C. Each rabbit underwent neurologic examination and assessment of food intake before SAH and daily thereafter until sacrifice. Neurologic deficits were graded using a four-point system (Table 1) by observing the rabbit on a flat surface. Paresis of the legs or abnormal gait (circling movement or difficulty walking) were noted. The rabbit's food intake was also classified using a four-point system: full (100%), >50% but <100%, <50%, and 0%.

Aortovenous angiography was carried out in 11 of the 13 Group 1 rabbits using 3.0 ml Renografin 60 hand-injected into an indwelling catheter inserted retrograde through the carotid stump into the aortic arch. One anteroposterior film obtained during the arterial phase performed 2 weeks after carotid ligation and before the first SAH was used as a baseline. The diameter of the basilar artery was measured on each film (magnification 1:1) at three points using a magnifier; the average of the three measurements was expressed as percentage reduction from baseline and defined as <9%, no spasm; 10–29%, mild spasm; and >30%, severe spasm. In six rabbits, sequential angiograms were obtained on Days 0, 3, 4, 7, and 10. In five rabbits, angiography was performed on Day 0 and at the time of sacrifice.

Rabbits were killed between Days 5 and 12 for pathologic examination of the cerebral arteries. The brain with cerebral vessels was removed immediately after death and examined grossly. The brain was then fixed in formalin for light microscopy.

### Results

The neurologic and angiographic findings in the 13 Group 1 rabbits and the five Group 2 rabbits are summarized in Table 2. The angiographic and symptomatic time course of one Group 1 rabbit (Rabbit 4) is shown in Figure 1.

Before induction of SAH on Day 0 five Group 1 rabbits showed minimum neurologic deficit with no laterality (Grade 2), which was considered to be the result of the bilateral carotid ligation; the remaining eight Group 1 rabbits and the five Group 2 rabbits showed no neurologic deficit (Grade 1). In all 18 rabbits, full food intake was observed on Day 0. All 13 Group 1 rabbits showed a progressive aggravation of neurologic deficits after SAH. These changes were initially observed on Days 1–4 and became most severe on Day 4 or 5. On their worst day, 12 Group 1 rabbits showed definite neurologic deficits of Grades 3 or 4, and one rabbit showed a minimum neurologic deficit of Grade 2. After Day 5, the neurologic deficits gradually improved, and complete recovery was observed in four of eight Group

### Table 1. Grading of Neurologic Deficit in 18 Rabbits With Experimental Subarachnoid Hemorrhage

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No neurologic deficit (normal)</td>
</tr>
<tr>
<td>2</td>
<td>Minimum or suspicious neurologic deficit</td>
</tr>
<tr>
<td>3</td>
<td>Mild neurologic deficit without abnormal movement</td>
</tr>
<tr>
<td>4</td>
<td>Severe neurologic deficit with abnormal movement</td>
</tr>
</tbody>
</table>

### Table 2. Summary of Results in 18 Rabbits Before and After Experimental Subarachnoid Hemorrhage

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Group 1 (carotid ligation)</th>
<th>Group 2 (control)</th>
<th>Neurologic deficit grade</th>
<th>Worst day</th>
<th>Neurologic deficit grade</th>
<th>Food intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>&lt;50%</td>
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<td>1</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>&lt;50%</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>2</td>
<td>Full</td>
</tr>
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<td>1</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>Full</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>Full</td>
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<td>6</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>2</td>
<td>5</td>
<td>Full</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>4 &lt;100%, 50%</td>
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<td>Full</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>4 &lt;100%, 50%</td>
<td>3</td>
<td>Full</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>1</td>
<td>5</td>
<td>4 &lt;100%, 50%</td>
<td>3</td>
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</tr>
<tr>
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<td>2</td>
<td>1</td>
<td>5</td>
<td>3 &lt;100%, 50%</td>
<td>2</td>
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<td>3 &lt;100%, 50%</td>
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<tr>
<td>13</td>
<td>2</td>
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<td>4</td>
<td>4 &lt;100%, 50%</td>
<td>4</td>
<td>Full</td>
</tr>
</tbody>
</table>

Food intake 100% (full) before subarachnoid hemorrhage.
1 rabbits followed for >10 days. In contrast, of the five Group 2 rabbits, two showed no neurologic deficits and remained at Grade 1 and three showed minimum or mild deficits of Grades 2 or 3; no Group 2 rabbit became Grade 4.

A significant decrease of food intake was seen in nine of the 13 Group 1 rabbits. The most striking decrease was observed during Day 1 (Figure 2). After initial recovery, eight of the nine rabbits showed a second decrease of food intake between Days 3 and 7. A good correlation was found between the neurologic deficit grade and food intake class on Days 4 and 5. All surviving Group 1 rabbits returned to normal eating after Day 8. Three of the five Group 2 rabbits showed a decrease of food intake on Days 1 and 3. No decrease of food intake, however, was observed after Day 4.

The mean ± SEM diameter of the basilar artery on the baseline angiograms for the 11 Group 1 rabbits studied was 1.34 ± 0.08 mm. In 10 of the 11 rabbits, mild or severe spasm of the cerebral vessels was observed on Days 3–6. The change of the basilar artery was most remarkable, and the average constriction of its diameter was 23%. Visualization of the anterior part of the circle of Willis was poor on one angiogram showing severe spasm of the basilar artery. In six rabbits angiographed sequentially, the maximum change (mean reduction from baseline 21%) was observed on Days 3 or 4 and gradually decreased after that (Figure 3). Angiographic changes correlated well with the neurologic deficit grade time course but not severity.

Gross pathologic examination revealed accumulated blood in the prepontine and chiasmal cisterns, the basal surface of the brain, and in the lateral ventricles of all rabbits killed within 8 days after SAH. The subarachnoid blood was absent in rabbits killed on Days 10 or 12. The ventricles were mildly dilated in most rabbits in both Groups 1 and 2. Cerebral infarction was seen in two Group 1 rabbits, but these lesions did not correlate with severity or laterality of neurologic deficits.
In an attempt to understand the pathophysiology of the delayed ischemia that occurs after SAH, a wide variety of laboratory experiments have been performed. These include the topical application of a vasoconstrictor agent, \(^1^2\) injection of autologous blood into the subarachnoid space, \(^3^2^1\) cutting arteries, \(^2^2^3\) puncturing vessels with a needle, \(^2^4^2^9\) or a combination of these methods in dogs, \(^4^6^9^1^4^1^7^1^9\) cats, \(^2^1^6^1^8^2^5^2^6\) rabbits, \(^2^8\) pigs, \(^2^0\) rats, \(^2^1^2^7\) monkeys, \(^1^3^7^8^1^0^1^3^1^5^2^3^2^4^3^0^3^1\) or baboons, \(^2^2^2^9\) The resultant vasospasm has been evaluated using a wide variety of techniques, including direct observation of vessels, \(^1^2^2^8\) angiography, \(^6^7^9^1^4^1^5^1^7^1^9^3^1\) cerebral blood flow, \(^3^8^1^0^1^2^1^3^1^8^2^1^2^2^5^2^5^2^8^2^9\) neurologic deficits, \(^3^7^1^0^1^2^1^3^2^2^2^6^2^7\) and pathologic change in the vessel wall, \(^1^2^4^1^7^1^9^2^0^2^3\). Unfortunately, no model truly duplicates the human situation because the animals do not demonstrate clinical signs of delayed ischemia. A recently reported model using primates \(^3^2^2^2^4\) apparently produces delayed neurologic deficit in some animals. Many animals are needed, however, to draw any conclusion from the experimental investigation of methods to prevent vasospasm. The primate model is too cumbersome and expensive for this purpose.

**Discussion**

**FIGURE 2.** Number of rabbits in each neurologic deficit grade and food intake class before (Day 0) and during 7 days after subarachnoid hemorrhage (SAH). Group 1, 13 rabbits with bilateral carotid ligation 2 weeks before SAH; Group 2, five rabbits without carotid ligation.

**FIGURE 3.** Changes in basilar artery diameter following subarachnoid hemorrhage (SAH) 2 weeks after bilateral carotid ligation in 11 rabbits. Arrows, time of SAH; •, sequential change in six rabbits; O, single investigation on Day 5 or 6 in five rabbits.
Therefore, we have tried to make a new laboratory model using rabbits with bilateral carotid ligation performed before the induction of SAH. Bilateral carotid ligation has been used in the past as a method of producing experimental cerebral ischemia. In most rabbits, however, the deficiency of cerebral blood flow that results from carotid ligation is compensated for by a well-developed collateral circulation through the circle of Willis. In our study, six of 21 rabbits that underwent bilateral common carotid artery ligation showed definite symptoms or died acutely. The remaining 15 rabbits (13 of which comprise Group 1) showed no or only minimum neurologic deficits and had a well-developed collateral circulation through the vertebrobasilar system. Two injections of autologous blood 48 hours apart into the cisterna magna were performed in an attempt to produce arterial narrowing of the vessels of the vertebobasilar system and secondary cerebral ischemia due to the already-compromised collateral blood flow. The experimental SAH was induced 2 weeks after carotid ligation to allow time for development of the basilar artery dilation that occurs in response to occlusion of the anterior circulation.

Our results showed progressive neurologic deficit, most severe on Days 4 and 5, in Group 1 rabbits with previous carotid ligation. These neurologic deficits were definitely severe and prolonged compared with the minimal deficits observed in Group 2 rabbits without carotid ligation. Narrowing of the basilar artery and other vessels was observed on angiograms performed between Days 3 and 6 in 10 of the 11 Group 1 rabbits studied. A decrease in food intake on Days 1 and 3 (just after the induction of SAH) was observed in both Groups 1 and 2, but the delayed decrease in food intake on Days 4–6 was restricted to Group 1 rabbits with previous carotid ligation. Pathologic investigations of the arterial wall revealed the morphologic changes of vasospasm, which are well known from many previous reports. Mild hydrocephalus was observed in most rabbits; however, no relation between the neurologic deficit grade and hydrocephalus was seen.

Our findings suggest that the changes in neurologic deficit grade and food intake class seen may be biphasic. The first phase is secondary to the acute effect of SAH and the delayed, more severe, phase is secondary to vasospasm and cerebral ischemia. Only the Group 1 rabbits demonstrated severe neurologic deficits, which were worst on Days 3 and 4. We conclude, therefore, that the changes are definitely a result of vasospasm in animals with compromised collateral blood flow.

There was a good correlation between neurologic deficit grade and angiographic findings. We did not measure cerebral blood flow. Our model, using rabbits with previous carotid ligation, offers a new animal model of symptomatic vasospasm that can be created easily, consistently, and inexpensively.

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