A new rat model of chronic cerebral hypoperfusion associated with arteriovenous malformations

JIAN HAI, M.D., MEIXIU DING, M.D., ZHILIN GUO, M.D., AND BINGYU WANG, M.D.

Department of Neurosurgery, Tongji Hospital, Tongji University; and Department of Neurosurgery, The Ninth People’s Hospital, Shanghai Second Medical University, Shanghai, China

Object. A new experimental model of chronic cerebral hypoperfusion was developed to study the effects of systemic arterial shunting and obstruction of the primary vessel that drains intracranial venous blood on cerebral perfusion pressure (CPP), as well as cerebral pathological changes during restoration of normal perfusion pressure.

Methods. Twenty-four Sprague–Dawley rats were randomly assigned to either a sham-operated group, an arteriovenous fistula (AVF) group, or a model group (eight rats each). The animal model was readied by creating a fistula through an end-to-side anastomosis between the right distal external jugular vein (EJV) and the ipsilateral common carotid artery (CCA), followed by ligation of the left vein draining the transverse sinus and bilateral external carotid arteries. Systemic mean arterial pressure (MAP), draining vein pressure (DVP), and CPP were monitored and compared among the three groups preoperatively, immediately postoperatively, and again 90 days later. Following occlusion of the fistula after a 90-day interval, blood–brain barrier (BBB) disruption and water content in the right cortical tissues of the middle cerebral artery territory were confirmed and also quantified with transmission electron microscopy. Formation of a fistula resulted in significant decreases in MAP and CPP, and a significant increase in DVP in the AVF and model groups. Ninety days later, there were still significant increases in DVP and decreases in CPP in the model group compared with the other groups (p < 0.05). Damage to the BBB and brain edema were noted in animals in the model group during restoration of normal perfusion pressure by occlusion of the fistula. Electron microscopy studies revealed cerebral vasogenic edema and/or hemorrhage in various amounts, which correlated with absent astrocytic foot processes surrounding some cerebral capillaries.

Conclusions. The results demonstrated that an end-to-side anastomosis between the distal EJV and CCA can induce a decrease in CPP, whereas a further chronic state of cerebral hypoperfusion may be caused by venous outflow restriction, which is associated with perfusion pressure breakthrough. This animal model conforms to the basic hemodynamic characteristics of human cerebral arteriovenous malformations.

KEY WORDS • carotid-jugular fistula • arteriovenous malformation • venous hypertension • rat

Primary hemodynamic alterations in patients with cerebral AVMs decrease CPP in the adjacent brain as a result of intracranial arteriovenous shunting. The common underlying mechanism seems to be chronic cerebral hypoperfusion caused by regional arterial hypotension and venous hypertension. Although several animal models have been developed in attempts to recreate the hemodynamic changes that occur in cerebral AVMs, the roles of feeding artery hypotension and draining vein hypertension in the pathogenesis of cerebral AVMs are less clear.

In clinical settings, resection of human AVMs can be complicated by postoperative edema and/or hemorrhage in adjacent brain parenchyma, despite the complete excision of the malformation. This phenomenon has been termed NPPB or occlusive hyperemia. The precise underlying mechanism remains controversial. Existing animal models were designed to reproduce brain edema and/or hemorrhagic complications seen in human AVMs by creating systemic hypertension rather than a normotensive state, which was different from the clinical situation.

The purpose of our study was to create a new animal model of chronic cerebral hypoperfusion in an attempt to investigate whether systemic arterial shunting and ligation of the vein draining the transverse sinus affects CPP, and to determine whether brain edema and/or hemorrhage occur following restoration of normal perfusion pressure.

Materials and Methods

Animal Preparation and Pressure Measurements

This study conforms to the rules set by the Animal Experimentation Committee at Shanghai Second Medical University. Twenty-four Sprague–Dawley rats, weighing between 200 and 250 g, were used in the experiment. On Day 0, the rats were anesthetized with an intraperitoneal injection of pentobarbital (40 mg/kg), which was...
supplemented as needed throughout the procedure. At the beginning of each surgery, the right femoral artery was cannulated with polyethylene tubing coupled to a pressure transducer and chart recorder to monitor systemic mean arterial pressure (MAP).

Anatomical dissections revealed that the EJV is the primary vessel draining intracranial venous blood in rats. To assess the DVP, the left EJV was exposed. A heparinized, saline-filled, flexible silastic catheter (0.3-mm inner diameter, 0.65-mm outer diameter), which was coupled to a pressure transducer and chart recorder, was advanced into the vein to a depth of approximately 4 mm. The catheter tip was directed rostrally to permit measurement of DVP; both MAP and DVP were recorded throughout each experiment.

The rats were randomly divided into a sham-operated group, an AVF group, and a model group (Fig. 1) to permit the evaluation of MAP and DVP preoperatively, immediately postoperatively, and again 90 days later. The CPP is defined as MAP minus DVP.

The Three Protocols

The Sham-Operated Group. Eight rats were assigned to this group. Anatomically the rat has a vein that drains the transverse sinus, which is separate from the petrosal sinus. This vein is located superiorly and posteriorly to the petrosal sinus and drains into the jugular vein. The left vein draining the transverse sinus was exposed through a 5-mm horizontal incision centered at the level of, and 4 mm posterior to, the external auditory meatus. Once the draining vein of the transverse sinus was exposed, the incision was closed. A second midline incision was made anteriorly over the neck. The right EJV and bilateral ECAs were exposed and ligated. This incision was then closed.

The AVF Group. This protocol involving eight rats included creation of an AVF and ligation of bilateral ECAs. The right CCA was exposed, and the ipsilateral EJV was divided 5 mm rostral to the subclavian vein. A fistula was created by distal end-to-side EJV–CCA anastomosis with interrupted 10-0 nylon sutures. The left draining vein of the transverse sinus was only exposed.

The Model Group. The protocol for this group of eight rats included creation of an AVF, occlusion of the left vein draining the transverse sinus, and ligation of bilateral ECAs. These animals underwent the same anastomosis as the AVF group.

We used TCD ultrasonography (model CDS; Medasonics, Freemont, CA) to assess the patency of the fistula and the direction of flow within the right ICA after formation of the fistula and again 90 days later.

Evaluation of BBB Integrity

The integrity of the BBB was investigated using Evans blue dye extravasation, according to Belayev, et al. The fistula was occluded after an interval of 90 days, and five rats in each of the three groups were subjected to intravenous injection with Evans blue dye (2% in saline, 4 ml/kg); the chest was opened 1 hour later. The rats were perfused with saline through the left ventricle until colorless perfusion fluid was obtained from the right atrium. After decapitation of the animals, cerebral cortical samples of the right MCA territory were obtained for local measurement of Evans blue dye. Samples were weighed and placed in 50% trichloroacetic acid solution. After homogenization and centrifugation, the extracted dye was diluted with ethanol (1:3) and its fluorescence was determined (excitation at 620 nm and emission at 680 nm) with a luminescence spectrometer (model F-3010; Hitachi Corp., Tokyo, Japan). Calculations were based on external standards in the same solvent (100–500 ng/ml). The amount of Evans blue dye in the tissue was quantified from a linear standard curve derived from known amounts of the dye and was expressed per gram of tissue. The pattern of Evans blue dye uptake in rat brains was also assessed and recorded photographically before quantitative measurements were performed.

Brain Water Content

For the measurement of brain water content, brain samples were obtained in the same area as the site of Evans blue dye assessment, placed in preweighed crucibles, and weighed. The samples were then dried to a constant weight in an oven at 95°C for 24 hours. The percentage of water content in the tissue was calculated using the following formula: \[ \% \text{ water} = 100 \times \frac{(\text{wet weight} - \text{dry weight})}{\text{wet weight}}.\]

Light Microscopy Examination

The right brain tissue blocks obtained at the coronal level (bregma 1–3 to 4 mm) in the study animals were cut, fixed in formalin,
The mean MAP, DVP, and CPP values in eight rats in each of the three groups*  

<table>
<thead>
<tr>
<th>Factor</th>
<th>Sham-Op</th>
<th>AVF</th>
<th>Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>preop</td>
<td>113.6 ± 7.2</td>
<td>112.0 ± 6.8</td>
<td>112.9 ± 6.6</td>
</tr>
<tr>
<td>MAP</td>
<td>2.3 ± 0.7</td>
<td>2.1 ± 0.8</td>
<td>2.4 ± 1.1</td>
</tr>
<tr>
<td>DVP</td>
<td>111.4 ± 6.9</td>
<td>109.9 ± 6.8</td>
<td>110.5 ± 6.3</td>
</tr>
<tr>
<td>postop Day 0</td>
<td>111.4 ± 6.9</td>
<td>90.0 ± 5.0†</td>
<td>87.1 ± 7.1†</td>
</tr>
<tr>
<td>MAP</td>
<td>2.1 ± 0.6</td>
<td>16.4 ± 1.7†</td>
<td>21.1 ± 2.2†‡</td>
</tr>
<tr>
<td>DVP</td>
<td>109.8 ± 5.9</td>
<td>73.6 ± 4.4†</td>
<td>66.0 ± 6.8†‡</td>
</tr>
<tr>
<td>postop Day 90</td>
<td>113.4 ± 5.1</td>
<td>111.1 ± 5.2</td>
<td>108.4 ± 5.9</td>
</tr>
<tr>
<td>MAP</td>
<td>2.3 ± 0.9</td>
<td>14.5 ± 1.9†</td>
<td>19.6 ± 2.1†‡</td>
</tr>
<tr>
<td>DVP</td>
<td>111.1 ± 4.5</td>
<td>96.6 ± 4.6†</td>
<td>89.9 ± 6.2†‡</td>
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</table>

* Values are expressed as the means ± SD.
† p < 0.05, postoperative compared with preoperative pressure.
‡ p < 0.05, pressure in the model group compared with the AVF group.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Group (5 rats each)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evans blue (µg/g tissue)</td>
<td>3.74 ± 1.44</td>
</tr>
<tr>
<td>water content (%)</td>
<td>78.74 ± 0.89</td>
</tr>
</tbody>
</table>

* Values are expressed as the means ± SD.
† p < 0.05, the model group compared with the other groups.

Results

All animals survived the procedure with no evidence of neurological deficits. The TCD ultrasonographic examinations confirmed that all fistulas were patent after 90 days, and that flow through the arteriovenous shunt was retrograde through the ICA on the side of the fistula.

Pressure Measurements

Preoperative values of MAP, DVP, and CPP in the rats were not significantly different among the three groups. The sham operation had no significant effect on MAP or DVP. Formation of a fistula resulted in significantly decreased MAP and CPP and increased DVP in the AVF and model groups compared with the sham-operated group (p < 0.05). In the model group, ligation of the left vein draining the transverse sinus was accompanied by a further pronounced rise in DVP and a decrease in CPP.

At the 90-day reevaluation, MAP, DVP, and CPP remained unchanged in the sham-operated group, and MAP had returned to the normal range in the other groups. In the AVF and model groups, the DVP had decreased, although it remained significantly above normal (p < 0.05). Compared with the AVF group, a further significant increase in DVP and a decrease in CPP could be observed in the model group (p < 0.05). The changes in MAP, DVP, and CPP in the three groups of rats throughout the experiments are summarized in Table 1.

Disruption of the BBB and Water Content in the Brain

Following restoration of normal perfusion pressure by occlusion of the fistula after 90 days, Evans blue dye extravasation and water content in rat brains in the model group were significantly increased compared with results in the other groups (p < 0.05). There were no significant differences, however, in Evans blue dye extravasation and water content between the sham-operated and AVF groups (Table 2). The degree of Evans blue dye leakage in rat brains in the AVF and model groups is shown in Fig. 2.

Histopathological Findings

On gross examination of the rat brains stained with hematoxylin and eosin in the AVF and model groups, no evidence of infarction, swelling, or hemorrhage was apparent (data not shown). Electron microscopic examination revealed no differences in the cerebral capillaries ob-

Fig. 2. Photographs of rat brains obtained in animals that had undergone occlusion of the AVF after a 90-day interval of patency. No prominent staining by Evans blue dye was demonstrated in the specimen from the AVF group (left). On the contrary, Evans blue dye extravasation is grossly visible in the specimen from the model group (right).
Rat model of chronic cerebral hypoperfusion

Anatomical dissections revealed that the primary vessel draining intracranial venous blood in rats is the EJV through the posterior facial vein and the transverse sinus vein, with no direct communication between the lateral sinus and the internal jugular vein. The predominance of the EJV in the rat makes it most suitable for creation of an AVF designed to affect the intracranial circulation. We thus performed an end-to-side anastomosis between the distal EJV and the CCA, combined with ligation of bilateral ECAs in rats, which resulted in significant decreases in MAP and CPP and an increase in DVP. Our model produced a substantial arterial shunting effect from the cerebral circulation, because cerebral cortical artery pressure is proportionate to systemic arterial pressure. On TCD ultrasonographic examinations we confirmed that the direction of flow within the right ICAs was retrograde through the AVF.

It has been demonstrated that draining vein abnormalities and venous hypertension play an important role in the pathogenesis and pathophysiology of cerebral AVMs. Angiographic evidence of limited venous outflow and aberrant venous patterns is a common accompaniment of both symptomatic and asymptomatic AVMs. Interference with venous drainage represents a single factor responsible for spontaneous hemorrhage of cerebral AVMs. Increased pressure of the draining vein in cerebral AVMs is regarded as an important cause of nonhemorrhagic focal symptoms previously thought to be due to steal. Occlusive hyperemia can be attributed to obstruction of the venous drainage system during resection of cerebral AVMs. Bederson, et al. found that permanent obstruction of the contralateral EJV immediately after creation of a cervical arteriovenous shunt caused all their rats to die. Based on the characteristics of the intracranial drainage vein in the rat, we ligated the vein draining the transverse sinus to induce occlusion of the primary vessel draining intracranial venous blood. All animals survived the procedure, and furthermore a cerebral hypoperfusion state may be caused by restriction of venous outflow. This result indicated that the combination of systemic arterial shunting and obstruction of the intracranial venous drainage system is necessary for a significant decrease in CPP.

A sensitive quantitative fluorescence method has been used for detecting the BBB disruption by extravasation of Evans blue dye, which has the useful property of binding to serum albumin in vivo and in vitro. Following occlusion of the arteriovenous shunt after 90 days, there were significant increases in Evans blue dye extravasation and water content in the brains of rats in the model group compared with the other groups. Electron microscopy demonstrated cerebral vasogenic edema and/or hemorrhage in varying degrees, which may be responsible for NPPB. Nevertheless, no prominent brain swelling or hemorrhage was identified using light microscopy. We think that NPPB may exist in a subclinical state in this animal model of chronic cerebral hypoperfusion. It has been reported that the incidence of NPPB in patients with larger AVMs is as high as 40% during surgical procedures. The patho-

![Fig. 3. Electron micrographs of capillaries in the cerebral cortex of the right MCA area obtained in animals in the sham-operated group (left) and model group (right).](image)

Left: Tissue from the sham-operated rats shows a well-defined basal lamina (arrow) and the clear cytoplasm of the astrocytic foot process layer (Fig. 3 left). In specimens from the model group, some capillaries were notable for an absence of astrocytic end feet, which correlated with cerebral vasogenic edema and hemorrhage (Fig. 3 right).

**Discussion**

The main hemodynamic alterations in cerebral AVMs decrease CPP, which is caused by the presence of feeding artery hypotension and draining vein hypertension due to intracranial arteriovenous shunting. In the experiment reported by Spetzler, et al., a CCA–EJV end-to-end anastomosis was constructed in such a way that blood flowed in a retrograde direction through the cephalic carotid artery system to enter the fistula and thus it flowed caudally through the jugular vein. Venous drainage is primarily orthograde into the systemic circulation so that intracranial venous hypertension is underemphasized. In addition, incorporation of the ECA circulation may be presumed to have contributed a continuing flow of unknown volume through the AVF. In the model of Morgan, et al., an anastomosis of the distal carotid artery to the distal EJV produced an AVF fed by retrograde flow through the ICA and very likely also caused venous hypertension. Their model, however, does not decrease systemic MAP when the fistula is opened.

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physiological mechanisms of this entity are less well understood; some authors have suggested that NPPB may be caused by multiple factors, such as arterial autoregulatory override, venous occlusion, or severely hypoxic situations in the cortex adjacent to AVMs.1,7,12,17,23,25

In this study, we found that there were absent astrocytic foot processes surrounding some capillaries in the brains of animals in the model group, which may be related to the breakdown of the BBB. Sekhon, et al.,20 postulated that these vessels had developed as a result of neovascularization in response to chronic cerebral ischemia, and that their anatomical configuration made them prone to mechanical weakness after the increase in perfusion pressure that occurs in adjacent brain parenchyma after AVM excision.

Conclusions

Our results indicate that this new animal model simulates the basic hemodynamic properties of human AVMs with impaired venous outflow, and that occlusion of arteriovenous shunting may lead to NPPB. This study will be of considerable utility in further exploring the pathophysiological mechanisms of AVMs and the prevention or treatment of complications after resection of large, high-flow intracerebral AVMs.

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References


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