A Modified Transorbital Baboon Model of Reperfused Stroke

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Background and Purpose—Although pathophysiological studies of focal cerebral ischemia in nonhuman primates can provide important information not obtainable in rodent models, primate experimentation is limited by considerations of cost, availability, effort, and ethics. A reproducible and quantitative model that minimizes the number of animals necessary to detect differences between treatment groups is therefore crucial.

Methods—Eight male baboons (weight, 22 ± 2 kg) underwent left transorbital craniectomy followed by 1 hour of temporary ipsilateral internal carotid artery occlusion at the level of the anterior choroidal artery together with bilateral temporary occlusion of both anterior cerebral arteries (A1) proximal to the anterior communicating artery. A tightly controlled nitrous oxide–narcotic anesthetic allowed for intraoperative motor evoked potential confirmation of middle cerebral artery (MCA) territory ischemia. Animals survived to 72 hours or 10 days if successfully self-caring. Outcomes were assessed with a 100-point neurological grading system, and infarct volume was quantified by planimetric analysis of both MRI and triphenyltetrazolium chloride–stained sections.

Results—Infarction volumes (on T2-weighted images) were 32 ± 7% (mean ± SEM) of the ipsilateral hemisphere, and neurological scores averaged 29 ± 9. All animals demonstrated evidence of hemispheric infarction, with damage evident in both cortical and subcortical regions in the MCA vascular territory. Histologically determined infarction volumes differed by <3% and correlated with absolute neurological scores (r=0.9, P=0.003).

Conclusions—Transorbital temporary occlusion of the entire anterior cerebral circulation with strict control of physiological parameters can reliably produce reperfused MCA territory infarction. The magnitude of the resultant infarct with little interanimal variability diminishes the potential number of animals required to distinguish between 2 treatment regimens. The anatomic distribution of the infarct and associated functional deficits offer comparability to human hemispheric strokes. (Stroke. 2000;31:3054-3063.)

Key Words: baboons ■ cerebral ischemia ■ disease models, animal ■ reperfusion

The use of various rodent and large-animal models has contributed significantly to our current understanding of the pathophysiological mechanisms underlying focal cerebral ischemia. In addition, recent strides in transgenic mice technology have enabled the systematic and targeted study of isolated gene products as they pertain to tissue damage in cerebral ischemia. Unfortunately, efforts to translate many of these pathophysiological findings to the clinical realm have failed, in part because of a growing realization of significant species-specific differences in responses to ischemic injury. Nonhuman primate models offer distinct advantages when it comes to comparability with humans, particularly when developing clinically useful strategies to improve patient outcome after stroke. Functional deficits in nonhuman primates can be easily assessed before animal euthanasia and represent clinically relevant outcome measures that are limited with rodent subjects. In addition, both ratios of white matter to gray matter and the degree of microvascular collaterals are more comparable, especially between baboons and humans. Cerebral hemispheric and brain stem blood flow measurements in baboons have been extrapolated to humans with the use of 133Xenon inhalation techniques. Finally, humanized antibodies can be tested in primates and effective dosing determined with greater accuracy.

As with experimental rodent stroke models, primate models need to be reproducible and to minimize interanimal variability. This is especially crucial in the context of the tremendous cost and effort, restricted availability, and ethical considerations associated with primate studies. Until recently,
these considerations were incompletely addressed by primate models of middle cerebral artery (MCA) occlusion, which were plagued not only by considerable interanimal variability in terms of infarct size but often by the exclusion of significant numbers of animals from analysis, qualitative rather than quantitative outcomes, suboptimal imaging of infarcted tissue, and perhaps most importantly, extremely small infarcts in the basal ganglia that may be pathophysiologically distinct from the combined cortical and subcortical human infarction seen with many hemispheric stroke syndromes.

In an effort to address several of these issues, we modified a model of reperfused stroke that uses, via a transorbital approach, unilateral internal carotid artery (ICA) and ipsilateral and contralateral anterior cerebral artery (A1) occlusion. By limiting collateral variability to the posterior circulation alone, we hypothesized that larger, more consistent infarction would occur, thereby allowing experiments involving fewer animals to achieve statistically significant results. In addition, experiments examining the pathophysiology of hemispheric cortical infarction along with the reperfusion injury seen in well-collateralized tissues might also be examined.

### Materials and Methods

#### Animals

Eight adult male baboons (Papio anubis, Biomedical Resources Foundation) with a mean body weight of 22.1±2.1 kg (range, 13 to 31 kg) were included in this series. Mean hematocrit was 40.5±1.0%, and total white blood cell count was 8.2±1.0×10^3 cells per deciliter. All animals were observed for 2 consecutive 45-day quarantine periods to be certified as free of disease and neurologically normal before use. All animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee and performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

#### Anesthesia

Awake animals were initially anesthetized with ketamine (Fort Dodge Animal Health) at an intramuscular dose of 5 mg/kg. The head, neck, forearm, and femoral areas were shaved with an electric clipper. Two 18- or 20-gauge peripheral venous catheters were placed, and intravenous fluid administration of 0.9% normal saline was begun. Propofol (Zeneca Pharmaceutical) was given as a bolus infusion before oropharyngeal intubation with a size 6 or 7 endotracheal tube. Animals were transferred to an operating room where hair of the head, neck, forearm, and femoral areas was shaved with an electric clipper. Two 18- or 20-gauge peripheral venous catheters were placed, and intravenous fluid administration of 0.9% normal saline was begun. Propofol (Zeneca Pharmaceutical) was given as a bolus infusion before oropharyngeal intubation with a size 6 or 7 endotracheal tube. Animals were transferred to an operating room where

#### Physiological Monitoring

An intra-arterial catheter was introduced into the femoral artery to provide continuous systemic blood pressure monitoring and to facilitate multiple blood specimen collections. Blood pressure was monitored (Datascope) to maintain a mean arterial pressure of 60 to 80 mm Hg. Hypotensive responses were treated with intravenous bolus injections of phenylephrine hydrochloride (Gensia Laboratories). Central venous pressures were monitored via a femoral vein catheter (Arrow International) and sustained at 5±2 mm Hg. An indwelling, transurethral Foley catheter (Baxter) permitted monitoring of urinary output to guide management of fluid balance and central venous pressures.

Arterial blood gas analysis was performed at regular intervals (Stat Profile 3, Nova Biomedical), and the respiratory rate and tidal volume were adjusted to keep PCO2 between 35 and 40 mm Hg. Monitoring of core body temperatures with an esophageal probe (Datascope) and of the brain with a parenchymal probe (Mon-a-Therm 70B, Mallinckrodt Medical) allowed body temperatures to be maintained at approximately 37°C with a warm air heating blanket (Mallinckrodt Medical). Continuous intracranial pressure (ICP) monitoring was accomplished with a parenchymal sensor (Neuro-monitor, Codman). Sustained ICP of >20 mm Hg for >5 minutes was the indication for treatment with mannitol at a dose of 0.5 g/kg, administered as an intravenous bolus infusion.

Before intubation and the administration of anesthesia, baseline complete peripheral blood cell counts were performed. Systemic blood pressure, central venous pressure, cerebral perfusion pressure, and core and brain temperatures were maintained at constant levels throughout the operative procedures and during the first 24 hours of reperfusion. During ischemia, ventilation was adjusted so that PCO2 was maintained at levels similar to those at baseline. These are summarized in Table 1.

#### Motor Evoked Potentials

Motor evoked potentials (MEP) were monitored by applying transcortical electric stimulation to the motor cortex and recording compound muscle action potentials from the forelimbs. Stainless steel needle electrodes were placed bilaterally into the scalp overlying the posterior frontal regions and at the vertex for stimulation and into the forearm extensor and flexor muscles for recording. Controlled, partial neuromuscular blockade was maintained by titrating the vecuronium infusion rate so that a train of 4 supramaximal stimuli delivered to the median nerve at 2 Hz produced only 2 thumb twitches. Stimulation was performed by delivering series of three 50- to 70-mA 0.2-millisecond pulses at 2-millisecond interpulse intervals between vertex (cathode) and ipsilateral scalp electrodes. Compound muscle action potentials recorded after 5 to 20 such trains were averaged to produce the MEP (Viking 2E Electrodiagnostic System, Nicolet Biomedical).

### TABLE 1. Physiological Characteristics at Baseline, During Arterial Occlusion, and During Reperfusion

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Occlusion</th>
<th>Reperfusion</th>
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<tbody>
<tr>
<td>Weight, kg</td>
<td>22.1±2.1</td>
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<td>...</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>61.4±3.3</td>
<td>63.4±3.9</td>
<td>65.4±2.5</td>
</tr>
<tr>
<td>CPP, mm Hg</td>
<td>63.2±5</td>
<td>63.7±6.2</td>
<td>49.3±3</td>
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<tr>
<td>ICP, mm Hg</td>
<td>0.9±0.5</td>
<td>1.7±1.7</td>
<td>16.1±1.9*</td>
</tr>
<tr>
<td>pH</td>
<td>7.48±0.03</td>
<td>7.46±0.02</td>
<td>7.45±0.06</td>
</tr>
<tr>
<td>Pco2, mm Hg</td>
<td>36.6±0.6</td>
<td>36.8±2.2</td>
<td>30.3±2</td>
</tr>
<tr>
<td>Brain temperature, °C</td>
<td>36.7±0.2</td>
<td>36.4±0.5</td>
<td>37±0.1</td>
</tr>
<tr>
<td>Esophageal temperature, °C</td>
<td>36.5±0.2</td>
<td>36.8±0.2</td>
<td>36.9±0.1</td>
</tr>
<tr>
<td>CVP, mm Hg</td>
<td>0±1.7</td>
<td>2.5±2.5</td>
<td>1.9±1</td>
</tr>
</tbody>
</table>

MAP indicates mean arterial blood pressure; CPP, cerebral perfusion pressure; and CVP, central venous pressure.

*P<0.05, but baseline and occlusion ICP values are measured with the dura opened.
As the transorbital approach was begun, the isoflurane concentration was decreased to permit the recording of stable, consistent MEP (typically 0.1% to 0.2%), and preischemic baseline MEP were obtained. Adequacy of cerebral ischemia was ascertained by stimulating the ipsilateral ischemic hemisphere and noting contralateral limb MEP dropout with the use of stimuli strong enough to also stimulate the contralateral (nonischemic) hemisphere and produce ipsilateral limb MEP.

Operative Technique

Positioning

After the insertion of all indwelling catheters and before placement of the ICP monitor, brain temperature probe, and MEP electrodes, animals were positioned prone in an adjustable stereotaxic frame, with 2-point head fixation via the external auditory canals. The skull base was positioned parallel to the floor, with the anterior skull base was elevated approximately 15° and turned slightly to the right. Dependent pressure points were padded to prevent tissue necrosis. Subdermal scalp and MEP recording electrodes were inserted before sterile draping of the operative field was accomplished.

Placement of ICP Monitor

A right frontal approach via a paramedian linear skin incision and burr hole was used for insertion of the intraparenchymal ICP monitor and brain temperature probe. A hand-held twist drill (Neurocare) was used to create the skull opening. The dura was cauterized and sharply incised to allow passage of the fiberoptic pressure sensor and temperature probe. The wound was sutured closed.

Transorbital Approach

Infiltration of the medial and lateral canthi of the left orbit with 0.5% lidocaine with epinephrine 1:100,000 (Abbott Laboratories) was performed before their incision in the plane of the palpebral fissure. A self-retaining lid retractor was placed. An 18-gauge needle was inserted into the anterior and posterior chambers of the globe for aspiration of the vitreous and aqueous humors. This internal decompression of the globe allowed it and the periocular soft tissues to be circumferentially dissected from the orbital walls. Transection of the optic nerve and ophthalmic artery enabled removal of the globe. The residual periorbital fat and extracocular muscles were removed with additional bipolar cauterization and curettage. The operating microscope (Super-Lux 40 to 2 Illuminator, Zeiss) was used for the remainder of the procedure. The high-speed pneumatic drill with a coarse diamond bit (Midas Rex) was used to remove the bone of the postero medial orbit. The dura of the anterior cranial fossa was incised with a No. 11 blade, and the dural edges were cauterized to reveal the anterior circle of Willis.

Vessel Occlusion

Microvascular technique was used to identify the cerebral arteries and clear them from their surrounding arachnoid membranes. After reconfirmation of the stability of the physiological variables, vessel occlusion was accomplished by the sequential placement of 3 micro-Yasargil (Aesculap) aneurysm clips: (1) on the proximal segment of the left anterior cerebral artery, proximal to the anterior communicating artery (left A1), (2) on the proximal right anterior cerebral artery (right A1), and (3) across the left ICA at the level of the anterior choroidal artery so that the clip incorporated and occluded the anterior choroidal artery (left ICA). Aneurysm clip placement is demonstrated in Figure 1.

Vessel occlusion was sustained for a test period while a series of MEP were elicited bilaterally to confirm impairment of left hemisphere electric activity. Failure of the MEP to fall constituted exclusion criteria for the animal, since this suggested an intrinsic resistance to ischemic insult, and exclusion would thereby prevent the inclusion of an animal with anatomic variation of its cerebral vasculature. If there was a fall in the MEP after the trial occlusion period, the 3 aneurysm clips were removed, and the hemisphere was allowed to reperfuse until the MEP were observed to return to baseline. The 3 aneurysm clips were then replaced for 1 hour of vessel occlusion. Once again the animal’s MEP were monitored to confirm ipsilateral ischemia. After the 1-hour period of occlusion, the clips were removed to permit reperfusion. A layer of gel foam was placed over the dural defect, and the retractor was removed. Radiolucent methylmethacrylate (Codman Cranioplastic, Johnson & Johnson) was used to fill the orbital defect, and the eyelids were sutured closed with a running 3-0 nylon.

Postoperative Care

Animals were removed from the surgical head frame and placed supine on a padded mattress with 30 degrees of head elevation. The dose of fentanyl was lowered to 20 µg/kg per hour, and the isoflurane was increased to 0.1% to 0.6%. The nitrous oxide was replaced with a balanced air and oxygen mixture. The animals remained intubated and sedated with continuous monitoring by a trained member of the operative team for the first 18 hours of reperfusion. Vecuronium and midazolam were continued. Physiological parameters including ICP, central venous pressure, PCO₂, core and brain temperature, blood pressure, and pH were closely regulated during this period. Sustained ICP >20 mm Hg was treated with intravenous infusions of mannitol (0.5 g/kg) as required. Pulmonary toilet was achieved with suctioning and chest physical therapy. By 18 hours of reperfusion, the inhalation anesthetic and narcotic agents were tapered, and the baboons were permitted to regain consciousness. If the arterial blood gases demonstrated satisfactory gas exchange (PCO₂ <45, PO₂ >95) without assisted respiration, all of the indwelling catheters and monitors were removed to allow extubation and return to housing cages for observation in the intensive care unit. Animals were monitored for their continued ability to self-care, eat, and drink. The wounds were examined for signs of infection.

Neurological Evaluation

Daily neurological assessments were performed by 2 investigators blinded to all imaging data using a 100-point neurological scale developed by Spetzler and associates,18 with higher scores reflecting better neurological function. Motor function was graded from 1 to 75, according to severity of hemiparesis in the extremities (10 = severe, 25 = mild, 55 = favors normal side, and 70 = normal) and face (1 = facial paresis and 5 = normal facial strength). Behavior and level of alertness were scored from 0 to 20 (0 = dead, 1 = comatose, 5 = aware but inactive, 15 = aware but less active, and 20 = normal), and visual field deficits were assigned 1 if present or 5 points if absent.

Animals were assigned an absolute score based on the maximum score of 100 if neurological evaluation was possible in all functional categories. To account for the inability to assess cranial nerve function in animals that were more severely impaired from the

Figure 1. Transorbital approach demonstrating placement of occlusive aneurysm clips on the ICA and both anterior cerebral arteries (A1 segments).

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ischemic insult, a relative neurological score was also calculated by expressing the absolute score as a percentage of the corrected maximum score of 90, which eliminated the possible 5 points each for full visual fields and intact facial nerve function from the denominator.

Radiographic Imaging
After 48 to 72 hours of reperfusion, animals were anesthetized with ketamine and sedated with an intravenous pentobarbital bolus and propofol infusion that was titrated to allow independent respiratory function for up to 6 hours while the airway was maintained with the endotracheal tube. Brain MRI was performed (Signa Advantage 1.5 T, General Electric) at this “early” time point, with the acquisition of coronal T2-weighted, gradient echo, diffusion/perfusion, fluid activation inversion recovery (FLAIR), and MR angiography sequences. The T2-weighted images were acquired with a slice thickness of 3 mm and without intervening space between images (Figure 2). If the animal’s neurological function score was >25 and the animal was deemed to be self-caring by the veterinarian, the animal was allowed to survive to day 10, at which time a repeated, “late” MRI was obtained before the animal was killed. Animals were euthanatized with an intravenous injection of pentobarbital (Veterinary Laboratories) at 72 hours of reperfusion if the neurological score was <25 or if the veterinarian determined that continued survival would be unethical secondary to devastating functional impairment.

Infarct Volume
Brains were removed intact with surrounding dura. Three coronal sections of 5-mm thickness were collected from the ischemic ipsilateral and stereoanatomically equivalent, normal contralateral hemispheres. The first section was obtained from the medial portion of the most posterior aspect of the precentral gyrus and immersed in a solution of 2% 2,3,5-triphenyltetrazolium chloride (TTC) (Sigma) in 0.9% phosphate-buffered saline for histological confirmation of infarct location and correlation with MRI. Additional sections were obtained immediately anterior and posterior to the initial section and embedded in Tissue-Tek compound for further histological processing. Infarcted tissue was visualized as nonstained portions of brain.19,20

Infarct volume was determined by 2 different blinded observers. Areas of ischemic damage showed high signal intensity on the T2-weighted images. With the use of commercially available graphics software Adobe Photoshop 4.0 and NIH Image 1.61 (National Institutes of Health), infarction volume was quantified by planimetric analysis and expressed as the percentage of the total volume of the ipsilateral hemisphere.

Statistical Analysis
Values are expressed as mean±SEM. Comparisons between means and groups were performed with the 2-tailed Student’s t test and 1-way ANOVA, respectively. Statistical significance is defined by probability value <0.05.

Results
Morbidity and Mortality
Each animal underwent MCA territory ischemia via the left transorbital approach for vessel occlusion without intraoperative complications. At 12 to 18 hours of reperfusion, 6 of the 8 animals were successfully weaned and extubated. Two animals (animals 1 and 4, Table 2) remained dependent on ventilatory support; they were scanned and killed at 72 and at 48 hours, respectively. The earlier euthanasia was performed to obtain brain tissue for histological analysis after a shorter period of reperfusion. One other animal (animal 5) was killed at 48 hours, also for issues pertinent to tissue harvest. This animal was initially extubated at 18 hours of reperfusion and was self-caring before euthanasia and harvest. Two animals (animals 2 and 7) were followed until day 10 because of good neurological function. The remaining animals (animals 3, 6, and 8) were killed at the 72-hour reperfusion time point in accordance with established ethical guidelines because they were unable to self-care.

Motor Evoked Potentials
No animal was excluded on the basis of MEP criteria. Confirmation of focal ischemia was established by contralateral MEP decline in each case. This occurred at a mean of 4.9±0.7 minutes during the initial test period of vessel occlusion. Normalization of MEP occurred at 12.5±3.3 minutes during the initial reperfusion period, thereby demonstrating the reversible nature of the ischemic insult when the duration of vessel occlusion was brief. The decrement in MEP during the subsequent 1-hour occlusion period occurred at 6.0±1.3 minutes, which is similar to the amount of time required for the MEP decline (P=NS) during the initial trial period. A representative MEP tracing is shown in Figure 3.

Neurological Impairment
The baseline neurological score of this cohort was 100±0. Early in the reperfusion period, from 24 to 72 hours, the absolute score was 29±9 and the relative neurological score was 30±9, revealing that there were minimal differences...
between the absolute and relative neurological scores. At 72 hours of reperfusion, neurological function correlated significantly with infarct volume (Figure 4), with the use of either the absolute or relative neurological scores ($r = 0.89$, $P = 0.003$ or $r = 0.88$, $P = 0.004$). This correlation was sustained even when prolonged survival was included ($r = 0.85$, $P = 0.007$ and $r = 0.85$, $P = 0.008$). Individual scores are listed in Table 2.

**Infarct Volumes**

Early brain MRI scans were obtained at 48 hours of reperfusion ($n = 2$) and at 72 hours ($n = 6$), while late MRI scans were obtained at 10 days of reperfusion ($n = 2$). No intracerebral hemorrhages were detected on review of the T2-weighted, gradient echo, and FLAIR images by an independent neuroradiologist. The T2-weighted images demonstrated hyperintense signal characteristics in areas of cortical, subcortical white matter, and basal ganglia infarction. At early reperfusion, the infarct volume was 32±7% ($n = 8$). In the subgroup of animals that survived to 10 days of reperfusion ($n = 2$), late infarct volume was 9±8%, which was not significantly different from the early infarct volume in this cohort (13±9%). No correlation was found between infarct volume and the weight of the animal. Measurements of infarct volumes correlated closely between 2 independent observers and varied by only 4.3±0.7%.

Reperfusion after clip removal was observed intraoperatively in all animals by direct visualization of vessel patency, blood flow, and lack of focal vasospasm. In

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**Table 2. Early and Late Infarct Volumes and Neurological Function Scores for Individual Baboons**

<table>
<thead>
<tr>
<th>Baboon No.</th>
<th>Self-Caring*</th>
<th>Infarct Volume†</th>
<th>Neurological Score‡</th>
<th>% Ipsilateral Hemisphere</th>
<th>cm³</th>
<th>Early §</th>
<th>Late</th>
<th>Absolute</th>
<th>Relative</th>
<th>Early</th>
<th>Late</th>
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<tr>
<td>Mean±SEM</td>
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<td>32±7</td>
<td>9±7</td>
<td>28±6</td>
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<td>63±31</td>
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<td>63±31</td>
<td>31±30</td>
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*Animal judged by veterinarian to meet institutional criteria to allow survival to day 10 time point ("late"); Spetzler score ≥25.
†As measured by volumetric T2-weighted MRI.
‡As measured by Spetzler 100-point scale. 18
§Early = 48 to 72 hours after operation.
∥Late = 9 to 10 days after operation.
¶Animal was neurologically stable and met criteria for late survival but was killed early for histological analysis.
addition, reperfusion was demonstrated on MR angiography by filling of the anterior cerebral arteries and MCAs distal to the sites of occlusion (Figure 5). At brain harvest, no gross evidence of intracerebral hemorrhage was detected. Comparison was made between the TTC-stained gross sections and the corresponding coronal MR image to identify a pathological correlate for the radiographic image of infarcted brain tissue. These 2 methods yielded infarct volumes with excellent correlation, with a difference of only 2.5 ± 0.5% between the infarct volumes identified by these different strategies.

Discussion

While detailed studies of cerebral injury are possible in rodents and are critical in helping to elucidate mechanisms (especially with the availability of several relevant genetically modified strains), the pathophysiology of disease may exhibit considerable interspecies variability. In the context of stroke, it has historically not been uncommon for a therapeutic agent conferring cerebral protection in rodents to fail to translate into clinical benefit.11,22 While this may be due in part to deficient clinical study design, several investigators have pointed to the considerable degree of interspecies variability in cerebrovascular physiology.

To address this issue, several models of primate stroke have been reported, dating back to the early 1970s (Table 3).18,22–34 Most of these are based on occlusion of the MCA in either macaques or baboons and use several techniques, including the following: intraluminal M1 embolization,26,31,35 extraluminal M1 ligation,25 lenticulostriate interruption,28–30 and either permanent or temporary M1 clipping.22,23,27,32–34 Unfortunately, the vast majority of the reports before 1980 involved qualitative assessments of neurological dysfunction and infarct volume, making assessment of their utility difficult. It was, however, evident from these early efforts that embolization/ligation techniques and lenticulostriate interruption had major limitations when it came to elucidating not only the pathophysiology of stroke but also the effect of therapeutic manipulations.25,26,28–31,35 Equally apparent was the fact that animal losses on the order of 20%, seen with some efforts at permanent M1 occlusion, were simply unacceptable.23

In an effort to improve matters, Spetzler et al18 reported in 1980 on 12 animals subjected to variable durations of cerebral ischemia using an inflatable cuff placed on M1. Advantages of this technique included the ability to induce ischemia in awake animals. In addition, that report contained the first quantitative system for scoring neurological deficits that showed a high degree of correlation with the degree of cerebral tissue damage. Although other groups have made minor modifications in this model, resulting in several important pathophysiological observations,24 the ability of this model to yield useful data on putative therapeutic strategies has been severely limited by small infarct size, tissue damage restricted to the basal ganglia, and marked interanimal variability (many animals fail to experience stroke, while others die), likely resulting from the inconstant nature of collateral blood flow in unanesthetized animals.18,24,27,31,36–38 In addition, subsequent work has called into question the actual degree of reflow obtained on release of the occluder and the accuracy and reproducibility of early methods of infarct volume analysis by CT or histological analysis alone. Although histopathological detection of infarcted tissue is reliable, it is an impractical method of calculating total infarct volume. Faced with these shortcomings, we modified a triple-vessel occlusion model developed by Zabramski and coworkers,39–42 on which the model presented here is based, while others have developed models of acute focal ischemia in territories other than the MCA: the anterior cerebral,43–45 posterior choroidal,46,47 or proximal basilar arteries.48 While these other models have added much to the understanding of primate vascular anatomy, they have generally been considered inferior for a variety of reasons. The use of electric coagulation to create occlusion of the lenticulostriate arteries29 or the posterior choroidal artery46 was associated with potential damage to adjacent cerebral tissue. The unpredictable localization of embolic material within MCA branches resulted in variable deep or cortical infarcts.31 In addition, outcomes were highly variable if unanesthetized monkeys were used.25

In contrast, we believe that this new model of reperfused MCA territory stroke involving occlusion of the ICA and both anterior cerebral arteries (A1 segments) for 1 hour has several easily discernible advantages. Unlike previously established nonhuman primate models that deliver isolated basal ganglia,29,49 subcortical,27,44–47 or brain stem58,59 infarcts, this model of MCA occlusion results in infarction of both cortical and subcortical gray matter as well as subcortical white matter (Figure 2). This is technically possible because of the exclusion of collateral circulation contributed by the anterior cerebral arteries. This triple-vessel occlusion was principally designed to reduce collateral circulatory variability via the luxuriant semiazygous anterior cerebral artery43 and the robust anterior-posterior connections provided by the anterior communicating and choroidal arteries. This strategy has enabled us to create larger and more consistently sized cerebral infarcts involving cortex, white matter, and nuclear structures in the MCA distribution.

Figure 5. MR angiography demonstrated restoration of blood flow distal to the sites of vessel occlusion after clip removal. Normal filling of the ipsilateral M1 (small arrow) distal to the site of ICA occlusion and of the contralateral A1 and distal A2 segments (large arrows) distal to the bilateral A1 segment clips are shown. Patency of bidirectional flow leads to turbulence in the ipsilateral A1 segment (arrowhead).
Achieving consistent hemispheric infarcts is challenging because of the dependence of cortical tissue on highly variable pial-pial collateral blood flow, which contrasts with the dependence of the basal ganglia on end-arteries for its blood supply. The 1-hour duration of vessel occlusion produced large areas of ischemia, but they were not severe enough to incite hemorrhagic transformation of the infarcts.

We have added further consistency and reproducibility to this model by ensuring the adequacy of the ischemic insult with MEP and a careful neuroanesthetic protocol that does not use barbiturates, which may have a neuroprotective effect of their own, so that cerebral/core temperature, partial pressure of carbon dioxide, cerebral perfusion pressure, and central venous pressure are tightly controlled.

We believe that this model is a significant improvement over previously published models because larger, more consistent infarcts will permit a reduction in the number of animals that need to be tested to establish the efficacy of a given therapeutic agent/strategy. For instance, when compared with the only other baboon study of temporary MCA occlusion in which neither animal exclusion nor quantitative outcome assessment was an issue, we see both a 4-fold increase in infarct size and a 41% reduction in the predicted number of animals needed to demonstrate a 50% reduction in infarct size. Economically this translates into a savings of nearly $200 000 over the course of a study (power=0.90, \( \beta=0.10, \alpha=0.05 \)).

In addition to the statistical advantages presented by this model, there are pathophysiological advantages because the type of ischemia and reperfusion it creates more closely mimics that seen with major hemispheric infarction in humans. This is in contrast to the lacunar or basal ganglia infarction seen with cuff-occluder infarcts. Incorporation of cortical areas into the experimentally produced regions of ischemic injury confers greater comparability to the type of human stroke that is encountered in clinical practice, for which understanding the underlying pathophysiological mechanisms governing the penumbra would most likely result in patient benefit. This model exemplifies reperfused MCA territory infarction, as demonstrated by both the swift return of MEP and the normal filling of the anterior cerebral arteries and MCAs on MR angiography when vessel occlusion was terminated. Therefore, it provides a substrate for the

<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>Primate Species</th>
<th>Technique*</th>
<th>No. Studied</th>
<th>No. Infarct, %</th>
<th>Died/Excluded, %</th>
<th>Infarct Size, cm³ or % of Hemisphere</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molinari et al (1974)</td>
<td>Macaque</td>
<td>M1 embolization (P)</td>
<td>16</td>
<td>20</td>
<td>‡</td>
<td>‡</td>
</tr>
<tr>
<td>Crowell et al (1981)</td>
<td>Macaque</td>
<td>M1 ligature (T: variable; awake)</td>
<td>38</td>
<td>26</td>
<td>29</td>
<td>‡</td>
</tr>
<tr>
<td>Yonas et al (1981)</td>
<td>Baboon</td>
<td>Lenticulostriate coagulation (P)</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>‡</td>
</tr>
<tr>
<td>Permanent M1 clipping</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hudgins and Garcia (1970)</td>
<td>Squirrel</td>
<td>Proximal M1 clip (P)</td>
<td>16</td>
<td>0</td>
<td>6</td>
<td>‡</td>
</tr>
<tr>
<td>Symon (1975)</td>
<td>Baboon</td>
<td>Proximal and distal M1 clips (P)</td>
<td>10</td>
<td>‡</td>
<td>20</td>
<td>‡</td>
</tr>
<tr>
<td>Temporary M1 balloon occluder (awake)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spetzler et al (1980)</td>
<td>Baboon</td>
<td>M1 cuff (T: variable)</td>
<td>12</td>
<td>66</td>
<td>0</td>
<td>‡</td>
</tr>
<tr>
<td>del Zoppo et al (1986)</td>
<td>Baboon</td>
<td>M1 cuff (T: 3 h)</td>
<td>6</td>
<td>0</td>
<td>CT: 16 Pathology: 33</td>
<td>CT: 3.2–1.5 cm³ Pathology: 3.9–1.9 cm³ 1.4–5.4 cm³</td>
</tr>
<tr>
<td>Temporary M1 clipping</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crowell et al (1970)</td>
<td>Macaque</td>
<td>Proximal M1 alone (T: variable)</td>
<td>43</td>
<td>21</td>
<td>9</td>
<td>‡</td>
</tr>
<tr>
<td>Henry and Chandy (1998)</td>
<td>Macaque</td>
<td>Proximal M1 alone (T:4 h)</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>TTC: 22.1±6.7% 5.5–44.1%</td>
</tr>
<tr>
<td>Frazee et al (1998)</td>
<td>Baboon</td>
<td>Proximal M1 alone (T: 3.5 h)</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>Pathology: 8.8±6.9% 2.5–17%</td>
</tr>
<tr>
<td>Young et al (1997)</td>
<td>Baboon</td>
<td>Proximal M1 and orbitofrontal (T: 6 h)</td>
<td>11</td>
<td>40</td>
<td>9</td>
<td>Pathology: 0.58±0.31 cm³ 0–0.9 cm³</td>
</tr>
<tr>
<td>Temporary ICA-ACA (×2) clipping</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Huang et al (2000)</td>
<td>Baboon</td>
<td>Bilateral A1/distal ICA clips (T: 1 h)</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>MRI: 32±19% MRI: 4–59% TTC: 4–52 cm³</td>
</tr>
</tbody>
</table>

ACA indicates anterior cerebral artery; N/a, not applicable due to lack of quantitative data or exclusion of animals.

*Length of occlusion either permanent (P) or transient (T).

†Power analysis using study’s infarct size (mean±SD) to estimate the number of animals required per cohort to detect a 50% reduction in infarct size (power=0.90, \( \beta=0.10, \alpha=0.05 \)).

‡Not assessed in study.
§Based on 100-point scale.†
||Not based on volumetric analysis.
elucidation of the pathophysiological mechanisms that underlie reperfusion injury and does so in a cerebral several times larger than that of the macaque.

Finally, our use of MRI in living animals to detect infarcts is superior to CT and postmortem imaging because of its greater anatomic resolution and ability to detect ischemic cerebral tissue injury shortly after the insult. Enough time has elapsed by 48 to 72 hours of reperfusion to permit the detection of infarcted cerebral tissue on MRI scans; we have confirmed this by histological analysis with TTC staining, which offers not only more obvious direct visualization of infarcted tissue compared with hematoxylin and eosin staining of whole brain sections but is also easier to perform. Performing MRI with a small slice thickness approximates a true volumetric determination of infarction volume. Furthermore, in using MRI scanning techniques identical to those performed on humans and managing these large infarcts with the identical intensive care unit medical protocols used in humans, successful experimental reductions in infarct volume and the highly correlated functional deficits produced by this model can more likely be translated into the clinical arena.

Conclusion

Temporary aneurysm clip occlusion of both A1 segments and the ICA at the level of the choroidal artery for 1 hour together with evoked potential confirmation of ischemia allows for large hemispheric infarcts involving both cortical and subcortical regions. All animals demonstrate infarction, but with aggressive intensive care unit management all can be kept alive for 72 hours, and one third recover their ability to self-care. Infarct volumes as determined by MRI and neurological deficit scores correlate well with one another and with histological evidence (TTC) of tissue injury. Compared with M1 occlusion alone, this model appears to be associated with a 4-fold increase in the degree of tissue damage and predicts a 40% reduction in the number of animals needed to demonstrate cerebral protection via a therapeutic strategy that reduces infarct volume by 50%.

Acknowledgments

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14. Polmar SH, Sherman DS. Double-blind, randomized, placebo-controlled, parallel-group trial of the efficacy and safety of enlimomab (anti-ICAM-1) compared to placebo administered within 6 hours of the onset of symptoms for the treatment of acute ischemic stroke. In: Programs and abstracts of the

<table>
<thead>
<tr>
<th>Neurological Examination</th>
<th>No. per Cohort</th>
<th>(50% Reduction)†</th>
</tr>
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<tr>
<td>Qualitative</td>
<td>N/a</td>
<td></td>
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<tr>
<td>Graded 0–3</td>
<td>N/a</td>
<td></td>
</tr>
<tr>
<td>Qualitative</td>
<td>N/a</td>
<td></td>
</tr>
<tr>
<td>Graded 0–4</td>
<td>N/a</td>
<td></td>
</tr>
<tr>
<td>66±14§</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>29±6§</td>
<td>MRI: 15</td>
<td></td>
</tr>
</tbody>
</table>

TABLE 3. Continued


21. Boehringer Ingelheim drops enlimomab for stroke. SCRIP World Pharm. 1993;97, No. 500534360F.


Editorial Comment

The gold standard of a good experimental model for focal ischemic stroke, regardless of the species or strain, is to achieve highly reproducible infarction volume, as determined by histopathologic analysis of brain tissue. If, in addition, brain injury evaluated histologically could be successfully validated by noninvasive MRI and neurological impairment quantified on a sensitive motor-deficit scale, as in the study by Huang et al, it should be generally accepted that the model is well characterized functionally and may have excellent potential for development of clinically useful strategies to improve patient outcome after stroke.

Over last 30 years, numerous models in rodents and nonhuman primates have been developed. These different models have helped us in many different ways to understand a complicated pathophysiological cascade of ischemic tissue damage and cellular and molecular mechanisms implicated in neurovascular injury during cerebral ischemia/hypoxia. The focus of many studies has been to uncover new neuroprotective approaches that could be successfully applied in clinical trials in stroke patients. Stroke agents that may experimentally curb ischemic neuronal damage include “clotbusters” (ie, tissue plasminogen activator), antagonists of calcium channels, anti-inflammatory drugs, and agents that may antagonize cell death, to name a few.

Unfortunately, a number of experimental stroke drugs have proved ineffective in human trials. This has raised the question of whether pathophysiological changes and the time course of neurovascular injury in an animal model are different from those in humans. Although the most obvious explanation for the failures is that humans often get to the hospital when it may simply be too late (eg, most of the trials have involved drugs as late as 6 hours after a stroke), the possibility that species-related and even strain-related differences may also play a role cannot be ruled out.

It is well known that the same surgical procedure for focal or global ischemia may produce very different neuropathological outcome in different strains of mice and rats. In some cases, this can be attributed to differing circulatory patterns that vary greatly between different strains of a given species; in other cases, however, no obvious vascular difference can be identified, but the genetic susceptibility to ischemic neuronal injury simply may vary for reasons not yet well understood (as, for instance, between C57BL/6 background and mixed 129Sv and C57BL6/6 mice). The strain variability in response to ischemic challenge should in no way discourage studies in different transgenic and knockout mouse stroke models, but the importance of using genetically matched control mice cannot be overemphasized.

The present stroke model in baboons utilizes transorbital approach and the 3-vessel occlusion model; ie, the internal carotid artery and both anterior cerebral arteries are temporarily occluded to limit collateral vascular variability to the posterior circulation alone. The authors have reported highly reproducible infarctions both by MRI and histological analysis. Based on the infarction volume in the control group and statistical power analysis, Huang et al suggest that in a prospective preclinical trial, the number of animals needed to demonstrate a 50% decrease in the infarction volume will be reduced by 41% in the present model, in comparison to previous trials that used other nonhuman primate stroke models.

The authors should be congratulated for assembling a professional neurosurgical team to perform this procedure in baboons, as well as for performing clinically relevant functional evaluations of ischemic damage. Because stroke models in nonhuman primates may have some important clinical similarities with human stroke, such as ratios of white matter to gray matter, the degree of circulatory collaterals, and a sensitive scale to measure neurological impairment, it is likely that the time course of ischemic collaterals, activation of a coagulation cascade, and neutrophil invasion may also correspond well to the ischemic response in human brain. Therefore, we look forward to seeing future applications of this valuable model used in designing human trials for stroke drugs.

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A Modified Transorbital Baboon Model of Reperfused Stroke
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