Chronic Cerebral Blood Flow Changes Following Experimental Subarachnoid Hemorrhage in Rats

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Experimental subarachnoid hemorrhage was induced in 52 adult male Wistar rats by microsurgical transclival basilar artery puncture. Telencephalic blood flow measured in 24 rats with subarachnoid hemorrhage was compared with that in 23 sham-operated rats and 10 unoperated control rats using the $[^{14}C]$butanol indicator fractionation technique. Telencephalic blood flow was significantly less in the rats with subarachnoid hemorrhage than in the sham-operated rats 3 (78.7±6.9 [n=7] and 112.0±8.5 [n=8] ml/100 g/min, respectively; p<0.01), 7 (74.9±5.1 [n=9] and 112.6±4.6 [n=8] ml/100 g/min, p<0.001), and 14 (81.9±6.0 [n=8] and 104.1±5.4 [n=7] ml/100 g/min, p<0.01) days after surgery. Telencephalic blood flow in unoperated controls (114.7±4.9 ml/100 g/min) did not differ significantly from sham-operated rats. Clinically, the 52 rats with subarachnoid hemorrhage were indistinguishable from 32 sham-operated rats. Postmortem examinations in 10 rats used in a preliminary investigation demonstrated significant blood clot in the basal cisterns 2 hours after basilar artery puncture. Intracranial pressure was slightly elevated (23 mm Hg over baseline) 30 minutes after the hemorrhage (n=7), but when measured 3 (n=3) or 7 (n=3) days after surgery it had returned to baseline. Histologic examination of the brains from 10 rats subjected to subarachnoid hemorrhage 7 (n=5) or 14 (n=5) days before sacrifice revealed no evidence of cerebral ischemia or vasculopathic changes in the cerebral arteries. Basilar artery puncture is a simple method of inducing experimental subarachnoid hemorrhage that has advantages over other rat models; this model causes vessel wall injury, minimal damage to the dura, extensive blood clot formation in the basal cisterns, and documented chronic changes in cerebral blood flow. (Stroke 1990;21:577–581)
calculation of the transcervical basilar artery puncture technique. General anesthesia was induced with 1.0 mg/kg ketamine and 10 mg/ml acepromazine, a 3-cm ventral neck incision was made, the rats were orally intubated, the cervical musculature and carotid arteries were retracted, and the clivus was exposed. A window 2 mm in diameter was drilled in the midline, exposing the basilar artery under the intact dura. Using an operating microscope, 54 rats the basilar artery was then punctured with a 20-μm tungsten wire attached to a micromanipulator. The neck incision was closed with skin staples. Thirty-two sham-operated rats were treated identically, except the basilar artery was gently manipulated with the wire but not punctured. No surgery was performed in 10 control rats. For histology studies 15 rats that underwent basilar artery puncture and nine that underwent sham operations were divided into three groups of five SAH and three sham-operated rats each. The rats in these groups were killed 3, 7, or 14 days after surgery. They were perfused through the aorta with 100 ml of normal saline followed by 200 ml of 10% formalin. The brains were then removed and placed in the same fixative for 2 weeks before sectioning. Segments of the brainstem containing the basilar artery were separated from the forebrain structures, and both tissue blocks from each rat were then embedded in paraffin, cut into 10-μm sections, and stained with hematoxylin and eosin for light microscopy.

Telencephalic blood flow (TBF) was measured in 24 awake SAH rats, 23 awake sham-operated rats, and the 10 control rats using the [14C]butanol indicator fractionation technique as previously described.21-23 Ten seconds after the intravenous injection of 5 μCi of [14C]butanol (New England Nuclear, Boston, Massachusetts), the rat's head was placed in a stereotactic frame, with the nose pointing down. A posterior midline incision was made to expose the atlantooccipital membrane. A 27-gauge needle cut 5 mm from its tip was placed on the stereotactic arm and then slowly lowered until it pierced the membrane. The needle was connected to a Grass polygraph recorder (Quincy, Massachusetts) through a Statham pressure transducer (Gould, Inc., Cleveland, Ohio).

Results are given in the text and figure as mean±SEM. Groups were compared using Student's unpaired t test.

**Results**

The 52 rats subjected to experimental SAH were clinically indistinguishable from the 32 sham-operated rats, displaying normal activity and feeding habits 24 hours after surgery. Mortality rates for the two groups were 16% (eight of 52) and 6% (two of 32), respectively. Most rats that died (89%, eight of nine) did so ≤1 hour after surgery from respiratory complications.

In 10 rats used for preliminary studies, autopsy 2 hours after transcervical basilar artery puncture revealed extensive SAH involving the basal cisterns in 100%. These hemorrhages were consistently larger (judged by visual inspection) than those achieved with injection of blood into the cisterna magna.21 No SAH was seen in rats subjected to sham operations.

Three days after basilar artery puncture, the brains of three rats showed gross evidence of old hemorrhage. This was confirmed by microscopic examination of the leptomeninges. Neither sham-operated nor SAH rats killed 7 or 14 days after surgery showed histologic evidence of hemorrhage.

Microscopic examination of the brains from 15 SAH and nine sham-operated rats revealed no evidence of thrombosis, medial necrosis, or intimal proliferation of the basilar artery or any other major cerebral arteries. In addition, no histologic signs of
ischemia were seen in the hippocampus of any brain examined.

Physiologic parameters for the SAH, sham-operated, and control rats are shown in Table 1. No significant differences existed among the three groups.

TBF was significantly lower in SAH than in sham-operated rats at all times after surgery (Figure 1). At 3 days, TBF in seven SAH rats was 78.7±6.9 ml/100 g/min compared with 112.0±8.5 ml/100 g/min in eight sham-operated rats (p<0.01). At 7 days, TBF was 74.9±5.1 (n=9) and 112.6±4.6 (n=8) ml/100 g/min in SAH and sham-operated rats, respectively (p<0.001). At 14 days, TBF in eight SAH rats was 81.2±6.0 ml/100 g/min compared with 104.1±5.4 ml/100 g/min in seven sham-operated rats (p<0.01). Control TBF was 114.7±4.9 ml/100 g/min.

ICP was 2.3±1.8 mm Hg greater than baseline (3.8±2.0 mm Hg) in seven SAH rats 30 minutes after basilar artery puncture. At 3 and 7 days after puncture, ICP was not different from baseline in the six rats studied.

Discussion

Although cerebral vasospasm appears to be the primary cause of delayed cerebral ischemia, vasospasm alone is not sufficient to induce clinically symptomatic cerebral ischemia.8,13,19,24–34 After ruptured aneurysm, as many as 70% of patients develop angiographically evident signs of cerebral vasospasm, yet only one third of patients develop clinical symptoms of delayed cerebral ischemia.4 This indicates that delayed cerebral ischemia after SAH must be a multifactorial process. Modern techniques that measure CBF in awake subjects have demonstrated chronic global CBF changes in patients after ruptured aneurysm that do not correspond with the distribution of angiographic vasospasm.5,24,31 Physiologic studies in patients with SAH have also demonstrated marked autoregulatory disturbances of CBF.34 The magnitude of both the global CBF changes and the autoregulatory disturbance predict patients at high risk for ischemia and poor outcome after SAH.30,34 These data indicate that alterations of the brain microcirculation may play a role in the development of delayed cerebral ischemia.

We demonstrate a chronic decrease in CBF in rats following experimental SAH due to transclival basilar artery puncture. These animals did not develop signs of delayed cerebral ischemia. In this sense our model is not an exact representation of the clinical entity of ruptured aneurysm. Nonetheless, this model may prove useful in the study of the diffuse microvascular alterations that follow SAH and in part contribute to the syndrome of delayed cerebral ischemia.

Our model provides significant improvements over previous techniques to produce SAH in rats. Unlike in models in which blood is placed in the cisterna magna,17,18,20 basilar artery puncture results in extensive blood clot formation in the basal cisterns. Also, because of the vascular injury, the endothelium is exposed to the subarachnoid space and potentially vasoactive substances are released into the cerebrospinal fluid. These features may explain why basilar artery puncture successfully produced chronic CBF changes whereas injection of blood into the cisterna magna did not.

The surprisingly small rise in ICP accompanying experimental SAH was likely related to the opening in the clivus, which served as a site of decompression. Although an undetected acute rise in ICP immediately after the puncture may have caused some degree of global ischemia, it seems highly unlikely that this phenomenon could be responsible for the chronic changes in TBF. Previous work in this laboratory21 in which either autologous blood or saline was injected into the cisterna magna produced an immediate decrease in CBF 3 hours after injection but no changes in CBF 1, 2, 3, 7, or 14 days later. It has been postulated that the initial changes in CBF were due to an acute elevation of ICP caused by the injectate. In spite of that sudden rise in ICP, there were no chronic changes in CBF, and therefore it is unlikely that in the basilar artery puncture model an acute rise in ICP is responsible for the TBF changes observed 3–14 days later. In addition, the absence of ischemic changes in the hippocampus on histologic sections 7 and 14 days after SAH strongly argues against the theory that an acute elevation of ICP may have caused some degree of global ischemia that affected CBF days later.

Other models of cerebral vasospasm in higher mammals have shown evidence of vasculopathy after...
experimental SAH. However, induced SAH in rats has not produced vasculopathic changes in the major arteries in the subarachnoid space. This failure may be related to the lack of interadventitial spaces in rats’ arterial walls. It has been suggested that impidement of arterial wall nutrition resulting from obstruction of the interadventitial spaces by blood in the subarachnoid space may contribute to the vasculopathic changes following SAH. As a result, rat basilar arteries may not be susceptible to the development of these degenerative changes in the vessel wall following SAH. It is unlikely that the chronic decrease in TBF in our model resulted from arterial spasm. Although the initial vasospastic response to SAH probably involves simple contraction of smooth muscle, the secondary vasospastic response lasting several days is believed to reflect alterations in vessel wall morphology such as intimal proliferation and medial necrosis. As we found no such vasulopathy, it seems more likely that the chronic alterations in TBF were caused by an increase in cerebrovascular resistance secondary to constriction of the brain microvasculature rather than by spasm of the major arteries.

Further evidence for the absence of vasospasm in this model comes from the measurement of basilar artery diameters in photographs taken by Barry et al. Although a significant degree of vasospasm was apparent 1 and 2 days after SAH, the basilar artery actually showed a significant dilation (15–25%) 8 days after SAH. Photographs taken during our investigation duplicate these results (unpublished data).

Histologic examination of the basilar artery in rats subjected to experimental SAH failed to demonstrate basilar artery thrombosis. This finding rules out the possibility that localized trauma from the arterial puncture explains the TBF changes observed. Similarly, normal ICP 3 and 7 days after SAH exclude the possibility that decreased TBF is related to hydrocephalus or chronic elevation of ICP.

In summary, the basilar artery puncture method of producing experimental SAH in rats is shown to have potential for future studies of aneurysmal SAH. The chronic changes in TBF that have been documented correspond closely with global changes in CBF that have been observed in numerous studies of patients following ruptured aneurysms. Studies outlined in this paper indicate that these blood flow changes are not secondary to epiphenomena of the experimental technique such as transient global ischemia, sustained increased ICP, or basilar artery thrombosis. Therefore, chronic decreases in TBF most likely are related to the enduring presence of blood in the subarachnoid space. This model does not produce chronic cerebral vasospasm and delayed cerebral ischemia, and in this sense it has significant limitations in the study of the clinical entity of ruptured cerebral aneurysm. However, this model does seem to hold promise for study of the secondary pathophysiologic events that follow aneurysmal SAH.

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