Local cerebral blood flow autoregulation following “asymptomatic” cerebral venous occlusion in the rat

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Object. Maintenance of cerebral blood flow (CBF) autoregulation in the brain is of major importance for patient outcome in various clinical conditions. The authors assessed local autoregulation after “asymptomatic” cortical vein occlusion.

Methods. In Wistar rats, a single cortical vein was occluded photochemically by using rose bengal and fiberoptic illumination. In rats with bilateral carotid artery occlusion, mean arterial blood pressure (MABP) was lowered in 5-mm Hg increments down to 40 mm Hg by using hypobaric hypotension. Local CBF at each pressure level was assessed by performing laser Doppler (LD) scanning at 25 (5 × 5) locations within bilateral cranial windows. In this manner, the lower limit of autoregulation (LLA) was detected. The LLA was 60 mm Hg in both right and left hemispheres in Group A (five rats), in which the animals received illumination without rose bengal and had no venous occlusion. Of the 11 rats that underwent vein occlusion, three developed severe reductions in local CBF and/or a growing venous thrombus and were distinguished as Group C (symptomatic; three rats); from previous work we know that those animals are bound to experience venous infarction. The remaining rats formed Group B (asymptomatic; eight rats). In this group the LLA remained at 60 mm Hg in the left hemisphere without occlusion, whereas, in the right cortex with the occluded vein, the LLA was found to be 65 mm Hg. Below a carotid stump pressure of 25 mm Hg regional CBF in the affected hemisphere dropped more abruptly to a possibly ischemic range than that in the opposite normal hemisphere.

Conclusions. The results of the present study suggest that cerebral venous circulation disorders are manifested via additional pathways, that is, from a partially impaired local autoregulation in the vicinity of the occluded vein, even under conditions in which the vein occlusion itself does not cause brain damage. Care should be taken in the control of blood pressure in patients with this pathological condition.

Key Words • autoregulation • cerebral blood flow • cerebral perfusion pressure • cerebral vein occlusion • laser Doppler scanning • rat

Although brain ischemia caused by arterial occlusion has been a focus of attention for decades, currently there is considerable interest in the study of cerebral injury following cerebral venous occlusion. Recent publications on this topic have documented that, in different animal models of venous occlusion, two outcome groups form spontaneously: animals with brain damage and those with intact brain tissue. This experimental evidence correlates well with the high variability of symptoms observed in patients after sinus–vein or venous occlusion. Furthermore, the phenomenon occurs at a quite similar injured/uninjured ratio, approximately 1:1 in a rat sinus–vein thrombosis model and 2 to 3:7 after occlusion of one cortical vein in the rat. Brain damage could be predicted by performing repeated angiography and cerebral blood flow (CBF) and tissue hemoglobin oxygen saturation monitoring in the early stage. Clinically, intraoperative CBF monitoring has already been used to predict the safety of occlusions in sections of the venous system. Therefore, CBF changes that occur after cerebral venous circulation disorders (CVCDs) should be evaluated in more detail. Thus, the pathophysiological characteristics of brain damage that occur as a result of CVCDs are being gradually understood. However, little is known about the condition of the brain after “asymptomatic” cortical vein occlusion.

In this experiment, photochemical cortical vein occlusion was used as a model of intraoperative sacrifice of cortical veins, which occurs during neurosurgical operations. The technique was established originally in experimental arterial occlusion, and the vessels were occluded with platelet aggregates by means of a dye-mediated photodynamic reaction. We have reported that this model is minimally invasive, clinically relevant, and reproducible. In this model, animals with asymptomatic cortical vein occlusion could be selected by using fluorescence angiography and regional (r)CBF data. Autoregulation of CBF is the intrinsic ability of the brain to maintain a constant perfusion in the face of blood pressure changes; it is defined as the dilation and constriction of cerebral resistance vessels in response to changes in cerebral perfusion pressure (CPP), resulting in a constant CBF. Cerebral blood flow autoregulation is impaired in the presence of severe head injury,
Autoregulation of CBF after asymptomatic vein occlusion

**TABLE 1**

Data obtained from arterial blood gas analyses sampled during initial control condition phase of experiments

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Animals</th>
<th>PO₂ (mm Hg)</th>
<th>PCO₂ (mm Hg)</th>
<th>pH</th>
<th>MABP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sham-operated</td>
<td>5</td>
<td>125.1 ± 7.5</td>
<td>42.9 ± 2.7</td>
<td>7.30 ± 0.04</td>
<td>101.9 ± 8.0</td>
</tr>
<tr>
<td>Group A</td>
<td>5</td>
<td>112.4 ± 13.6</td>
<td>43.5 ± 2.0</td>
<td>7.30 ± 0.03</td>
<td>115.0 ± 13.8</td>
</tr>
<tr>
<td>Group B</td>
<td>8</td>
<td>124.6 ± 14.0</td>
<td>38.7 ± 2.5</td>
<td>7.31 ± 0.10</td>
<td>110.5 ± 10.9</td>
</tr>
</tbody>
</table>

* Values are expressed as means ± standard deviation. There were no statistical differences between groups (by ANOVA at the p = 0.05 level of significance).

The current experiment was designed to examine local autoregulation (the lower limit of autoregulation [LLA]) in brains tolerating the occlusion of a solitary cortical vein. To do so, we used laser Doppler (LD) scanning\(^{24,29}\) and the hypobaric hypotension technique\(^{15}\) and we examined rCBF and local (l)CBF changes in both hemispheres at various blood pressure levels in the rat cortical vein occlusion model using a photochemical thrombosis technique.\(^{22-24}\)

**Materials and Methods**

The present study was conducted according to animal experiment guidelines approved at the 80th General Assembly of the Japan Science Council (1980).

### Animal Preparation

Twenty-one male Wistar rats, each weighing 280 to 370 g (mean 326.4 ± 29.3 g) were premedicated with 0.5 mg atropine and anesthetized by an intraperitoneal injection of chloral hydrate (36 mg/100 g). Anesthesia was maintained by hourly administration of chloral hydrate (12 mg/100 g) through a peritoneal catheter. The animals were intubated via a tracheostomy after relaxation had been achieved, and ventilation was controlled by using a small animal respirator with an atmosphere of 70% nitrous oxide/30% oxygen. During the experiment, temporal muscle and rectal temperature were maintained at 37°C by a feedback-controlled homeothermic lamp and a blanket control unit. Aided by the use of an operating microscope, both carotid arteries were exposed and carefully isolated from surrounding tissue. Special care was taken not to damage the vagus nerve or peripheral nerve tissue. No. 5-0 thread was looped around the left carotid artery for later occlusion by attaching a 15-g weight to the thread. Polyethylene catheters were inserted into the femoral vein and guided bidirectionally into the right carotid artery. The centrally directed catheter was used for continuous measurement of mean arterial blood pressure (MABP) and for blood gas analysis; the arterial line directed toward the circle of Willis was used to estimate carotid artery stump pressure (CSP). Venous lines were inserted for administration of fluid and drugs. The rats’ PaO₂, PaCO₂, and arterial pH were measured using a blood gas analyzer. Blood pressure and CSP were continuously monitored via an intrarctal catheter that was connected to a pressure transducer. Each rat was mounted on a stereotactic frame. After a 1-cm midline skin incision had been made, bilateral cranial windows (3 × 4 mm) were created over the bilateral frontoparietal regions by using a high-speed drill with the aid of the operating microscope. During the craniotomy, the drill tip was cooled continuously with physiological saline to avoid thermal injury to the cortex. The dura was left intact and the frontoparietal cortex was exposed bilaterally. Saline with a fixed temperature (37°C) was perfused over the dura.

#### Local CBF Measurement by Laser Doppler Scanning

Local CBF was measured by laser Doppler (LD) flowmetry in which a 0.8-mm needle probe was used. Local CBF was expressed in LD units (LDU) and was measured at 25 (5 × 5) locations in a scanning procedure that was accomplished using a motor-driven and computer-controlled micromanipulator connected to a personal computer. The scanning field included the distal area affected by the vein occlusion (Fig. 1 left). Placement of the field was decided on the basis of a preliminary study: ICBF was examined over a large area in the cranial window at 48 (8 × 6) locations, each at a distance of 400 μm to investigate the topographical relationship between CBF change and an occluded vein (Fig. 1 right). Here, the random registration of 25 individual measurements results in one scanning procedure with information being obtained from 25 different locations, each at a distance of 300 μm. To avoid artifacts caused by measurements recorded with a probe that was still moving, a delay of 2 seconds was allowed before each measurement. Twenty data points measured for the next 2 seconds were averaged and used as one ICBF measurement. Regional CBF was determined by calculating the median value from the 25 locations. Therefore, the stop time in one position was 4 seconds, and one scan took approximately 2 minutes. The technique permits repeated scans to be obtained from a given set of locations.

#### Fluorescence Angiography

Fluorescence angiography was performed to examine epicortical vessel structures by intravenous injection of 2% Na-fluorescein solution and by using an excitation source at a wavelength of 450 to 490 nm. A photomicroscope with magnifications ranging from 5.8 to 35, furnished with a 50-W mercury lamp and fluorescence filter, was used for fluorescence angiography performed before and 30 minutes after induction of venous occlusion. The images were recorded on video tape to permit careful reevaluation. To minimize damage by fluorescence excitation or environmental light, illumination was restricted to the angiography. Complete occlusion of the targeted cortical vein, together with other undamaged intact vessels around the illumination point, were identified by the second fluorescence angiography.

#### Cortical Vein Occlusion by Using the Photochemical Thrombosis Technique

Single cortical vein occlusion was induced by using intravenous rose bengal (50 mg/kg) and fiberoptic illumination connected to a 100-μm fiber. The diameter of veins chosen for occlusion was approximately 100 μm. The methods used here have been described in detail previously.\(^{15}\)

#### Hypobaric Hypotension

The lower portion of the rat body was placed in a negative pressure chamber, connected to an electronically controlled vacuum pump for induction of hypobaric hypotension. The barometric pressure within the chamber could be reduced, and hypotension was caused by the pooling of venous blood in the lower half section of the body.\(^{15}\)

#### Experimental Protocol

After a 30-minute cortical vein occlusion, the second fluorescence angiography and LD scanning were performed. Following this, the MABP was reduced by hypobaric hypotension in 5-mm Hg increments down to 40 mm Hg. The MABP was reduced to the intended level immediately and was then maintained constant for 5 minutes. During this plateau phase, the MABP was continuously measured and the ICBF was recorded bilaterally from 25 locations over each cortex. At the end of the experiment, the animals were killed by an overdose of anesthesia. The biological zero of the LD was then determined. Eleven animals underwent the experiment.
This experiment was designed to study brains that had no primary parenchymal damage from CVCDs. Accordingly, those animals with indications of later brain damage following venous occlusion were omitted from Group B. Selection was based on the fact that brains severely affected by cortical vein occlusion show an interruption of venous blood flow and/or a growing venous thrombus in the second fluorescence angiography and significant CBF decrease during the experiment. Following those criteria, three rats were identified as belonging to Group C (symptomatic) (see Results).

Five rats (Group A) that received a craniotomy had illumination and induced hypotension performed in the same fashion as described earlier but without rose bengal injection. Another five rats only underwent a craniotomy (sham-operated controls).

Statistical Analysis

Results are expressed as the means ± standard deviation (SD) for physiological variables. Regional CBF is expressed as the median of ICBF data obtained from each location. The unpaired t-test or the Kruskal–Wallis test was used for analysis of rCBF in the right or left cortex and of physiological variables such as blood gas levels (PaO₂ and PaCO₂) and pH. Differences in sequential rCBF were evaluated using analysis of variance (ANOVA; Dunnet’s test) for repeated measures. The chi-square test was used for discrete variables (Table 2). Statistical significance was assumed at a probability level of less than 0.05. Statistical analysis was performed using commercially available statistical computer software.

Sources of Supplies and Equipment

Ventilation of animals was controlled by using a Harvard rodent ventilator (model 683) purchased from Harvard Apparatus, Inc. (S. Natick, MA) and their temperatures were controlled by using a homeothermic lamp (model IFR 100) purchased from Unique Medical, Tokyo, Japan) and a blanket control unit (CMA 150) from Carnegie Medicine AB (Stockholm, Sweden). The operating microscope was obtained from Zeiss (Wetzlar, Germany). Measurements of PaO₂, PaCO₂, and arterial pH were made by using the ABL 300 blood gas analyzer available from Radiometer (Copenhagen, Denmark). The pressure transducer (Polygraph system RM-600) was obtained from Nihon Koden (Tokyo, Japan) and the stereotactic frame (SR-6) from Narishige Inc. (Tokyo, Japan).

Laser Doppler flowmetry was performed by using model ALF-21 available from Advance (Tokyo, Japan). The motor-driven and computer-controlled micromanipulator (XYZ scanning stage) was provided by Scholar Tec (Osaka, Japan) and connected to a 89 Note SX personal computer from NEC (Tokyo, Japan).

Table 2: Observation frequency of the LLA of each experimental animal in Groups A and B*

<table>
<thead>
<tr>
<th>MABP (mm Hg)</th>
<th>Group A (5 animals)</th>
<th>Group B (8 animals)†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rt Hemi</td>
<td>Lt Hemi</td>
</tr>
<tr>
<td>70</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>65</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>60</td>
<td>3$§$</td>
<td>3$§$</td>
</tr>
<tr>
<td>55</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>50</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>45</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Hemi = hemisphere.
† Proportion of LLAs in both sides of Group B is significantly related (p = 0.037, chi-square test).
‡ Side where cortical vein occlusion was induced.
§ Shows LLA in each group, calculated from the median rCBF of each animal.
Autoregulation of CBF after asymptomatic vein occlusion

Fluorescence angiography was performed using Na-fluorescein solution available from Nacalai Tesque (Kyoto, Japan) and a I2-filter block excitation source purchased from Leitz (Wetzlar, Germany). A photomicroscope (M 420) was purchased from Wild (Heerbrugg, Switzerland) and the fluorescence angiograms were recorded on video tape (BR-S600) obtained from Victor (Tokyo, Japan).

Rose bengal was purchased from Katayama Chemicals (Osaka City, Japan) and the fiberoptic illumination system (L4887 fiberoptic system) from Hamamatsu Photonics (Hamamatsu, Japan). Statistical analysis was performed using Sigma-Stat software available from Jandel Scientific (Erkrath, Germany).

Results
In three of the 11 animals in Group B, CBF and angiographic studies indicated that occlusion of a solitary vein would lead to parenchymal damage. In these rats, significant reductions in CBF were observed within 30 minutes after cortical vein occlusion, as was a growing venous thrombus. Accordingly, these three rats were categorized as Group C (symptomatic) and the remaining animals formed Group B (asymptomatic; eight rats).

Physiological Variables
Under control conditions the physiological variables in sham-operated animals (five rats), animals that did not receive rose bengal (Group A; five rats) and animals that later had vein occlusion (Group B; eight rats) showed no significant changes in blood gases (PaO_{2} and PaCO_{2}), pH, and MABP between groups (Table 1).

Relationship Between CSP and MABP
The correlation between CSP and MABP is shown in Fig. 2. During the control phase (MABP 106.2 \pm 13.4 mm Hg), CSP remained stable (36 \pm 2.3 mm Hg; Fig. 2, filled squares) and was unrelated to MABP; CSP dropped slightly (31.2 \pm 3.4 mm Hg) after occlusion of the left carotid artery caused by using the previously placed snare, and then decreased with MABP reduction (Fig. 2 empty circles).

Lower Limit of Autoregulation of Normal Brain
The calculation of median rCBF values from the 25 locations in the five sham-operated control animals showed no significant changes during the experiment, or between the right and left hemispheres. Regional CBF (mean \pm SD) was 25.8 \pm 8.9 LDU and 24.8 \pm 9.9 LDU (right and left hemispheres, respectively) at the beginning of the experiment and then remained constant. At the end of the experiment, the rCBF was 26.5 \pm 11.5 LDU and 26.3 \pm 11.7 LDU, respectively. In Group A, the rCBF was 21.8 \pm 10.9 LDU in the right and 23.3 \pm 9.9 LDU in the left hemisphere at 115.0 \pm 13.8 mm Hg MABP (baseline conditions). There were no differences between hemispheres with respect to rCBF following the reduction in MABP. The rCBF was stable until the MABP was reduced below 60 mm Hg (LLA). A detailed analysis of individual cases revealed that the LLA was at 60 mm Hg in three rats and at 55 mm Hg in two rats (Table 2). The LLA was identical for the right and left hemispheres in each animal.

Averaging the median rCBF values in animals from Group B (Fig. 4 left) revealed 24.3 \pm 9.8 LDU and 24.1 \pm 7.9 LDU (right and left hemispheres, respectively) as the baseline data at 110.5 \pm 10.9 mm Hg MABP. With induced hypobaric hypotension, the rCBF of the unaffected left cortex behaved similarly to that of Group A; there was a plateau at an MABP of greater than 60 mm Hg and, below that threshold, rCBF decreased with MABP (LLA).
Opposed to this, the rCBF of the right cortex with an occluded vein fell earlier, and an averaged autoregulation curve suggests that autoregulation was abolished after asymptomatic vein occlusion (Fig. 4 left). An analysis of the pressure–flow relationship for individual animals, however, revealed still existing LLA between an MABP of 66 to 70 mm Hg in one rat, 61 to 65 mm Hg in four rats, and 56 to 60 mm Hg in three rats (Table 2, Fig. 4 center). Significant differences between the right and left hemispheres were observed between 61 and 65 mm Hg (p < 0.05, unpaired t-test). There was a significant relationship between the LLAs of the left and right hemispheres: the uninjured left hemisphere had a lower LLA than the right hemisphere with vein occlusion. Figure 4 right shows alterations in rCBF in the right hemisphere in Groups A and C that can be compared with the rCBF of Group B in Fig. 4 center. The rCBF in Group A remained constant until the MABP was 61 to 65 mm Hg, whereas the rCBF in Group C already decreased before induced hypotension and continued to drop with the hypotension. The rCBF at an MABP of 61 to 65 mm Hg in Group B (13.5 ± 3.2 LDU) was significantly lower than that in Group A (22.2 ± 5.8 LDU) (p < 0.05; unpaired t-test). The rCBF at an MABP of 61 to 65 mm Hg in Group C (6.9 ± 1.6 LDU) could not be compared with other groups because of the small number of rats in that group.

The correlation between rCBF and CSP in Group B animals showed that the rCBF in the right side was lower than that in the left hemisphere below 25 mm Hg CSP (Fig. 5).

**Discussion**

Until recently, understanding of the pathophysiological characteristics of CVCD-associated disorders was limited. The lack of appropriate animal models, in particular, partly hindered progress. The photochemical thrombosis model for cortical vein occlusion is quite attractive from the standpoint of its minimal invasiveness and technical ease. In previous communications,22,24 we reported that this experimental approach is characterized by histological brain injury that has a fixed probability of nearly 30% following single vein occlusion. These animals showed acute extension of venous thrombus, local critical ischemia, and severe brain damage. The remaining 70% of animals, which tolerated a solitary vein occlusion, did not have major flow disturbances or neurological damage. This subgroup of animals was brought into focus in the current study. The selection/discard criteria of asym-
Autoregulation of CBF after asymptomatic vein occlusion

Cerebral perfusion pressure (CPP) is the pressure difference between inflow and outflow pressure within the subarachnoid space; in most instances intracranial pressure (ICP) reflects outflow pressure. Therefore, CPP is usually calculated as the difference between MABP and CSP, rCBF in the brain affected by vein occlusion at normal blood pressure. Neverthless, autoregulatory dysfunction was observed. Nameely, there exist functional differences between intact brain and brain affected by CVCDs, although both of these are intact histologically.

Cerebral perfusion pressure (CPP) is the pressure difference between inflow and outflow pressure within the subarachnoid space; in most instances intracranial pressure (ICP) reflects outflow pressure. Therefore, CPP is usually calculated as the difference between MABP and ICP (MABP – ICP). Cerebral blood flow is normally preserved at a CPP greater than 60 mm Hg.28 Carotid stump back pressure has been widely used to determine selective shunting during carotid endarterectomy.2 As shown in Fig. 5, below a threshold of 25 mm Hg CSP, rCBF in the brain affected by vein occlusion decreased to a low flow more suddenly than that in the opposite normal brain. Taking into account that flow is determined by local cerebrovascular resistance and local CPP (arterial blood pressure – local ICP), then it may not be surprising that the flow in the potentially affected tissue may well remain unchanged at normotension, whereas the flow decreases earlier at the hypotension state than in normal tissue. Most likely, venous occlusion increases venous pressure in the distal portion, resulting in increased local ICP and then in decreased local CPP. Hartmann, et al.,13 reported that reduction in blood pressure that is caused by drugs that dilate peripheral and intracranial vessels during surgery or intensive care management, particularly in patients with increased ICP, may cause deleterious effects on tissue perfusion. The findings in this study also bear significant importance for clinical application. We should know that the breakpoint at which CBF starts to decrease is at a higher level in the brain with CVCDs than in a normally autoregulating brain.

The recruitment of collateral pathways occurs during the early phase of venous occlusion. The severity of CVCDs depends on the availability of individual venous collateral vessels, which is a possible explanation for the differences in the extent of the upward shift of the LLA (as shown in Table 2) among animals of Group B with durable CVCDs.

The brain may be able to tolerate the first insult of cortical vein occlusion; however, the effects of the first stress will become manifest by a subsequent distress (for example, brain retraction during surgery, excessive changes in systemic blood pressure). The combination of insults results in a disproportionate enlargement of the affected hemisphere, which could not be explained by the increased infarction size alone. Intraoperative compression in a brain with venous circulation disorders, caused by a spatula shifting the brain for hours, is well known to be very harmful and often causes hemorrhagic venous infarction. The fact that the brain with CVCDs is very fragile has been underestimated so far. Extreme care should be taken in such an ailing brain.

A question arises whether the upper limit of autoregulation is also affected by CVCDs. This issue is of much scientific interest per se and also of great importance in the management of patients with CVCDs. Strict control of postoperative hypertension is one of the key ways to prevent intracerebral hemorrhage. Patients with CVCDs should therefore be kept in the intensive care unit to maintain normotension.

Conclusions

The autoregulatory capacity of CBF is influenced by vein occlusion even in the face of normal CBF. Therefore, care should be taken in the control of blood pressure in patients with this borderline critical condition.

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