An Experimental Model of Intraoperative Venous Injury in the Rat

ABSTRACT—Intraoperative obliteration of cerebral veins occasionally causes unexpected severe complications, especially in elderly patients. However, very little information is available on the pathophysiology of cerebral venous circulation disturbance. Occlusion of cortical veins in rats by a photochemical thrombotic technique is a less invasive, clinically relevant and reproducible model that is suitable for the study of venous circulation disturbance. In the present study, 54 male Wistar rats were used. We examined changes of the cerebral venous flow pattern by fluorescence angiography and brain damage histologically in a one- or two-(cortical) vein occlusion model using a photochemical thrombotic technique. Approximately 30% (9 of 27) of animals in the single-vein occlusion group and 90% (15 of 17) of those in the two-vein occlusion group had microcirculation perturbation, which results very soon in the formation of venous thrombus accompanied by severe venous infarction. In addition, infarction size in the two-vein occlusion group ($9.7 \pm 3.2\%$) was significantly larger than in the single-vein group ($2.9 \pm 1.3\%$) (unpaired T-test, $P < 0.01$).

In conclusion, the photochemical dye technique of attaining cerebral venous occlusion is a worthwhile addition to the study of circulation perturbations of the brain.

Brain damage following intraoperative obliteration of cerebral veins with subsequent increase of intracranial pressure, brain edema, brain infarction, and intracerebral hemorrhage has been described (Fig. 1). Nevertheless, venous infarction has received relatively little experimental and clinical attention so far, and the pathophysiology of venous circulation disturbance is still poorly understood.

Various experimental models using different techniques and animals (i.e., rats, cats, pigs, or dogs) have been utilized to investigate cerebral venous occlusion.¹⁻⁷ However, the results of these studies vary widely, suggesting the difficulty of developing a standardized and reproduc-

ible experimental model. Therefore, research on cerebral venous circulation disorder has been limited partly because of lack of animal models that produce consistent venous infarction.

Photochemically induced arterial occlusion has been developed and utilized in a considerable number of laboratories.⁸⁻¹⁰ The methodology is simply and quickly performed, minimally invasive, and—owing to the photochemical potency of rose bengal—virtually assured of producing a recognizable lesion. We apply this technique to cortical vein occlusion and introduce it as a less invasive (locally and systemically), uncomplicated, and clinically relevant model.

Figure 1. A: Intraoperative sacrifice of cortical vein (wide arrow). B: Hemorrhagic infarction was observed postoperatively.

MATERIALS AND METHODS

This study was conducted according to German animal protection legislation or the animal experimentation guidelines approved at the 80th General Assembly of the Japan Science Council (1980).

We used 54 male Wistar rats (weighing 240 to 390 g). They were given free access to food and water under the same environmental conditions prior to surgery. These animals were premedicated with 0.5 mg atropine and anesthetized with chloral hydrate (36 mg/100 g, intraperitoneally). Anesthesia was maintained by chloral hydrate (12 mg/100 g) given hourly, and the body (rectal) temperature was kept at close to 37.0°C. Spontaneous ventilation was maintained during the experiment. Polyethylene catheters were placed into the tail artery and the right femoral vein. The arterial line served for continuous registration of arterial blood pressure and blood gas analysis; the venous line served for administration of fluids and drugs. PaO_2 , PaCO_2 , and arterial pH were monitored. The heads of the animals were placed in a stereotactic frame. A 1.5-cm midline skin incision

and a cranial window (4.5 mm $\times$ 6 mm) were made over the right frontoparietal region by a high-speed drill under an operating microscope. Continuous physiological saline cooled the drill tip during the craniotomy. The dura was left intact, and the right frontoparietal cortex was exposed. Saline of fixed temperature (37°C) was frequently perfused over the dura.

Fluorescence angiography was carried out in all rats. Epicortical vessel structures were studied using 2% Na⁺-fluorescein solution (E. Merck, Darmstadt, Germany) and an excitation source with a wavelength of 450 to 490 nm and a I2-filter block (Leitz, Wetzlar, Germany). A photomicroscope furnished with a 50-W mercury lamp and fluorescence filter (I2; Leitz, Wetzlar, Germany) was used for fluorescence angiography before, and 30 and 90 minutes after, induction of venous occlusion. The images were recorded on videotape (HS-S5600E (RS), Mitsubishi, Japan). In order to minimize the damage by fluorescence excitation, illumination was restricted to angiography.

Cortical vein occlusion was induced by rose bengal and fiberoptic illumination (100 μm). Rose bengal was injected slowly without decreasing the systemic pres-

sure at 50 mg/kg. Care was taken to avoid illumination of tissue and other vessels near the target veins. The fiber was pointed at the prearranged location of the vein with approximately 100 μ m diameter for 10 minutes for the first vein (one vein occlusion; $n=27$) (Fig. 2) and another 10 minutes with additional injection of a half dose of rose bengal for the second vein (two-vein occlusion; $n=17$). Complete occlusion of the cortical vein was confirmed later by fluorescence angiography. After the third fluorescence angiography, the resected bone flap was repositioned and the skin wounds were closed. The rats were returned to their individual cages and allowed free access to water and food.

In addition, five rats serving as sham-operated controls (sham A) received a similar craniotomy and intravenous injection of the rose bengal solution without illumination, and another five serving as sham-operated controls (sham B) received a similar craniotomy and illumination without rose bengal.

Two days after the operation, clinical manifestations were observed, and the rats were each given an injection of 2.0% Evans blue solution (1ml/kg) under general anesthesia with chloral hydrate. After one hour, the rats were submitted to perfusion fixation with 4% paraformaldehyde. The brain was then removed from the skull and embedded in paraffin to obtain coronal section of the frontoparietal region. Sections were stained with hematoxylin and eosin.

We examined the influence of photochemical technique on endothelial cells using cultured endothelial cells derived from bovine artery in N-(2-hydroxyethyl) piperazine-N'-2-ethanesulfonic acid (HEPES) buffer and rose bengal at 1.6 mg/cc of medium. After 10-min irradiation, cell viability was measured by the trypan blue exclusion test (Boehringer). One hundred cells were counted three times under the microscope and the percentage of blue cells (number of blue cells per hundred) was determined.

Figure 2. Fluorescein angiography of the vein after photochemically induced thrombosis in one-vein occlusion group. The thrombus is indicated by arrows (mag. $\times 30$).

For a quantitative assessment of brain injury, the picture of the brain through a light microscope was projected onto the screen and a genlock interface. The area of the injured brain obtained in three parts (the largest infarcted lesion, its 0.4 mm anterior and posterior) was measured over the video image using a software developed in this laboratory or OS/2 (J 2.1 operating system, IBM Japan, Japan), and expressed on the average as a ratio to the contralateral hemispheres. Dimensions of the grids were calibrated with a microscope ruler (Ernst Leitz, Wetzlar, Germany).

RESULTS

Physiological Variables

There were no significant changes in physiological parameters of blood gases (PaO_2 and PaCO_2) and mean arterial blood pressure (MABP) before and after venous occlusion or among all study groups (data not shown).

Fluorescence Angiographical Findings

Fluorescence angiography was performed to examine the condition of the cerebral venous bloodstream and the thrombus after vein occlusion. Complete occlusion of the targeted cortical vein together with other undamaged intact vessels around the irradiation point were identified by the second fluorescence angiography.

No changes were apparent in both sham-operated groups (A and B) estimated from three angiographies, including occlusion of the vessels and extravasation of fluorescein. The second angiography showed a flow reversal, a dilatation of the distal portion of the occluded vein, and a deceleration of flow. The third angiography verified an arrest of flow, further extension of thrombus, and the extravasation of fluorescein into the parenchyma close to the occluded vein. In the two-vein group, basically similar findings were observed, although both of the two veins did not always show the same reactions. Earlier and more extensive extravasation of fluorescence occurred in the parenchyma between two occluded veins in the two-vein group than in the single-vein group.

Histological Studies

No cell injury was apparent in the test of direct damage by photochemical thrombotic technique using cultured endothelial cells with cell viability measured by trypan blue dye exclusion test (Boehringer).

Histopathological observations demonstrated that the brains of both sham-operated controls appeared nor-

Figure 3. A: Histology of a coronal section through the frontoparietal area in one-vein group. Note venous infarction with petechial hemorrhage in the hemisphere. (mag. $\times 20$) B: Histology of a coronal section through the frontoparietal area in two-vein group. Note more severe and larger infarcted brain with edema than single-vein group. (mag. $\times 20$)

mal. In the single-vein group, nine of 27 rats had parenchymal damage such as multiple petechial hemorrhages surrounding the dilated capillaries and edematous areas in the white and gray matter (Fig. 3A). Fifteen of 17 rats in the two-vein group showed more extensive petechial hemorrhage and infarcted lesion (Fig. 3B).

Quantitative assessment of brain injury, which was expressed as a percentage ratio of contralateral hemisphere, showed a significantly larger infarction size in the two-vein group ($9.7 \pm 3.2\%$) than in the single-vein group ($2.9 \pm 1.3\%$) (T-test, $P < 0.01$) (Fig. 4).

DISCUSSION

Recently much attention has been paid to the study of cerebral injury following venous circulation disturbances.^{1-7,11,12} Skull base surgery has become routine in neurosurgery, and sinuses and bridging veins are more often exposed, with an increased risk of postoperative complications following interruption of the venous circulation than in earlier times. Also, neurosurgical operations are performed more frequently in elderly patients, and mild venous circulation disturbances that are without consequence in young patients often cause unexpectedly severe complications in the elderly. Nevertheless, very little is actually known about the pathophysiology of venous circulation disturbances in the brain, and until recently, the technically challenging experimental procedures necessary to occlude sinus and veins in small animals.

The methodology used here is less invasive (locally and systemically), non-tricky, and clinically relevant. We confirmed no injury to cultured endothelial cell by this photochemical technique. Furthermore, we have already showed the absence of any influence on the brain (e.g., CBF, CBV fraction, and histology) and

physiological parameters (e.g., systemic blood pressure, pulse, arterial pH, PO_2 , PCO_2 , hematocrit) using irradiated or injected dye control.^{5,11}

Recent experiments on sinus-vein thrombosis have demonstrated that occlusion of the superior sagittal sinus (SSS) alone is not sufficient to block venous outflow, and that a cerebral venous thrombosis becomes critical for the parenchymal blood supply only if draining cortical veins are occluded.^{3,4,7,12,13} In the present study also, fluorescein angiography showed that growing thrombus was observed in cases with brain damage after single- and two-vein occlusion, whereas thrombus did not extend in cases without brain damage.

We have already reported this animal model.^{5,11,14} In previous studies using this technique,^{11,16} we reported that cerebral venous occlusion causes local ischemia adjacent to the occluded veins without sufficient collateral flow, and this circulation perturbation appears even in the very early period following the venous occlusion and results in the extension of venous thrombus, local critical ischemia, and severe brain damage. In this study, we re-examined the rate of venous infarction after single- and two-vein occlusion by more animals, and also showed *in vitro* study using cultured endothelial cells.

The key observation of the present study is that this experimental approach is characterized by a wide variety of symptoms with histological brain injury (30%) following one-vein occlusion but certainly brain damage (90%) after occlusion of two adjacent veins. Therefore, the occlusion of a single vein is an appropriate model for studying the variable pathophysiology of venous occlusion observed experimentally as well as clinically, and the occlusion of two adjacent veins is a reproducible and quite useful approach for the study of the pathophysiology of cerebral venous circulation disturbance.

Figure 4. Box plots of quantitative assessment of infarction size, which was expressed as a percentage ratio of contralateral hemisphere, in one-vein and two-vein occlusion groups. Data are shown as box plots. The mean value is indicated as a dotted line, the median as a horizontal line, boxes mark the 25th and 75th percentile, error bars the 10th and 90th percentiles, hollow circles the 5th and 95th percentiles. Infarction size is significantly larger in the two-vein than in the one-vein group (*: $P < 0.01$).

Though it is widely accepted that surgical obliteration of cerebral veins and additional brain compression by retractors is dangerous, little is known about the mechanism of brain injury. Therefore, studies of the mechanism underlying brain injury after cortical vein occlusion with brain compression are currently examined using this model.

In conclusion, the photochemical dye technique for cerebral venous occlusion is less invasive, technically easy, and useful for the study of circulation perturbations of the brain.

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