Subarachnoid Hemorrhage Fails to Produce Vasculopathy or Chronic Blood Flow Changes in Rats

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Cerebral blood flow was measured by a $[^{14}C]$butanol indicator fractionation technique in rats subjected to subarachnoid hemorrhage, in control rats, and in rats given injections of buffered saline into the subarachnoid space (sham hemorrhage). Cerebral blood flow was significantly decreased in both the subarachnoid hemorrhage and sham hemorrhage rats 3 hours after injection. However, blood flow returned to control levels by 24 hours, and measurement for 14 days after subarachnoid hemorrhage failed to show any delayed decrease in cerebral blood flow. Electron microscopic studies of basilar arteries from rats subjected to subarachnoid hemorrhage 72 hours before killing failed to show any of the morphologic changes that have been associated with vasospasm in humans or in higher animal models. Our studies indicate that the rat model of subarachnoid hemorrhage has limited applicability to the study of subarachnoid hemorrhage following ruptured cerebral aneurysms in humans. However, although rats are not a perfect model of this clinical condition, some pathophysiologic changes similar to those observed in human subarachnoid hemorrhage have been demonstrated in this model and deserve further investigation. (Stroke 1988;19:878–882)

Animal models of aneurysmal subarachnoid hemorrhage (SAH) have been developed in numerous species. The most successful models have been in primates,1–3 dogs,4 and cats.5,6 However, recent fiscal considerations and social concerns voiced by animal rights groups have limited the availability of these models, and these factors will only increase in the future. To counteract this trend and to create a more efficient animal model of aneurysmal SAH, rats have recently gained popularity.7–10 Several recent investigations have used rats for experimental SAH, with results indicating that rats have many features found in the higher animal models.7,9,11,12 Nonetheless, to be an exact model of a clinical condition, animals must reproduce the pathophysiologic defects found in patients.

Following aneurysmal SAH, the most significant cause of morbidity in survivors is the delayed onset of cerebral ischemia.13,14 This problem has been related to vasospasm of the cerebral arteries as well as to diffuse disturbances of cerebral blood flow (CBF).15 In an attempt to reinforce the validity of the rat model of aneurysmal SAH we studied the time course of CBF following acute experimental SAH. Electron microscopic studies of the basilar arteries of rats subjected to SAH were also performed to determine if there were chronic changes in the blood vessel morphology similar to those seen in other models of aneurysmal SAH or in the clinical condition of cerebral vasospasm.

Materials and Methods

Experimental Subarachnoid and Sham Hemorrhages

Male Sprague-Dawley rats weighing approximately 400 g each were used. SAH was produced using our previously described techniques.7 After induction of general anesthesia, each rat was immobilized in a stereotactically headholder (David Kopf Instruments, Tujunga, California). A 2-mm burr hole was then drilled in the skull in the midline of the interparietal-occipital suture just rostral to the occipital bone. Fresh arterial blood (0.6 ml) was then withdrawn from a femoral artery catheter into a syringe fitted with a 30-gauge needle. The needle was passed through the burr hole and advanced along the inner table of the occipital bone toward the cisterna magna. Using an operating microscope,
the needle tip was seen to enter the cisterna magna. The autologous blood was then slowly injected into the subarachnoid space over approximately 60 seconds. Sham hemorrhages were created by injection of 0.6 ml mock cerebrospinal fluid (CSF) (buffered saline, pH 7.30) into the subarachnoid space. Following SAH or sham hemorrhage, the rats were allowed to fully recover in individual cages.

Determinations of Cerebral Blood Flow

CBF was determined 3, 24, 48, and 72 hours, 7 and 14 days after SAH in 40 rats. Seventeen rats with sham hemorrhage had CBF determined 3, 24, and 48 hours after mock CSF injection. CBF was determined in these two groups and in 14 control rats using a modified indicator fractionation technique with [14C]butanol as the tracer.16,17 Each rat was anesthetized with 0.5% halothane in 70%-30% N2O-O2, to allow placement of femoral artery and femoral vein catheters. The rat was immobilized on a lead brick warmed to 37° C and allowed 2 hours to fully recover from anesthesia. Temperature was monitored by a rectal probe and maintained at 37° C by means of a heating lamp. Arterial blood pressure was constantly recorded using a Grass multichannel polygraph recorder (Quincy, Massachusetts), and arterial blood gases were monitored with a blood gas analyzer (model 213, Instrumentation Laboratories, Dayton, Ohio). Five microcuries [14C]butanol dissolved in 0.2 ml 0.9% NaCl was then injected into the femoral vein in <1 second. A reference arterial sample was withdrawn from the femoral artery catheter at a constant rate using a Harvard infusion-withdrawal pump (South Natick, Massachusetts) so that 135 µl blood was removed during the 10 seconds after the intravenous injection of [14C]butanol. At the end of 10 seconds, the pump was disconnected and the rat was simultaneously decapitated. The brain was quickly removed and dissected on ice bilaterally into the frontal cortex, parieto-occipital cortex, diencephalon, brainstem, and cerebellum.

The brain sections were partially homogenized by forcing the tissue through a 26-gauge needle. The homogenates were then injected into preweighed counting vials containing 1 ml of a 1:2 vol:vol mixture of Protosol (New England Nuclear, Boston, Massachusetts) and ethanol. After the vials were reweighed they were capped and heated to 50° C for at least 12 hours to solubilize the tissue. The arterial blood was expelled into a scintillation vial containing 0.5 ml Protosol-ethanol and heated for 15 minutes; 0.25 ml H2O2 was then added to the blood samples, and they were reheated for 15 minutes. All samples were then cooled, and 10 ml Omnifluor scintillation fluid (New England Nuclear) was added. Radioactivity of all samples was assayed in a Packard liquid scintillation counter (Downer’s Grove, Illinois), and corrections were made for quenching and background contamination using internal standards. CBF was calculated as

\[
\text{CBF} = \frac{(Q_r \times F_r \times 100)}{(Q_r \times M_r)}
\]

where \(Q_r\) = radioactivity in the tissue sample in counts per minute, \(F_r\) = rate of withdrawal of the reference sample in milliliters per minute, \(Q_r\) = radioactivity in the reference sample in counts per minute, and \(M_r\) = mass of the tissue sample in grams.

Mean regional CBF at each time after SAH or sham hemorrhage was determined, and the significance of the difference between mean regional CBF and the control regional value was tested using Student’s t test for independent samples. Whole-brain blood flow for each rat was calculated as the sum of the regional CBFs divided by the sum of the weights of all brain regions.

Electron Microscopic Studies

Ten rats underwent experimental SAH as described. Seventy-two hours later they were anesthetized and then perfusion-fixed with intracardiac glutaraldehyde at a pressure of 100 mm Hg for 10 minutes. The basilar artery of each brain was removed using an operating microscope, and several ring segments of the arteries were then embedded in Epon and subjected to transmission electron microscopy. These experimental arterial segments were compared with similarly prepared basilar artery ring segments from five control rats.

Results

Cerebral Blood Flow

Regional CBF in the SAH, the sham hemorrhage, and the control groups are presented in Table 1. There was a significant decrease in CBF 3 hours after SAH in all brain regions compared with controls. Similarly, there was a significant decrease in CBF 3 hours after sham hemorrhage. By 24 hours CBF began to recover and approached control levels. There was no significant difference in CBF 24 hours after SAH or sham hemorrhage compared with controls. CBF in the sham hemorrhage group appeared to recover more rapidly than in the SAH group, but the difference was not significant. All subsequent measurements of CBF for up to 14 days after SAH or sham hemorrhage failed to show any significant difference from controls.

Three hours after SAH, whole-brain blood flow was 82 ± 17 compared with 100 ± 11 ml/100 g/min in the control rats (p<0.02). Sham hemorrhage rats similarly displayed a significant decrease in whole-brain blood flow compared with controls, 78 ± 5 vs. 100 ± 11 ml/100 g/min (p<0.02). Whole-brain blood flow for the SAH and sham hemorrhage groups are graphically portrayed in Figure 1, showing the time course of changes following SAH or sham hemorrhage compared with control.

Electron Microscopy

Electron micrographs of the basilar artery ring segments subjected to SAH failed to show any morphologic difference from controls. No intimal
proliferation was identified, the smooth muscle cells appeared normal, and the adventitia was not disrupted.

### Discussion

Patients that survive an acute aneurysmal SAH often have delayed clinical deterioration due to cerebral ischemia, which develops several days after the initial rupture of the aneurysm.14,18 The exact etiology of this delayed cerebral ischemia is unknown, but certainly the contribution of vasospasm of the large arteries of the cerebral circulation is significant.13 As well, global reductions of CBF have been documented in patients after SAH regardless of their neurologic condition.19-23 The exact pathophysiology of this phenomenon is unknown, but the global changes in CBF correlate poorly with angiographic vasospasm.21 In addition, there is evidence that global decreases in CBF after SAH are predictive of delayed ischemia and poor neurologic outcome.22 It is, therefore, reasonable to theorize that global reduction of CBF in conjunction with angiographic vasospasm are both related to delayed focal ischemia. Laboratory support for this theory is lacking, in part because of the inadequacy of existing animal models for aneurysmal SAH. An ideal laboratory model of this clinical condition would induce both of these changes in experimental animals.

Our studies demonstrate that subarachnoid injections of arterial blood into the basal cisterns of rats do not produce chronic, diffuse changes in CBF. This finding is somewhat surprising, especially in light of our earlier work that documented an acute decrease in CBF in the first hour after SAH and a global depression of cerebral metabolic activity 48 hours after SAH. It should be noted that no rat undergoing SAH suffered any noticeable neurologic deficit.

Following SAH there is usually a biphasic change in the caliber of the cerebral blood vessels.24 In the

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**TABLE 1. Cerebral Blood Flow in Control Rats and After Experimental Subarachnoid or Sham Hemorrhage**

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Control (n = 14)</th>
<th>3 hours (n = 5)</th>
<th>24 hours (n = 8)</th>
<th>48 hours (n = 8)</th>
<th>72 hours (n = 14)</th>
<th>7 days (n = 3)</th>
<th>14 days (n = 4)</th>
<th>3 hours (n = 5)</th>
<th>24 hours (n = 8)</th>
<th>48 hours (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole brain</td>
<td>100 ±11</td>
<td>82 ±17</td>
<td>96 ±24</td>
<td>101 ±17</td>
<td>108 ±17</td>
<td>91.7 ±7</td>
<td>114 ±18</td>
<td>78 ±5</td>
<td>106 ±18</td>
<td>100 ±14</td>
</tr>
<tr>
<td>Right frontal cortex</td>
<td>114 ±17</td>
<td>89 ±16</td>
<td>110 ±37</td>
<td>113 ±23</td>
<td>125 ±23</td>
<td>104 ±9</td>
<td>129 ±32</td>
<td>90 ±8</td>
<td>118 ±22</td>
<td>112 ±15</td>
</tr>
<tr>
<td>Left frontal cortex</td>
<td>118 ±26</td>
<td>93 ±20</td>
<td>110 ±27</td>
<td>120 ±32</td>
<td>130 ±23</td>
<td>101 ±4</td>
<td>127 ±18</td>
<td>87 ±9</td>
<td>121 ±24</td>
<td>119 ±9</td>
</tr>
<tr>
<td>Right posterior cortex</td>
<td>105 ±15</td>
<td>87 ±17</td>
<td>92 ±28</td>
<td>112 ±23</td>
<td>118 ±19</td>
<td>101 ±10</td>
<td>129 ±20</td>
<td>82 ±9</td>
<td>117 ±23</td>
<td>111 ±25</td>
</tr>
<tr>
<td>Left posterior cortex</td>
<td>103 ±15</td>
<td>85 ±21</td>
<td>95 ±27</td>
<td>115 ±31</td>
<td>115 ±29</td>
<td>102 ±13</td>
<td>135 ±42</td>
<td>82 ±7</td>
<td>113 ±24</td>
<td>117 ±25</td>
</tr>
<tr>
<td>Diencephalon</td>
<td>95 ±23</td>
<td>81 ±17</td>
<td>103 ±14</td>
<td>95 ±14</td>
<td>102 ±18</td>
<td>89 ±13</td>
<td>104 ±10</td>
<td>77 ±8</td>
<td>111 ±15</td>
<td>103 ±24</td>
</tr>
<tr>
<td>Brainstem</td>
<td>95 ±13</td>
<td>71 ±17</td>
<td>87 ±18</td>
<td>86 ±9</td>
<td>93 ±15</td>
<td>87 ±17</td>
<td>96 ±8</td>
<td>67 ±4</td>
<td>95 ±18</td>
<td>74 ±16</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>81 ±15</td>
<td>64 ±11</td>
<td>80 ±21</td>
<td>74 ±6</td>
<td>76 ±12</td>
<td>69 ±4</td>
<td>89 ±7</td>
<td>60 ±4</td>
<td>74 ±12</td>
<td>71 ±8</td>
</tr>
</tbody>
</table>

Values are mean ± SD, ml/100 g/min.

*Significantly different from control (p<0.05).

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**FIGURE 1. Time course of whole-brain cerebral blood flow (ml/100 g/min) changes after experimental subarachnoid or sham hemorrhage (Time p SAH) in rats. •, rats with subarachnoid hemorrhage; ○, rats with sham hemorrhage.**
acute phase of SAH the blood vessels narrow due to constriction of the smooth muscle cells. However, several days after SAH there is a secondary constriction of the blood vessels, and this delayed constriction appears to be an irreversible change in blood vessel wall structure and not merely a change in smooth muscle tone. In fact, the results of several hundred studies aimed at treating cerebral vasospasm have universally demonstrated the failure of vasoactive agents to reverse the angiographic narrowing seen after SAH. Therefore, angiographic studies of animal models of cerebral vasospasm ideally should be correlated with both morphologic studies showing histologic changes in the blood vessel walls and physiologic studies showing pharmacologic irreversibility of experimentally induced vascular narrowing. The exact correlation of histologic changes with angiographic spasm has not yet been proven. However, postmortem specimens from patients dying of vasospasm have demonstrated histologic blood vessel changes, and the animal models that most closely reproduce the clinical entity of delayed cerebral ischemia after SAH also demonstrate blood vessel changes. Therefore, models that do not develop irreversible morphologic blood vessel changes after SAH may have only limited applicability to the clinical syndrome. Experimental designs must take these potential limitations into consideration.

Other investigators have shown some very mild degrees of angiographic vasospasm in rats following SAH. As well, sustained narrowing of the basilar artery following a direct basilar artery puncture has been noted by direct visualization. Our electron microscopic studies failed to show any of the proliferative vasculopathy that has been reported in successful models of vasospasm in other animals or in the clinical setting. It is possible that significant quantities of blood did not remain in contact with the blood vessels long enough to produce cerebral vasospasm. However, similar hemorrhages have been shown to cause diffuse decreases in metabolism and alterations in cerebral catecholamine content and synthesis. As well, all rats in our study had a dramatic Cushing response when the blood was injected into the cisterna magna. Many rats also had transient respiratory arrest. Previous studies in our laboratory have revealed that injection of >0.6 ml blood into the cisterna magna generally causes death (unpublished data). For these reasons we feel that we injected maximum volumes of blood. An alternative explanation for the lack of vascular changes seen in our study is that rat cerebral blood vessels are somehow more resistant to this insult than the vessels of other species. Previous studies have shown that the structure of rat blood vessels is different from that of humans and other species in that the rat basilar artery does not contain interadventitial spaces, which have been implicated in the development of vasospasm.

Our investigations were undertaken in an exhaustive effort to document delayed CBF changes after SAH in rats and to reproduce the morphologic changes of vasospasm that have been observed in the clinical setting. Our studies failed to show these responses in the rat model of SAH. However, our studies do not completely invalidate rats as a model for some aspects of the clinical condition of aneurysmal SAH. It is still probable that there are diffuse autoregulatory changes within the rat brain following SAH. This possibility is supported by our studies showing diffuse metabolic changes in the rat brain following SAH. Uncoupling of metabolism and blood flow is one of the primary physiologic changes observed when there is a defect in cerebrovascular autoregulation. As well, the locus coeruleus, which controls the norepinephrine content of the brain, has been shown to be intimately involved with CBF regulation and control of cerebrovascular autoregulation by other investigators. Previous studies in our laboratory have suggested that, following SAH, the locus coeruleus is activated and may contribute to the susceptibility of the brain to ischemia. Studies in rats should continue in an effort to determine if this model could be a viable experimental device in which to study the brain's capacity to withstand ischemic insults following SAH. Nonetheless, data from the rat model of SAH must always be interpreted with caution since this animal does not respond in a fashion identical to that of humans after SAH.

References


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**Key Words**: cerebral blood flow • cerebral ischemia • subarachnoid hemorrhage • rats
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Stroke. 1988;19:878-882
doi: 10.1161/01.STR.19.7.878

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