Neuropathologic Consequences of Internal Carotid Artery Occlusion and Hemorrhagic Hypotension in Baboons

D.I. Graham, PhD, FRCPATH, A.D. Mendelow, PhD, FRCSEd, Ursula Tuor, PhD, and W. Fitch, PhD, FFARCS

We studied eight anesthetized and physiologically monitored adult baboons (Papio cynocephalus); four were subjected to hemorrhagic hypotension alone and four to hemorrhagic hypotension plus unilateral carotid artery occlusion. Cerebral blood flow was measured using xenon-133, the electroencephalogram was recorded using silver-silver chloride epidural electrodes, and histologic examination was carried out after perfusion-fixation. In the baboons subjected to hypotension alone (mean arterial blood pressure of 28 mm Hg) cerebral blood flow was 28.5±5.0 ml/100 g/min, whereas in the baboons subjected to hypotension plus unilateral carotid artery occlusion it was 21.8±1.8 ml/100 g/min at a mean arterial blood pressure of 27 mm Hg. There was no ischemic damage in the former group, but in the latter group there was necrosis in the arterial boundary zones of three baboons and in the distribution of the middle cerebral artery in one. We conclude that, when combined with hypotension, unilateral carotid artery occlusion may lead to hemodynamic ischemia accentuated in the arterial boundary zones of the ipsilateral cerebral hemisphere. (Stroke 1990;21:428–434)

There is a well-known association between occlusion of the internal carotid artery (ICA) and cerebral infarction.1–5 One of the patterns of ischemic brain damage seen after ICA occlusion is infarction along the boundary zone between the territories of the anterior cerebral artery (ACA) and middle cerebral artery (MCA).6–8 This pattern is the consequence of a rapid and considerable reduction in cerebral perfusion pressure.6,9

When ischemic brain damage occurs along the arterial boundary zones, the critical factor is a reduction in the availability of tissue oxygen, usually because of a local reduction in cerebral blood flow (CBF). Because permanent occlusion of one or more of the large arteries in the neck may be symptomless,9 the aim of our study was to determine if hypotension of a degree that is normally well tolerated produces focal ischemic damage, particularly in the arterial boundary zones, when combined with occlusion of one ICA.

Materials and Methods

Eight adult baboons (Papio cynocephalus) weighing 7–16 kg were divided into two groups. Group 1 (control baboons, n=4) was subjected to hemorrhagic hypotension alone, and Group 2 (occlusion baboons, n=4) was subjected to hemorrhagic hypotension plus unilateral carotid artery occlusion.

The baboons were prepared in a manner identical to that established in our laboratory.10 Briefly, the animals were sedated with 10 mg i.m. phencyclidine hydrochloride, anesthetized with 7.5 mg/kg thiopental sodium, intubated, and connected to a positive-pressure ventilator supplied with a mixture of 70% N2O and 30% O2. Ventilation rate was adjusted such that the baboons were normocapnic (PacO2 of approximately 40 mm Hg) and normoxic (PaO2 of approximately 100 mm Hg). Phencyclidine (0.01 mg/kg/min) was infused intravenously, arterial blood pressure was monitored continuously, and body temperature was maintained at 37–38°C. A fine catheter was positioned in the superior sagittal sinus to permit the sampling of cerebral venous blood and the continuous measurement of sagittal sinus venous pressure. The electroencephalogram (EEG) was recorded from epidural silver-silver chloride electrodes positioned bilaterally over the cortex in the posterior frontal region 1 mm from the sagittal suture line, and these recordings were used to monitor and control the depth of anesthesia.11

CBF was measured from the right parietal area of the brain with a 1-in. collimated sodium iodide scintillation crystal and calculated by the height+area method12 after the intracarotid injection of xenon-133.
via a catheter in the right linguofacial trunk. The remaining branches of the right external carotid artery (ECA) were ligated, and the scalp and temporalis muscle on the same side were removed to eliminate errors caused by isotope clearance from extracranial tissues.

Staged hemorrhagic hypotension was achieved by the withdrawal and reinfusion of warmed autologous blood from the descending aorta via a cannula in one of the femoral arteries. Mean arterial blood pressure (MABP) was simultaneously monitored throughout the 10 minutes of the xenon-133 CBF measurement between predetermined limits (“bins” of 10 mm Hg) depending upon the initial baseline blood pressure after occlusion. Blood gas tensions were measured to ensure normoxia and normocapnia.

After the initial surgery, the baboons were allowed to stabilize for 30 minutes. Measurements of baseline CBF were made. Thereafter, in group 2 baboons the common carotid artery (CCA) was completely occluded by ligature. Because all branches of the ECA had been ligated, occlusion of the CCA corresponded to occlusion of the ICA, there being no collateral blood supply through the ECA. The CCA was occluded for 397 ± 15 minutes (mean ± SEM).

At the end of the procedure, all baboons were placed in the supine position before fixation. After heparinization with 1,000 IU/kg, a thoracotomy was performed and a cannula was secured in the proximal part of the aorta. After incising the right atrium and clamping the descending thoracic aorta, physiological saline was infused briefly at the existing MABP. Infusion was continued at the same pressure with 21% FAM fixative (40% formaldehyde:glacial acetic acid: absolute methanol, 1:1:8). After perfusion, the baboons were decapitated and the heads were stored in the same fixate for 12–24 hours. The brain was removed and immersion-fixed in FAM for a further 24 hours. The hindbrain was then detached by a cut at right angles to its long axis and sectioned into slices 6 mm thick, and the cerebellum was sectioned into two slices perpendicular to the folia of the dorsal surface of each hemisphere. Large representative bilateral blocks of brain were embedded in paraffin, and sections were stained with hematoxylin and eosin and with a combination of cresyl fast violet and Luxol fast blue. The histologic sections were examined by one of us without knowledge of the baboon’s surgical history. Microscopic abnormalities were recorded on a series of line diagrams.

In addition, a general autopsy was undertaken on each baboon, and representative blocks taken from multiple organs were immersion-fixed in 10% formalin and embedded in paraffin; the sections were stained with hematoxylin and eosin.

MABP was calculated as the diastolic pressure plus one third of the pulse pressure. All values are expressed as mean ± standard error of the mean. Comparisons were made using Student’s paired t tests (or nonpaired t tests as appropriate) with Bonferroni’s correction for multiple comparisons; p < 0.05 was taken as the level of significance.

<table>
<thead>
<tr>
<th>Group</th>
<th>MABP (mm Hg)</th>
<th>pH*</th>
<th>Paco2* (mm Hg)</th>
<th>Pco2* (ml/100 g/min)</th>
<th>CMRO2* (mg/100 g/min)</th>
<th>CMBGlu* (ml/100 g/min)</th>
<th>CBF§ (ml/100 g/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>28</td>
<td>108±7</td>
<td>37.7±1.3</td>
<td>3.2±0.4</td>
<td>0.22±0.05</td>
<td>28.5±5.0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>27</td>
<td>108±10</td>
<td>39.9±0.9</td>
<td>1.9±0.3</td>
<td>0.22±0.03</td>
<td>21.8±1.8</td>
<td></td>
</tr>
</tbody>
</table>

MABP, mean arterial blood pressure; CMRO2, cerebral metabolic rate for oxygen; CMRGlu, cerebral metabolic rate for glucose; CBF, cerebral blood flow; group 1, hemorrhagic hypotension alone; group 2, hemorrhagic hypotension plus unilateral carotid artery occlusion. Data are mean ± SEM.

*pMeasurements taken from abdominal aorta.
†Measurements taken from superior sagittal sinus.
‡Measurements taken from abdominal aorta and superior sagittal sinus.
§Measurements taken from right parietal region of skull.

Results

Values of the physiologic variables, MABP, and CBF for the two groups are given in Table 1.

While under anesthesia but before the induction of systemic hypotension, MABP was similar in the two groups. Following unilateral carotid artery occlusion, MABP increased in the group 2 baboons. Nevertheless, baseline CBF (before hypotension) did not differ significantly between the groups (group 1: 49±6 ml/100 g/min, group 2: 47±3 ml/100 g/min) at a mean Pao2 of 38±1 mm Hg.

Graded hemorrhage resulted in MABPs of 28±0 and 27±0.4 mm Hg in groups 1 and 2, respectively; CBF at these values of MABP are given in Table 1. Even though CBF was lower in group 2 than in group 1, there was no significant difference between the values. Hypotension lasted 224±15 minutes in group 1 and 231±13 minutes in group 2.

Arterial pH decreased in each baboon with acute decreases in MABP, but the overall change was relatively small and similar in the two groups. As indices of metabolic function, the cerebral metabolic rate for glucose was similar in the two groups, while that for O2 was lower in group 2 than in group 1 (Table 1, difference not significant).

The EEG reflected a stable depth of anesthesia (stages 1–3) throughout, except at the lowest MABP (30 mm Hg), when stage 4 was evident in two baboons and stage 5 in one. In no baboon did the EEG become isoelectric.
The brains of all baboons appeared to be normal both externally and in the standard slices of the cerebral and cerebellar hemispheres and the brainstem. As judged by the uniform hardening of the specimens and by the absence of blood in the vessels, perfusion-fixation was good in all baboons. The cyto logic artifacts dark cells and hydropic cell changes were not seen.

Abnormalities were few in group 1 baboons and were limited to small foci of superficial necrosis at the crests of the parasagittal gyri, which we interpreted as damage secondary to placement of the EEG electrodes. There were no other histologic abnormalities, and in particular there was no evidence of ischemic damage in other brain areas.

In addition to the EEG electrode artifacts seen in group 1 baboons, microscopy of group 2 baboons revealed neurons with features of the ischemic cell process in the brains of all four. The neurons showed potentially reversible microvacuolation and irreversible ischemic nerve cell change (both with and without incrustations) as described previously for FAM-fixed material (Figure 1). There was some associated swelling of the astrocytes but no recognizable changes in either the microglia or the blood vessels. In three of the four group 2 baboons, the lesions were localized to the boundary zone between the distributions of the right ACA and MCA and achieved their greatest extents particularly in the sides and depths of the intraparietal and parieto-occipital sulci (Figure 2, top left and top right). In one of the three group 2 baboons with arterial boundary zone infarction there was necrosis in the lateral part of the striatum (Figure 2, bottom left); in the fourth baboon there was extensive necrosis in the lateral part of the striatum and in the cortex of the lateral convexity of the right cerebral hemisphere, that is, within the distribution of the MCA (Figure 2, bottom right).

In no baboon were any abnormalities seen in the thalami, hippocampi, or hindbrain. There was no evidence that intracranial pressure had been high.

The mean±SEM weight of the hearts in groups 1 and 2 were 54±5 and 57±7 g, respectively. There were no histologic abnormalities in any organ examined, and in particular the vascular system was entirely normal.

Discussion

Factors that influence the size of an infarct include the functional efficiency of the anastomotic vessels over the surface of the brain, the configuration of the circle of Willis (which is of normal caliber and symmetry in only some 50% of adults) with an even higher incidence of variation noted at post mortem examination in patients with cerebral infarction), disease of the major neck arteries, and systemic arterial blood pressure. If all of these are normal, then the cortical lesion will be small; if one or more are abnormal, then the lesion will be large. Indeed, it has been shown in healthy experimental primates that division of the proximal MCA results in an infarct that may be limited to the internal capsule. In many other experimental models of focal ischemia the lesions have been larger, affecting the cortex, white matter, and basal ganglia, serving to emphasize the importance of two factors in the pathogenesis of cerebral infarction in humans, namely, some impairment of cardiac function and some degree of stenotic and/or occlusive vascular disease. Other factors that influence infarct size after focal cerebral ischemia include the species, glucose concentration, and evidence of preexisting arterial hypertension.

As cerebral infarction is rarely due to a single cause, it is not surprising that several patterns of ischemic brain damage can be identified following occlusion of an ICA. One such pattern is ischemic damage situated along the boundary zones between the distributions of the major cerebral and cerebellar arteries. It has been estimated that some 10% of all cerebral infarcts lie within the arterial boundary zones.

The pathogenesis of infarction within the arterial boundary zones has been variously ascribed to cerebral thromboangiitis obliterans, occlusion of the ICA, and microembolism. There is considerable evidence now, in both humans and experimental animals, that infarction within arterial boundary zones can be attributed to major decreases in cerebral perfusion pressure. The particular vulnerability of the boundary zones is due to the anatomy of the vascular supply of the cerebrum and cerebellum, blood being delivered through large vessels in the neck and ending in a system of small vessels that anastomose with their counterparts from adjacent arterial fields. Once the capacity for autoregulation of blood flow is lost as a result of reduced perfusion pressure, oxygen delivery decreases to a critical level in those parts of each arterial territory that are most remote from the parent stems, that is, the boundary zones.

This pattern of damage has been described in humans after dental anesthesia in a semirecumbent position, after the overzealous treatment of malignant hypertension with antihypertensive agents, following subarachnoid hemorrhage, and in the brains of patients dying of nonmissile head injuries.

Experimental studies in subhuman primates have shown that in the absence of hypoxemia or a significant reduction in blood pH, ischemic brain damage accentuated along the arterial boundary zones can be induced by reducing the cerebral perfusion pressure to 25 mm Hg (3.3 kPa) and maintaining an isoelectric EEG for at least 15 minutes. Under these conditions vasodilatation is maximal, CBF is pressure-dependent, and as a result of the unique anatomy of the cortical arterial supply, blood flow is reduced to a critical level first along the arterial boundary zones. A similar pattern of ischemic brain damage has been induced by Brierley et al in lightly anesthetized baboons spontaneously breathing an O₂/N₂ mixture with a Pao₂ of 22–25 mm Hg (2.9–3.3 kPa) for 20 minutes before occlusion of one and then
FIGURE 1. Photomicrographs from baboon brain. Top left: Ischemic neuronal necrosis in deep layers of cortex of right intraparietal sulcus. Top right: Deep layers of normal cortex of intraparietal sulcus; compare with top left. Both stained with hematoxylin and eosin, ×141. Bottom: Ischemic cell change in pyramidal neurons of deeper layers of cortex from right intraparietal sulcus. Cell bodies are shrunken, and nuclei are triangular and darkly stained. There is microvacuolation of cytoplasm and incrustation formation. Hematoxylin and eosin stain, ×1407.

the other CCA. This pattern of ischemic brain damage has also been induced in baboons with renovascular hypertension subjected to drug-induced hypotension\textsuperscript{10,37} and after the intercarotid injection of air.\textsuperscript{38}

Our principal aim was to determine if hypotension of a degree that is normally well tolerated produces focal cerebral infarction when there is also occlusion of the extracranial arteries. Our study reported previously\textsuperscript{13,39} compared CBF determined with xenon-133 and \textsuperscript{14}C]iodoantipyrine autoradiography. Our present study describes the nature and pattern of the neurohistologic changes in greater detail in fewer animals. A lower MABP limit of 30 mm Hg was
FIGURE 2. Line diagrams of coronal sections of baboon brain showing distribution of ischemic brain damage (speckled). Top left: Carotid occlusion for 390 minutes and graded hypotension for 210 minutes. Ischemic damage is limited to depths of right intraparietal sulcus. Top right: Carotid occlusion for 360 minutes and graded hypotension for 220 minutes. Ischemic damage is present in sides and depths of both superior frontal and intraparietal sulci of right cerebral hemisphere. Bottom left: Carotid occlusion for 440 minutes and graded hypotension for 270 minutes. Ischemic damage is present in sides and depths of superior frontal, intraparietal, and parietooccipital sulci and in upper pole of striatum. Bottom right: Carotid occlusion for 400 minutes and graded hypotension for 210 minutes. Ischemic damage is present throughout distribution of right middle cerebral artery.
chosen because previous studies had shown that brain damage occurred only when the cerebral perfusion pressure was decreased rapidly to < 25 mm Hg and was sustained at this value for at least 15 minutes. In group 1 MABP was reduced to 28 mm Hg and CBF to 28.5 ± 5.0 ml/100 g/min, that is, to a level not expected to produce ischemic damage. Indeed, histologic examination of the brains of group 1 baboons confirmed the complete absence of ischemic damage. In contrast, in group 2 when the same degree of hypotension (27 mm Hg) was superimposed upon unilateral carotid artery occlusion, CBF was reduced to 21.8 ± 1.8 ml/100 g/min in the ipsilateral parietal lobe. At this MABP, CBF is likely to have been inhomogeneous, critical levels being induced only in the sides and depths of the intraparietal and parieto-occipital sulci, that is, in the arterial boundary zones between the distributions of the ACA and MCA. Furthermore, because of the low metabolic requirements (presumably because of anesthesia), the MABP threshold for ischemic damage was probably lower than it would have been in awake baboons or humans. Evidence in support of the localized nature of the critical reduction in CBF is obtained from the EEG, which at no time became isoelectric because of the position of the electrodes on the surface of the hemisphere rather than in the depths of the sulci, corresponding to the arterial boundary zone. In this same model, asymmetric changes in ocular blood flow have also been described.

In one group 2 baboon there was infarction within the distribution of the MCA ipsilateral to the carotid artery occlusion. The exact cause of this pattern of damage is not clear, though presumably it was due to impairment of the anastomotic capacity of the circle of Willis, either singly or in combination with stenosis of the proximal part of the MCA. As arteriography was not undertaken in these animals and in the absence of any record of gross anomalies of the circle of Willis, the exact etiology of the pattern of cerebral infarction seen in this baboon is not clear.

Our results in subhuman primates indicate that, when combined with hypotension, unilateral carotid artery occlusion may lead to hemodynamic ischemia accentuated in the arterial boundary zones of the ipsilateral cerebral hemisphere.

References

28. Brierley JB: Ischameric necrosis along brain arterial boundary zones: Some aspects of its etiology, in Fahn S, Davis JN,


KEY WORDS • carotid artery diseases • cerebral blood flow • hypotension • baboons
Neuropathologic consequences of internal carotid artery occlusion and hemorrhagic hypotension in baboons.
D I Graham, A D Mendelow, U Tuor and W Fitch

doi: 10.1161/01.STR.21.3.428

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/21/3/428

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/