Histological studies of intracranial vessels in primates following transluminal angioplasty for vasospasm

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Percutaneous transluminal angioplasty for treatment of cerebral vasospasm was performed in primates. Chronic cerebral vasospasm was induced by placement of an autologous blood clot over the right internal carotid artery (ICA), middle cerebral artery (MCA), and anterior cerebral artery (ACA). Cerebral angiography on Day 7 showed that the diameters of the ICA, MCA, and ACA were reduced to 55.7% ± 1.3%, 55.3% ± 2.6%, and 59.6% ± 1.3%, respectively, of baseline. The angioplasty was carried out with a silicone microballoon attached to a microcatheter under somatosensory evoked potential (SEP) monitoring on Day 7. The angioplasty for ICA was performed satisfactorily; however, the balloon could not be advanced to the spastic M or A, portions of the cerebral artery. Following angioplasty, the diameters of the ICA, the M segment, and the A segment were 79.6% ± 2.9% (p < 0.001), 67.6% ± 4.3% (p < 0.05), and 61.7% ± 2.2% (not significant), respectively, of baseline. Histological studies demonstrated that the vessels were well dilated and patent without endothelial cell damage.

Key Words • angioplasty • cerebral vasospasm • subarachnoid hemorrhage • monkey

Cerebral ischemia caused by chronic cerebral vasospasm is a major source of morbidity and mortality following subarachnoid hemorrhage (SAH). Angiographic vasospasm has been reported to occur in 30% to 70% of patients between 4 and 12 days following SAH. The pathogenesis of this condition is poorly understood, and it has been hypothesized that several vasoconstrictor substances are putative spasmogens. Despite the numerous therapies that have been proposed to treat this condition, medical regimens involving pharmacological agents and hyperensive hypertensive therapy have achieved only limited success.

Since Zubkov, et al., first described the use of angioplasty for vasospasm following SAH in 1984, there have been a number of reports indicating that this procedure can offer marked improvement to patients with ischemic deficits due to vasospasm. Morphological changes after balloon dilatation in extracranial arteries have been described previously; however, intracranial transluminal angioplasty has not been studied. The present investigation in monkeys was designed to observe morphological changes after balloon dilatation of vasospastic vessels.

Materials and Methods

This experimental procedure followed the guidelines of the Japan Science Council and was approved by the Animal Experimentation Committee of Fukui Medical School.

Laboratory Preparation

Seven Japanese monkeys (Macaca fuscata) of both sexes, weighing from 8 to 12 kg each, were used in this study. After control angiograms were obtained to determine the baseline vessel caliber of the cerebral arteries, SAH was induced as described by Espinosa, et al., in cynomolgus monkeys (Day 0). The animals were again studied angiographically to observe the development of cerebral vasospasm on Day 7, and then underwent balloon angioplasty on the right side. Angiography was performed a third time following angioplasty. Somatosensory evoked potentials (SEP's) and arterial blood pressure were monitored before, during, and up to 2 hours following angioplasty. All the animals were killed by exsanguination under general anesthesia for microscopic study.

Cerebral Angiography

Anesthesia was induced with an intramuscular injection of ketamine hydrochloride (10 mg/kg) and maintained by intraperitoneal administration of sodium pentobarbital (10 mg/kg/hr). The animals were intubated, paralyzed with pancuronium bromide (0.1 mg/kg/hr), and ventilated with a Harvard respirator. Ventilation was adjusted to maintain normocapnia. Body temperature was kept constant with an electric blanket.

By use of sterile surgical technique, a No. 6 French transfemoral catheter was placed, with the aid of fluoroscopic guidance, in the right or left common carotid artery. The catheter was connected to a three-way stop
cock on a pressure transducer to measure arterial blood pressure and to an angiographic injector to administer contrast medium (Hexabrix).* The arterial phase of the cerebral angiograms, anteroposterior view, was obtained by injecting each with 8 ml contrast medium at 300 psi on both sides.

**Induction of Subarachnoid Hemorrhage**

A right frontotemporal craniectomy was carried out in all animals. With the use of an operating microscope, the sylvian fissure was split and the arachnoid membrane over the middle cerebral artery (MCA), the internal carotid artery (ICA), the anterior cerebral artery (ACA), and the posterior communicating artery was opened very carefully. Liliequist’s membrane was incised and cerebrospinal fluid was drained by suction. The clot, created from 10 ml of autologous arterial blood, was placed over the exposed arteries. The dura was closed in a watertight fashion and the incision was closed in layers. The paralysis was reversed with neostigmine bromide (Prostigmin) (0.07 mg/kg) and atropine sulfate (0.02 mg/kg) administered intravenously. After extubation, the monkey was given routine post-operative care and then returned to its cage.

**Angioplasty**

Angioplasty was performed with a silicone microballoon attached to a microcatheter in all animals on Day 7 following the second angiography to determine cerebral vasospasm under appropriate anesthesia. A No. 6 French catheter placed in the right common carotid artery for angiography was used as a coaxial catheter, which was connected to a continuous perfusion system containing heparinized saline. The silicone balloon was 0.5 mm in diameter and 2 mm in length uninfated.† It expanded to 2.1 mm in diameter and 4 mm in length when inflated with 0.01 ml iiodinated contrast material. The microcatheter with the balloon was advanced to the C1 portion of the ICA under fluoroscopic guidance, and angioplasty of the spastic ICA was performed with the balloon. The inflation time of the balloon was 5 to 10 seconds and the balloon was inflated two or three times. Arterial blood pressure was continuously monitored from a catheter placed in the brachial artery, and SEP’s were monitored before, during, and up to 2 hours after angioplasty. The third angiography was carried out following angioplasty in all monkeys.

**Angiographic Assessment**

The diameter of the cerebral arteries was measured on the angiogram on Day 0 and Day 7, before and following angioplasty. The angiographic caliber of both supracallosal portions of the ICA, both M1 segments of the MCA, and both A1 portions of the ACA was measured by an observer without knowledge of the animal’s history. The diameters of the ICA, MCA, and ACA as

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* Pressure transducer P-50 manufactured by Statham Instruments, Inc. Oxnard, California, and angiographic injector manufactured by Cordis Corp., Miami, Florida.

† Silicone balloon developed for this experiment by Dow Corning, Tokyo, Japan.

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**Fig. 1.** Somatosensory evoked potential recordings after electrical stimulation of the median nerve. The central conduction time (CCT) is defined as the interpeak latency between N2 and N6. The amplitude (AMP) is defined as the N2-P1 peak-to-peak amplitude. C3 = left sensory cortex; Cv2 = the C-2 spinous process; Fpz = frontopolar vertex.

percentages of the baseline value on Day 0 were calculated for each animal.

**Measurement of Somatosensory Evoked Potentials**

The SEP’s were monitored with a Neuropack 4 system.‡ Paired stimulation electrodes were placed on the median nerves at the wrist. Reciprocal electrical stimulation of the median nerve was performed bilaterally with a square wave of 0.1 msec duration and at a rate of 4 Hz, with sufficient intensity to produce slight twitching of the first digit. Recording subcutaneous needle electrodes were located at the C-2 spinous process and the bilateral sensory cortex (C3’ and C4’). A frontal reference electrode was used at the frontopolar vertex (Fpz). The SEP’s were obtained from the average of 200 responses with an analysis time of 50 msec. The N2-P1 peak-to-peak amplitude and the cortical N2 and subcortical N6 interpeak latency (central conduction time, CCT) were used to assess brain function (Fig. 1).1,6,9,28 The SEP measurements were performed before, during, and up to 2 hours after angioplasty.

**Microscopic Studies**

After a brief period of perfusion with heparinized saline solution, perfusion-fixation was started from the heart with 4% paraformaldehyde in 0.1 mol/liter phosphate buffer (pH 7.4) at a pressure of 110 mm Hg. The

‡ Neuropack manufactured by Nikon Kohden, Tokyo, Japan.
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Fig. 2. A: A selective baseline angiogram of the right carotid artery (Day 0). B: An angiogram obtained 7 days after the induction of subarachnoid hemorrhage. Severe vasospasm is evident. C: Angiogram showing a balloon-inflated artery on Day 7. D: Angiogram following angioplasty showing that the internal carotid artery and middle cerebral artery have returned to almost normal size.

The brain was removed and the bilateral ICA, MCA, and ACA were carefully prepared and divided into two pieces for scanning electron microscopy (SEM) and transulminal electron microscopy (TEM). The specimens for SEM were stored in cacodylate-buffered 2.5% glutaraldehyde for 3 hours and postfixed in 1% osmium tetroxide in the same buffer for 1 hour. They were dehydrated in graded alcohols and amyl acetate and critical-point dried with CO2, coated with gold in an evaporator and examined by a Hitachi S-450 electron microscope. The specimens for TEM were fixed, dehydrated like those for SEM, and embedded in Epon 812 resin. Semithin sections were cut with an ultramicrotome and stained with toluidine blue. Ultrathin sections of selected areas were stained with uranyl acetate and lead citrate and examined with a Hitachi H-7000 electron microscope.

Statistical Analysis

Data were expressed as mean ± standard error of the mean. Student's t-test was used for statistical evaluation. A p value < 0.05 was regarded as statistically significant in the analysis of all data.

Results

Angiographic Changes

All animals showed evidence of vasospasm in the cerebral arteries on angiograms obtained on Day 7 compared with Day 0 (Fig. 2A and 2B). The percent diameters of each vessel, averaged on Day 7 in relation to Day 0, are shown in Fig. 3. On Day 7, the diameters of the ICA, MCA, and ACA were 55.7% ± 1.3%, 55.3% ± 2.6%, and 59.6% ± 1.3%, respectively, on the right side, whereas those on the left side were 70.0% ± 3.6% (p < 0.05), 71.7% ± 2.5% (p < 0.05), and 71.4% ± 3.2% (p < 0.05). The arteries on the clot side were significantly more spastic than those on the other side.

The transluminal angioplasty of the right ICA was performed on Day 7 (Fig. 2C). We could not advance the silicone balloon to the spastic M1 or A1 portion of the cerebral artery because of the size of the balloon. The caliber of the ICA and MCA returned to normal following angioplasty (Fig. 2D). The diameters of the ICA, the M1 segment, and the A1 segment following angioplasty were 79.6% ± 2.9% (p < 0.001), 67.6% ± 4.3% (p < 0.05), and 61.7% ± 2.2% (not significant), respectively (Fig. 3). Significant dilatation of the arteries was obtained by the procedure.

Changes in Somatosensory Evoked Potentials

There were no significant differences in physiological parameters before compared with after angioplasty. There was no significant left-right difference in the N1 latency in C-2 Fpz. Before angioplasty, the CCT ob-
tained from the right hemisphere was significantly prolonged (p < 0.01) compared with the value from the left. The mean CCT's in the right hemisphere were 4.81 ± 0.17 msec (p < 0.05) during angioplasty, 4.50 ± 0.15 msec (p < 0.05) at 1 hour, and 4.45 ± 0.25 msec (not significant) at 2 hours following angioplasty, whereas those in the left hemisphere were 4.06 ± 0.10 msec (p < 0.05), 4.02 ± 0.09 msec (not significant), and 3.99 ± 0.15 msec (not significant), respectively. The mean SEP amplitude on the right decreased to 48.0% ± 9.7%, and recovered to 81.3% ± 2.8% at 1 hour following angioplasty and 86.4% ± 3.0% at 2 hours. In contrast, the mean SEP amplitude on the left showed no significant change during angioplasty. The SEP amplitude was significantly larger (p < 0.01) on the left side than on the right.

Morphological Changes

Scanning electron microscopy of the vasospastic arteries demonstrated endothelial convolutions covered with abnormal endothelium along the longitudinal axis. Areas with detached endothelium were observed in the vessels. After balloon dilatation, SEM of the arteries showed the convolutions flatter than those of vasospastic arteries. The degree of endothelial cell damage resulting from the angioplasty was slight without additional endothelial injury.

Transmission electron microscopy of the vasospastic arteries demonstrated marked corrugations of the internal elastic lamina and endothelial cells compressed between tight folds of the elastic lamina, and a small number of smooth-muscle cells with degenerative changes (Fig. 4 left). After balloon dilatation, TEM of the arteries showed that the corrugated internal elastic lamina was extended (Fig. 4 right). The degree of the endothelial cell damage resulting from the angioplasty was not as severe as that due to vasospasm itself. Most smooth-muscle cells appeared intact following angioplasty.

Discussion

Transluminal Angioplasty

Transluminal treatment of arteriosclerotic obstruction of vessels by means of percutaneously introduced catheters dates from a report in 1964 by Dotter and Judkins. In 1984, Zubkov, et al. introduced this technique for treating cerebral vasospasm following SAH, and demonstrated improved cerebral blood flow (CBF) with the use of 133Xe. Since then, a number of cases of intracranial vasospasm have been reported treated with angioplasty. The pressure required to dilate cerebral arteries seems to be much less than that needed to dilate atherosclerotic vascular lesions. In our study, the transluminal angioplasty of the right ICA was satisfactorily performed on Day 7, but we could not advance the silicone microballoon to the spastic M1 or A1 portion of the cerebral artery because of the size of the balloon. Following balloon dilatation of the ICA, angiography demonstrated an almost normal luminal diameter of the M1 portion of the MCA as well as the ICA. The dilation of the proximal portion of the M1 with the balloon, combined with increased blood flow resulting from angioplasty for the ICA, allowed the M1 portion to dilate to almost normal size.

Somatosensory Evoked Potentials

Hume and Cant initially proposed the concept of CCT, which measures the transmission of impulses in the SEP's in the brain. Symon, et al., revealed that the measurement of CCT can be used to evaluate the electrical activity of the brain in ischemic insult. In our study, the mean CCT on the right was significantly prolonged compared with the value from the left before angioplasty on Day 7. It was more prolonged during angioplasty. These results indicate that the CBF in the right hemisphere decreased because of vasospasm, and it decreased more during angioplasty compared with...
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that of the left hemisphere. It is also reported that the SEP amplitude was affected by a reduction in CBF.6,21,22 Dowman, et al.4 reported that changes in SEP amplitude and latency during and immediately following temporary MCA occlusion predict development of the somatosensory ischemic lesion. Symon, et al.27 reported that the amplitude of SEP was of less value than CCT latency as a perioperative monitor.

Morphological Changes

Cerebral vasospasm induced by blood within the subarachnoid space has been associated with structural and morphological changes in the vessel wall.3,16 These consisted of swelling of the endothelium with areas of displacement from basement membrane, formation of smooth-muscle cells to the subendothelium, fragmentation and corrugation of the internal elastic lamina, swelling and necrosis of smooth-muscle cells, and an inflammatory reaction of the adventitia containing lymphocytes, plasma cells, and macrophage.8,16 Chavez, et al.4 reported that histological changes of the normal canine basilar artery 1 hour after angioplasty consisted of denudation of endothelium, stretching and focal dehiscence of internal elastic lamina, and altered myocytes in the media. It has been reported in the normal aorta of rabbits that acute changes after transluminal angioplasty were related to the size of the balloon and the duration of inflation.31 Yamamoto, et al.30 have reported that the long-lasting effects of balloon dilatation may be caused by the disruption of connective tissues that proliferate in the vessel wall following SAH.

Using a pharmacologically constricted basilar artery in a dog model, Pile-Spellman, et al.23 dilated the artery to between 100% and 104% of its original size. Under light microscopy, they observed mild histological changes including medial thinning and dehiscence of the internal elastic lamina without intimal fracture. In our study, the same morphological changes were observed in the arterial wall. Mechanical dilatation of the spastic vessels with the microballoon resulted in stretched internal elastic laminae and tunica media without an additional endothelial injury. Endothelial cell damage or exposure of subendothelial tissue leads to platelet adhesion and may subsequently cause thrombus formation.13 There was no platelet deposition in our study. This may be partially related to the use of systemic heparinization in our experiment.

Microballoon angioplasty for the treatment of intracranial vasospasm is a promising technique. Further investigation will be needed to determine the following points: the optimum time to perform the procedure, patient selection, and how far distally in the intracranial arteries it is necessary to dilate. The microballoon selected should be small enough to preserve blood flow in the spastic cerebral arteries.

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References