Stable xenon-enhanced CT measurement of cerebral blood flow in reversible focal ischemia in baboons

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When the lateral striate arteries of the baboon are temporarily occluded for either 20 or 60 minutes, a near-cessation of blood flow is followed by a dramatic, transient local increase in blood flow values. These findings are evident from serial xenon (Xe)-computerized tomography (CT) measurement of cerebral blood flow (CBF). In this study, 20 minutes of vessel occlusion resulted in brief (< 1 hour) hyperemia, with no subsequent CT alteration and minimal random neuronal injury. Sixty minutes of occlusion resulted in a more prolonged hyperemia, a low-density area on CT images within 3 hours of reperfusion, and infarction of all cellular elements within the anterior lentiform nucleus. The Xe-CT method provides a sensitive, noninvasive technique for examining sequential alterations of CBF in small regions deep within the brain. This method of recording CBF also permits correlative studies of cerebral infarction, both clinically and experimentally, and allows reasonable inference about the probabilities of neuronal tissue damage with or without reperfusion.

KEY WORDS □9 ischemia □9 cerebral blood flow □9 infarction □9 xenon □9 computerized tomography □9 baboon

The xenon (Xe)-computerized tomography (CT) method of recording CBF has proved to be technically feasible,8,24,31 and a broad range of clinical applications for this method has been reported.4-6,16,20,25,34,41,42,44 This method provides quantitatively accurate CBF values that closely agree with values derived by other CBF techniques.10,28,39 Unlike other methods, however, the Xe-CT method can define CBF values near zero in even very small, centrally located brain regions, and these low flows have been consistently followed by infarction.4,5,16,40

To evaluate the potential value of Xe-CT measurement of CBF in the management of stroke victims, a model of acute focal cerebral ischemia and reperfusion was created in the baboon and repeatedly examined by this method. Temporary ischemia was produced for either 20 or 60 minutes in two groups of baboons by temporary clip-occlusion of the lateral striate arteries (LSA). The CBF and CT information obtained was then analyzed and correlated with the pathological changes observed by light and electron microscopy.

Materials and Methods

Animal Preparation

Nine baboons underwent temporary clip-occlusion of the right LSA, Monkeys 1 to 4 for 60 minutes and Monkeys 5 to 9 for 20 minutes. Each animal had two
Xenon-enhanced CT measurement of CBF

Xe-CT studies of CBF before occlusion, one or two studies during occlusion, and up to eight studies following clip removal. Monkey 1 was sacrificed on Day 35 and Monkey 8 on Day 23. The remaining seven animals were sacrificed 6 hours after the onset of occlusion. The brains were removed and prepared for light and electron microscopy.

To minimize the amount of dissection required within the CT facility, the right middle cerebral artery (MCA) of each animal was exposed in the animal laboratory 3 days before the planned vessel occlusion. At that time, after the orbital contents were excised, the LSA were exposed through a retro-orbital craniectomy, which extended medially to the rim of the optic canal. After identification and dissection of the MCA, LSA, and orbitofrontal arteries from the surrounding arachnoid, the dural defect was covered with a gelatin sponge and the skin was closed with suture. The orbital wound was then irrigated with streptomycin solution, and each animal received 10 mg/kg oxacillin sodium and 10 mg/kg chloramphenicol daily for the next 3 days.

Three hours before transfer to the CT facility where the LSA were to be re-exposed and temporarily occluded, the baboons received a single dose of ketamine hydrochloride. Thereafter, they received a balanced anesthetic of 0.2 mg/kg diazepam (Valium) and 0.2 mg/kg morphine sulfate every 2 hours, and 0.2 mg/kg pancuronium bromide (Pavulon) and 0.02 mg/kg propranolol hydrochloride (Inderal) every hour for the duration of the study (approximately 8 hours). Each agent was administered on a fixed schedule to maintain analgesia, and blood pressure and heart rate were recorded continuously so that the adequacy of surgical anesthesia could be assessed. Femoral arterial and venous catheters were placed after the animals received lidocaine hydrochloride about the surgical sites. A capnograph* continuously monitored end-tidal CO2, and arterial blood gases were measured at the beginning and the end of each CBF study. Carbon dioxide partial pressures varied between 33 and 36 torr for all nine baboons and each animal's mean blood pressure varied from 90 to 120 mm Hg between animals.

Lateral Striate Artery Occlusion

The baboon's head was secured within the CT scanner in a plane parallel to the orbitomeatal line. Monkey 1 was studied with a prototype CBF system in a GE 8800 CT scanner, and the other eight animals were examined with the newer clinical system integrated within the GE 9800 CT scanner. The latter system was equipped with standardized hardware and software modifications for Xe-CT CBF analysis. First, a baseline blood flow study was obtained; then, without moving the baboon within the head holder, the periorbital tissues were infiltrated with lidocaine, the orbital wound was reopened with sterile technique, and the MCA and LSA were exposed under magnification. The second baseline CBF study was then performed. Twenty minutes later, allowing time for Xe washout, two or three low-tension microvascular clips† were placed across the origins of the LSA, usually also occluding the orbitofrontal artery.

Two CBF studies were obtained in Monkeys 1 to 4 during 60 minutes of occlusion, and a single CBF study was performed in Monkeys 5 to 9 during a 20-minute occlusion period. The first CBF study was begun within 5 minutes of clip application. In both groups of animals, removal of the clips resulted in restoration of normal color and arterial pulsation within the orbitofrontal arteries and LSA. The orbit was irrigated with warm sterile saline, and the field was covered with moist, sterile dressings. In all cases, the first CBF study after clip removal was obtained 5 to 10 minutes after reperfusion of the LSA; studies were then performed until the 6th hour after occlusion of the LSA in Monkeys 1 to 7. For technical reasons, Monkeys 8 and 9 received fewer blood flow studies after documentation of a 20-minute low-flow challenge. Because Monkeys 1 and 8 were to be kept alive for examination of more long-term pathological effects, their orbital wounds were resutured between the 1st and 2nd hours after reperfusion. They then received the same antibiotic regimen prescribed after their first operation.

Analyses of CBF were obtained at three brain levels by CT scans 5 mm thick (Fig. 1). While the animals inhaled 33% Xe/67% O2 for 4½ minutes, four CT scans were obtained at each of the three levels of study. An indirect record of the arterial “build-up” curve was obtained by measuring the end-tidal Xe concentration recorded by a thermoconductivity analyzer. A weighted least-squares fit of the Kety-Schmidt equation18 was then performed, assuming a single compartment for this type of analysis. Using a standard technique, ROI's were sampled at 5-mm diameter within the striate nuclei and at 10-mm diameter from within the cortical mantle of each hemisphere (Fig. 2). The mean of 25 CBF values within each 5-mm ROI and the mean of 100 values within each 10-mm ROI were calculated using the CT computer. For all animals, a clear plastic

* Capnograph manufactured by Puritan-Bennett Corp., Los Angeles, California.

† Bemer low-tension microvascular clips manufactured by Aesculap Instruments Corp., Burlingame, California.

‡ Linde XeScan xenon in Oxygen USP, Linde Medical Gases, Somerset, New Jersey.
FIG. 1. Xenon-computerized tomography cerebral blood flow studies from Monkey 1 obtained at three brain levels before, during, and after temporary clip-occlusion of the right lateral striate arteries (LSA). Occlusion of the LSA resulted in an absence of flow only within the right lentiform nuclei. Reperfusion resulted in marked local hyperemia only within the right lentiform nuclei, which, by the 5th hour, returned to nearly normal. Gray scale represents 0 to 100 ml/100 cc/min, from black to white.

FIG. 2. Typical template used to acquire regional blood flow values. Regions of interest (ROI’s) 1 to 4 and 5 to 8 are 5 mm diameter and located within the right and left lentiform nuclei, respectively; ROI’s 9 to 18 are 10 mm in diameter and located within the cortical mantle of both hemispheres.

H. Yonas, et al.

Neuropathological Methods
Details of the neuropathological methods have been described previously. Briefly, all animals were anesthetized and killed by intra-aortic perfusion-fixation with buffered paraformaldehyde, pH 7.4 (2 liters at 2% and 2 liters at 4%). Multiple horizontal sections of the fixed brain were cut to delineate the gross lesions. Specimens for electron microscopy were obtained from the anterior, middle, and posterior putamen, and the inferior frontal gyrus bilaterally.

Results

Cerebral Blood Flow and CT Scanning
Monkeys 1 to 4 (60 Minutes of Occlusion). Temporary occlusion for 60 minutes in Monkeys 2 to 4 was
TABLE 1
Pathological findings in nine baboons after LSA occlusion and reperfusion

<table>
<thead>
<tr>
<th>Monkey No.</th>
<th>Duration of LSA Occlusion</th>
<th>Time From Reperfusion to Sacrifice</th>
<th>Gross Lesions</th>
<th>Light Microscopy Findings</th>
</tr>
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<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td>Neurons</td>
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<td>Acute Change</td>
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<td></td>
<td>Loss of Neurons</td>
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<td></td>
<td></td>
<td>Oligodendro-glioma</td>
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<td></td>
<td></td>
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<td>Astrocytes</td>
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<td></td>
<td>Capillaries</td>
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<td></td>
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<td></td>
<td></td>
<td>Cavitiation</td>
</tr>
<tr>
<td>1</td>
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<td>35 days</td>
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<td>0 ++ ++ ++ ++ ++</td>
</tr>
<tr>
<td>2</td>
<td>60 mins</td>
<td>5 hrs</td>
<td>soft caudate, putamen</td>
<td>++ 0 ++ 0 ++ 0 0</td>
</tr>
<tr>
<td>3</td>
<td>60 mins</td>
<td>5 hrs</td>
<td>soft caudate, putamen</td>
<td>++ 0 ++ 0 ++ 0 0</td>
</tr>
<tr>
<td>4</td>
<td>60 mins</td>
<td>5 hrs</td>
<td>soft caudate, putamen</td>
<td>++ 0 ++ 0 ++ 0 0</td>
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<tr>
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<td>20 mins</td>
<td>5 hrs 40 mins</td>
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<tr>
<td>6</td>
<td>20 mins</td>
<td>5 hrs 40 mins</td>
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<td>23 days</td>
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<tr>
<td>9</td>
<td>20 mins</td>
<td>5 hrs 40 mins</td>
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<td>++ 0 0 0 0 0 0</td>
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* Abbreviations: LSA = lateral striate artery; 0 = no abnormalities; + = minimal change; ++ = moderate change; +++ = maximal change; -- = no lesions.

accompanied by CBF values of 1 to 3 ml/100 cc/min within ROI's 1 and 2 (Table 1, Figs. 3 and 4), which were located within the caudate nucleus and the most anterior part of the putamen. Average flow values for ROI's 1 to 4 were 2 ± 1, 1 ± 1, 10 ± 1, and 6 ± 10 ml/100 cc/min, respectively (mean ± standard deviation). The average flow values obtained 40 minutes after occlusion were slightly higher, although they remained below 8 ml/100 cc/min in ROI's 1 and 2. In the first CBF study, begun within 5 minutes of clip removal, CBF consistently arose above the preocclusion CBF values in ROI's 1 to 4 by 201%, 267%, 191%, and 164%, respectively. Hyperemia was homogeneous within the striate nuclei. Monkeys 1, 2, and 4 demonstrated their highest flow values in the first study after clip removal, whereas in Monkey 3 the highest flow

**Fig. 3.** Sequential cerebral blood flow (CBF) values from regions of interest 1 to 4 obtained in Monkey 3 before, during, and for 5 hours after 60 minutes of lateral striate artery (LSA) occlusion. Note the drop in flow values to near zero with LSA occlusion, followed by hyperemia, most marked within anterior lentiform nuclei. BL = baseline blood flow.

**Fig. 4.** The average and standard deviation of the right regions of interest (ROI's) 1 to 4 and left ROI's 5 to 8 in the striate nuclei of Monkeys 2 to 4 (each shown by a different symbol), which sustained 60 minutes of lateral striate artery occlusion. Note the relative stability of the left and prolonged hyperemia within the right striate nuclei.
values were observed 40 minutes after clip removal. Blood flow values remained above the baseline level for at least 5 hours in Monkeys 1, 3, and 4, whereas in Monkey 2 they returned to nearly baseline levels by the 1st hour after clip removal. Monkey 2 also had the lowest flow values recorded in any of the studies performed during occlusion of the LSA. The CBF values in the cortical and left striate ROI's did not change significantly during either occlusion or reperfusion of the LSA. Changes in CT density were identified within the caudate putamen by the 2nd hour after reperfusion in Monkey 2 and by the 3rd hour after reperfusion in Monkeys 1, 3, and 4 (Fig. 5).

Monkeys 5 to 9 (20 Minutes of Occlusion). The CBF reduction recorded immediately after occlusion of the LSA was slightly less severe in the 20-minute occlusion group than in the 60-minute group. Although an intraocclusion blood flow of 2 ml/100 cc/min was found in Monkey 8, the average flow values for ROI's 1 to 4 were 8 ± 2, 5 ± 2, 9 ± 4, and 21 ± 7 ml/100 cc/min, respectively, for the single studies obtained during occlusion (Fig. 6). Flow studies immediately following occlusion of the LSA revealed that the average increase in CBF above baseline was by 41%, 137%, 214%, and 137% for ROI's 1 to 4, respectively. Hyperemia was homogeneous within the striate nuclei. In Monkeys 5 to 7, CBF returned to baseline values within 65 minutes after clip removal; no consistent pattern of flow change within the cortex and/or the left striate nucleus accompanied or followed temporary occlusion in these animals. No CT density alteration was identified in any animal.

Neuropathological Examination

Gross Pathology. Table 1 shows the duration of occlusion of the LSA and the time at which each animal was sacrificed. Monkey 1, killed 35 days after 60 minutes of occlusion, had frank cavitation in the corpus striatum and internal capsule. Monkeys 2 to 4, sacrificed 5 hours after 60 minutes of clip-occlusion, had only slight, often subtle softening in the right putamen and caudate nucleus. In the animals that underwent 20 minutes of occlusion followed by reperfusion, no gross lesions were seen whether sacrifice occurred at 6 hours or 23 days after occlusion.

Light Microscopy. Cytological and histological changes were related to the duration of occlusion (Table 1). In Monkey 8, sacrificed 23 days after 20 minutes of occlusion, there was a maximal (++++) loss of neurons, maximal (++++) reactive astrocytosis, and moderate...
Xenon-enhanced CT measurement of CBF

FIG. 7. Photomicrograph of the right middle putamen after 20 minutes of ischemia and 5 hours 40 minutes of reperfusion. Note the enlarged perivascular space and swelling of astrocytic foot processes. Epon araldite, 1 \( \mu \)m, toluidine blue O, \( \times \) 100.

FIG. 6. Average and standard deviation of right and left regions of interest (ROI's) 5 mm in diameter in the striate nuclei of Monkeys 5 to 7 (each shown by a different symbol), which sustained 20 minutes of lateral striate artery occlusion. Note the stability of the left striate ROI's and only a brief (< 60-minute) hyperemia after reperfusion within the right ROI's. BL = baseline blood flow.

(+ + ) neovascularization in the entire right putamen. In Monkey 1, sacrificed 35 days after 60 minutes of occlusion, there was moderate (+ + ) cavitation, moderate (+ + ) macrophage infiltration, moderate (+ + ) neovascularization, and reactive astrocytosis in the putamen and caudate nucleus. Surviving neurons, oligodendrocytes, and other cellular elements distant from the area of cavitation were normal. Monkeys 2 to 4, sacrificed 5 hours after 60 minutes of occlusion, had pallor of the affected corpus striatum. Within this pale zone, acute neuronal change of the type associated with severe ischemia was observed (Fig. 7).

In the brains of Monkeys 5, 6, 7, and 9, killed almost 6 hours after 20 minutes of occlusion, there were minimal changes in the corpus striatum (Fig. 8). Topographic variability was seen in the lesions that developed in Monkeys 1 to 4. The caudate nucleus and the adjacent white matter tracts were involved more extensively in Monkeys 1 and 2.

Electron Microscopy. A well-developed infarct was found in animals that had undergone 60 minutes of occlusion; after 5 hours of reperfusion, electron microscopic findings were consistent with the observations made previously following 5 hours of permanent occlusion of the LSA.43

In animals subjected to 20 minutes of ischemia followed by reperfusion, the minimal lesions detected via light microscopy showed changes at the ultrastructural level. Perivascular astrocytic foot processes were swollen and occasionally fragmented; neuronal synapses were swollen as well. Neuronal nuclear and cytoplasmic changes were less conspicuous than in the animals subjected to 60 minutes of ischemia, but swelling and fragmentation of the cristae in the mitochondria were present in some nerve cells. Only a rare, small myelinated fiber showed minor areas of splitting and vacuoles within myelin sheaths.

Discussion

Temporary occlusion of the origins of the LSA in the baboon produced a consistent model of reversible focal cerebral ischemia. In each animal, clip-occlusion
of the LSA resulted in CBF values of 8 ml/100 cc/min or less within the anterior striate nuclei. The consistency with which this change occurred was possible because most LSA arise from the fronto-orbital artery or from the MCA immediately adjacent to it.\(^{45}\) Therefore, by identifying this anatomical landmark within the initial segment of the MCA in the baboon, the origins of the LSA could be located rapidly and then isolated with minimal brain retraction. A similarly highly reproducible model of focal ischemia has also been reported to accompany permanent LSA occlusion of the baboon and cat.\(^{11,40}\)

Despite the small numbers of animals, each group was characterized by certain consistent changes. All animals with 60-minute occlusion developed infarction of all cellular elements of the anterior striate nuclei, whereas all animals with 20-minute occlusion had more subtle, incomplete lesions. Although CBF values during occlusion were slightly higher in the 20-minute group, a relatively severe ischemic challenge was produced in all animals. Consistent with other reports, the difference in the severity of pathological lesions suggests that a threshold for infarction at this level of blood flow exists between 20 and 60 minutes of severe ischemia.\(^{3,35}\) However, even the animals with 20-minute occlusion with CBF values at or below 8 ml/100 cc/min showed light and electron microscopic evidence of selective cellular injury. Although only one animal was studied histologically 23 days after 20 minutes of occlusion, the severity of injuries found at that time suggests that the injury may have been more serious than was indicated microscopically, and that there were deleterious factors in addition to the duration of ischemia.\(^{17,19,23}\) Dramatic hyperemia developed in all of our animals after clip removal, similar to the hyperemia that has been reported to follow relatively brief but severe reversible focal and global ischemia in the laboratory\(^{13,26,36}\) and in clinical practice.\(^{12,27}\) Although Heiss, \(\text{et al.}\)\(^ {13}\) suggested that hyperemia after an ischemic event was prognostic of a severe injury, in all of our animals occlusion of the LSA was followed by elevated blood flow values, but of different durations. In three of the animals with 60-minute occlusion, the hyperemia persisted for 4 to 5 hours after reperfusion, whereas the animals with 20-minute occlusion had a more transient hyperemia which ceased by the 2nd hour of reperfusion. Thus, hyperemia \textit{per se} may not carry a uniformly consistent prognosis.

The extent and duration of the CT-defined low density that occurs with reperfusion of an ischemic region provide valuable additional insight into the severity of the ischemic challenge. A well-defined reduction of CT density within the striate nuclei has previously been reported to follow 6 hours of permanent occlusion of the LSA.\(^{40}\) This was consistent with the reports of early mass effect and early CT density change,\(^ {33}\) as well as rapid edema formation which accompanies the most severe ischemic insult.\(^ {30}\) In this study, 60 minutes of severe ischemia was accompanied by a pathologically defined complete infarction and a well-defined CT density change, whereas 20 minutes of ischemia resulted in incomplete infarction and no CT-defined alteration. Sixty minutes of occlusion also resulted in the earlier appearance of low density (by the 3rd hour after occlusion) than was produced by permanent occlusion of the LSA.\(^ {40}\) This supports the observation that reperfusion followed by severe hyperemia may aggravate the edema that accompanies ischemia, and controlling hyperemia may, in fact, be therapeutic.\(^ {15}\)

Xenon-CT measurement of CBF has been a safe and well-accepted clinical examination at our institution for more than 4 years.\(^ {21}\) We have performed over 2500 clinical studies in patients with a broad spectrum of neurological disorders including vasospasm,\(^ {41}\) acute stroke,\(^ {14}\) and head injury.\(^ {6,20}\) Although caution concerning the use of this study has been urged due to the potential for Xe to elevate CBF,\(^ {9,27,35}\) neither neurological deficits\(^ {31}\) nor raised intracranial pressure have accompanied our Xe-CT CBF studies. We conclude that Xe-CT measurement of CBF is not only a valuable laboratory study but also a safe method for clinical CBF analysis, and that its unique quantitative capability within even small and centrally placed brain regions warrants continued experimental and clinical investigation.

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