Global Brain Ischemia: A Reproducible Monkey Model

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SUMMARY We developed a monkey model of 16 minutes global brain ischemia (GBI) resulting in reproducible, severe, permanent functional neurologic deficit with long term (7 days) postischemic (PI) survival made possible by standardized intensive care with 24 hour coverage by trained personnel. Quantitated neurologic deficit (ND) and brain histopathological examinations were developed. Fifteen minutes GBI resulted in rapid recovery within 12-24 hours PI without residual neurologic sequelae. Twenty minutes GBI caused severe neurologic deficit and within 4 days PI, a delayed Cushing response eventually leading to cardiac arrest. Sixteen minutes GBI resulted in severe neurologic deficit (monkeys unable to sit, stand, walk, or feed themselves), but with long term survival. Brain histopathological analyses revealed a combination of cortical and brainstem lesions. Severest changes were observed in the occipital (calcarine) cortex with less severe damage in the frontal and temporal regions. Oculomotor nuclei and medial longitudinal fasciculus in the midbrain were regularly affected. With this model we can test the efficacy of promising therapies in terms of clinically relevant variables.

UNDERSTANDING the pathogenesis of postischemic (PI) encephalopathy and its amelioration by promising therapies has been hampered by the variability in degree of neurologic recovery and brain histopathology after seemingly comparable durations of global brain ischemia (GBI). Earlier studies suggested that 6 minutes GBI caused severe permanent brain damage but recent studies indicate that the brain is capable of functional recovery after 20 and 60 minutes of GBI. Data of past studies are difficult to interpret because recovery was evaluated in terms of acute physiological, biochemical or neuropathological changes not necessarily related to functional neurologic recovery and the methods for arresting brain circulation were highly invasive and probably contributed to PI morbidity and mortality.

Using a simple, and noninvasive method for GBI in the rhesus monkey we determined the duration of ischemia resulting in severe ND with long-term PI survival. Our earlier observations in dogs and those of Myers, et al., in monkeys provided an estimate of the "threshold" of ischemic brain damage of 15 minutes. Sixteen minutes GBI resulted in severe neurologic deficit with survival, 15 minutes in complete recovery and 20 minutes in death within 7 days PI.

Methods

Anesthesia and Surgical Preparations

Prequarantined female rhesus monkeys 4 to 5 kg body weight (Primate Imports, Inc.) were fasted overnight with water ad libitum. Anesthesia was induced with 4% halothane (Fluothane, Ayerst, Inc.)/O2 followed by intubation and IM injection of 0.4 mg atropine and .06 mg/kg pancuronium bromide and mechanical ventilation (Harvard Apparatus, Inc.) on 1% halothane/33% O2/66% N2O plus CO2 to maintain end-tidal CO2 (continuously monitored by an LB-1 Beckman infrared analyzer) at about 5-6%. ECG, rectal temperature, and urine output were continuously monitored and 5% dextrose/0.45 NaCl infused IV at 3-5 ml/kg/hr. Femoral artery and vein catheters were inserted for monitoring of mean arterial pressure (MAP), blood sampling and drug infusion. Frontal, parietal and occipital bipolar EEG was monitored via stainless steel screw electrodes. A subdural suprastriatal catheter was inserted for intracranial pressure (ICP) monitoring.

Ischemia

A pediatric tourniquet (Zimmer Warsaw, Inc.) wrapped around the neck and inflated to 30 psi and trimethaphan camsylate (Arfonad, Roche Laboratories) induced hypotension (MAP about 50 torr) were used to arrest brain circulation with blood pressure control throughout the ischemic episode. The effectiveness of this method in completely arresting brain circulation has been tested by brain scan with 99mTc and lack of brain 133Xe clearance after intra-arterial injection. An isoelectric EEG (50 µV/cm) within 15 seconds after the start of ischemia verified complete ischemia in each study.

Postischemia

The monkey was continuously ventilated on 100% O2 for up to 6 hours and thereafter on 40% O2/N2 then weaned from the ventilator when: (a) level of consciousness — normal or stuporous; (b) vigorous response to carinal stimulation; (c) vigorous gag reflex; and (d) arterial PO2 of 350 torr or greater on 100% O2 and PaCO2 less than 40 torr. Five cm H2O PEEP was applied continuously while intubated and on spontaneous ventilation.

Fluid and electrolyte balance were maintained by IV infusion of 5% dextrose/0.45% NaCl (3-5 cc/kg/hr) for up to 24 hours PI, then switched to 10% dextrose/0.24% NaCl and nasogastric feeding of a prepared diet containing 1 calorie/ml (Sustacal, Meade Johnson). A total of about 250 calories/day was administered in the form of Sustacal and 10% dextrose/0.24% NaCl. IV fluid composition was altered according to serum electrolytes and osmolality.

The following pharmacologic interventions were used in the intensive care of the monkeys: (a) diphenylhydantoin (Dilantin, Parke Davis) 10 mg IM and phenobarbital (Elkins-Sinn, Inc.) 5 mg/kg, as needed for nausea; (b) trimethaphan camsylate for MAP > 125 torr; (c)
Cardiac irregularities; (e) regular insulin (Squibb) and 50% Astra Pharmaceuticals) in 10 mg boluses in 1% solution for furosemide, 1 mg/kg for oliguria; (d) lidocaine (Xylocaine, TABLE 1

<table>
<thead>
<tr>
<th>Table 1 Neurologic Deficit Scoring Sheet (Maximum Deficit = 500 Points)</th>
</tr>
</thead>
</table>

**I. LEVEL OF CONSCIOUSNESS**
- normal 0, clouded or delirious 30, stupor 60, coma 100

**COMMENTS:**

**II. CRANIAL NERVES**
- pupil size - normal 0, mod R*
- abnormal 2, severely abnormal 5 L
- light reflex - normal 0 R
- sluggish 2, absent 5 L
- eyes, resting pos. - normal 0, R
- mod. abnormal 2, severely abnormal 5 L
- eyelash reflex - normal O, sluggish 2, absent 5 R
- corneal reflex - normal 0, L
- sluggish 2, absent 5 L
- ciliopalpebral reflex - normal 0 L
- sluggish 2, absent 5 L
- oculcephalic reflex - normal 0, R
- sluggish 2, absent 5 L
- auditory response - normal 0, L
- sluggish 5, absent 10 L
- gag reflex - normal 0, R
- sluggish 5, absent 10 L
- corneal cough reflex - normal 0, R
- sluggish 5, absent 10 L

**COMMENTS:**

**III. MOTOR FUNCTION**

A. Absent grasping or pathologic grasp reflex 25
B. Response to toe pinch
- Quick appropriate response in all limbs 0
- Sluggish appropriate response in all limbs 5
- Inappropriate response in 1 or more limbs 10
C. Limb position in resting state
- Normal 0, Mod. abnormal 10
- Severely abnormal 25

**D. Obvious paralysis or spasticity** 25
E. Behavioral—Motor Function

1. cannot chew 15
2. cannot feed himself 15
3. cannot sit 15
4. cannot stand 15
5. cannot walk 15
6. walks with ataxia 15
7. does not clean himself 10

**COMMENTS:**

**IV. RESPIRATION**

- normal 0
- abnormal 50
- on ventilator 100

**COMMENTS:**

*R = Right, L = Left.

furosemide, 1 mg/kg for oliguria; (d) lidocaine (Xylocaine, Astra Pharmaceuticals) in 10 mg boluses in 1% solution for cardiac irregularities; (e) regular insulin (Squibb) and 50% dextrose-water (Travenol) at 2 units insulin/5 cc 50% dextrose in water for hyperkalemia unresponsive to furosemide therapy; (f) peritoneal dialysis with potassium-free Dianeal (Travenol) for hyperkalemia during oliguria unresponsive to insulin/dextrose and furosemide therapy.

Nursing and body care included: (a) bedding change once every 24 hours; (b) alcohol bath and rubdown once per day; (c) saline cleansing of mouth and eyes once per day; and (d) rotation from left to right lateral position once every two hours.

EEG recordings and neurologic deficit (ND) scores (table 1) were obtained every 2 hours in the first 24 hours then every 6 hours. The quantitated ND examination permitted quantitation of ND from coma with apnea (100%) to normal (0%). Record sheets for monitoring physiologic variables, blood and urine composition and fluid balance were maintained.

**Brain Histopathology**

At 7 days PI during pentobarbital (Nembutal, Abbott Laboratories) anesthesia (30 mg/kg body weight), the brain was perfused with 500 cc's 4% paraformaldehyde followed by 500 cc's 3% glutaraldehyde via the left ventricle. Two hours later the brain and cervical spinal cord (C4) were carefully removed and placed into 4% buffered paraformaldehyde solution for 2 weeks prior to gross and microscopic histological analysis after staining with hematoxylin and eosin, and in some cases Nissl and Bielschowsky silver stains. All sections were read and graded (JM and GR) according to the type and severity of histopathology and cytopathology (table 2). Three types of lesions were graded: (1) single or multifocal infarction; (2) neuronal necrosis; and (3) edema. The severity of these lesions was assessed on a 4-point scale: minimal, 1+; moderate 2+; severe 3+ and maximal 4+. The points indicating the severity of each type of lesion were then multiplied by a weighting factor depending upon the type of lesion (infarction 4 X, neuronal necrosis 2 X, and edema 1 X) for each region of the brain examined. A maximum score of 52 points was obtained if all three types of lesion occurred with maximal severity. Total neuropathological scores were obtained with a maximum possible score of 1,040 for all 20 brain areas.

**Results**

Of 4 monkeys subjected to 15 minutes GBI, 2 rapidly recovered and apparently suffered no neurologic sequelae and 2 died at 2 days PI with pulmonary edema (table 3). MAP was not sufficiently controlled PI in monkey number 22. Barring complications in providing intensive care, 15 minutes GBI did not result in detectable ND by 7 days PI.

**Table 2 Brain Histopathologic Weighted Scoring System**

<table>
<thead>
<tr>
<th>Weighting factor</th>
<th>Pathology</th>
<th>X4 Infarction</th>
<th>X4 Neuronal Changes</th>
<th>X1 Edema</th>
<th>Total pts*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal cortex (F)</td>
<td>+ +</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>26</td>
</tr>
<tr>
<td>Parietal cortex (P)</td>
<td>+ +</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>29</td>
</tr>
<tr>
<td>Occipital cortex (O)</td>
<td>+ +</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>29</td>
</tr>
<tr>
<td>Temporal cortex (T)</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>7</td>
</tr>
<tr>
<td>Insular cortex (I)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>17</td>
</tr>
</tbody>
</table>

*Total possible points = 52.
TABLE 3  15 Min. Global Brain Ischemia in Monkeys

<table>
<thead>
<tr>
<th>Monkey no.</th>
<th>Duration survival</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>&gt; 7 days</td>
<td>Rapid recovery, 9 hr PI fully awake, placed back in cage, no apparent neurologic sequel</td>
</tr>
<tr>
<td>18</td>
<td>49 hours</td>
<td>8 min rales; PaO₂ = 100, PaCO₂ = 60, MAP = 80</td>
</tr>
<tr>
<td>22</td>
<td>48 hours</td>
<td>No BP support, 30º PI MAP = 50 torr, hypertension during ischemia, pulmonary edema</td>
</tr>
<tr>
<td>24</td>
<td>&gt; 7 days</td>
<td>6 hours PI sitting up unsteadily 13 hours PI, ataxia, full recovery, no apparent neurologic sequel</td>
</tr>
</tbody>
</table>

PI = postischemia, BP = blood pressure, MAP = mean arterial pressure.

Of 7 monkeys subjected to 20 minutes GBI, ischemia was complete in 5 and incomplete in 2 as judged by EEG silence within 15 seconds (table 4). Of the two subjected to incomplete ischemia, one survived until sacrifice at 4 days PI with a final ND score of approximately 31%, the others survived for 60 hours PI but died after pulmonary edema. The 5 monkeys with complete ischemia for 20 minutes did not survive beyond 4 days PI. The typical sequence of events observed after 20 minutes of GBI was a secondary rise in ICP paralleled by a gradual rise in MAP followed by protracted arterial hypotension unresponsive to vasopressors, secondarily isoelectric EEG, and finally cardiac arrest. In 2 monkeys (numbers 27 and 2A) where ICP recordings were unreliable, the monkeys were hypotensive or hypoxic prior to cardiac arrest; both of these patterns could have resulted from a secondary increase in ICP. Twenty minutes GBI appears to be the “upper limit” of the desirable duration of ischemia since the monkeys invariably died before 7 days PI with severe brain edema.

Of 19 monkeys subjected to 16 minutes GBI, 9 died before 7 days (13 to 128 hours PI) from complications in PI intensive care. In 4/9 monkeys, oliguria, hyperkalemia, and hypotension led to cardiac arrest; 3/9 died because of intensive care “accidents” (primarily of respiratory care); 1/9 had incomplete ischemia and 1/9 died in protracted hypotension at 24 hours PI.

Ten of the nineteen monkeys survived until sacrifice at 7 days PI with severe ND. Mean ND immediately PI was 100% (i.e., 500 points maximal deficit) and from 70% to 6 hours PI, it decreased rapidly to 50% by 24 hours PI then remained at 50% thereafter (fig. 1). In 19 experiments EEG recovery time was 66 ± 39 minutes (mean ± SD) with a range of 30 to 156 minutes. Examination of components of ND (fig. 2) revealed severest deficits in motor function, while cranial nerve deficit and respiration recovered rapidly between 12 and 24 hours PI. Level of consciousness improved rapidly between 0 and 6 hours PI and was essentially unchanged thereafter.

Respiratory variables reflected mechanical ventilation at 0 and 5 minutes PI, and at 6 hours PI, spontaneous hyperventilation (50 breaths/min) with hypocarbia. Mean PaO₂ was maintained well above 100 torr throughout. Postischemic arterial pH was normal immediately after cuff deflation (0 hours PI), then indicated respiratory alkalosis at 150 hours PI.

Cerebral perfusion pressure (CPP) was 53 torr at time of cuff deflation but was subsequently maintained at about 100 torr. ICP was 8 torr preischemia, 11 torr immediately after cuff deflation and then fluctuated within normal limits (7–16 torr), throughout the 7 days PI. Serum electrolytes were maintained within normal limits throughout.

Histopathological analysis of 6 brains at 7 days PI revealed a pattern of cortical and brain stem lesions (figs. 3 and 4) with severest damage in the occipital (calcarine) cortex, brainstem and especially midbrain. The lesions in hippocampus and cerebellum were mostly neuronal necrosis but small infarcts were also present in hippocampus (1 of 6 brains) and cerebellum (1 of 6 brains). There was regional variation in the distribution and severity of damage (fig. 5). Neocortex was regularly damaged more severely than limbic cortex. The parietal and the occipital cortex, especially the calcarine area, showed the most consistent and severe lesions. Infarction and neuronal necrosis, though frequently present, were less severe in frontal and temporal cortex. There was no consistent distribution of brain damage along arterial border zones but rather the maximum damage appeared to be in the most distal distribution of the posterior cerebral artery. Damage to tissues in the distribution of the anterior cerebral artery followed in severity that of the posterior cerebral artery. The smallest number of lesions were in the distribution of the middle cerebral artery. The corpus striatum and thalamus contained fewer lesions than the neocortex or midbrain. A regular site of damage (6 of 6 monkeys), in the form of circumscribed infarcts with occasional microglial proliferation and early phagocyte formation, was the oculomotor nuclei and medial longitudinal fasciculus. Occasional infarcts were also seen in the substantia nigra in the midbrain and in the tegmentum of the pons and medulla.

TABLE 4  Twenty Minutes Global Brain Ischemia

<table>
<thead>
<tr>
<th>Monkey no.</th>
<th>Duration survival</th>
<th>Final neurologic deficit*</th>
<th>Cause of cardiac arrest</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>&gt; 7 days</td>
<td>31</td>
<td>sacrificed</td>
</tr>
<tr>
<td>26</td>
<td>60 hours</td>
<td>62</td>
<td>pulmonary edema</td>
</tr>
<tr>
<td>27</td>
<td>30 hours</td>
<td>59</td>
<td>hypotension</td>
</tr>
<tr>
<td>28</td>
<td>24 hours</td>
<td>52</td>
<td>increased ICP, hypotension, arrest</td>
</tr>
<tr>
<td>29</td>
<td>20 hours</td>
<td>90</td>
<td>increased ICP, hypotension</td>
</tr>
<tr>
<td>1A</td>
<td>90 hours</td>
<td>96</td>
<td>increased ICP, hypotension</td>
</tr>
<tr>
<td>2A</td>
<td>72 hours</td>
<td>100</td>
<td>hypoxia</td>
</tr>
</tbody>
</table>

*Maximum neurologic deficit = 100%.
Discussion

Our results suggest that the ischemic "threshold" of the brain in the rhesus monkey, is about 15 minutes. Rapid neurologic recovery was observed after 15 minutes GBI whereas 16 minutes GBI produced severe ND until sacrifice at 7 days PI. These results support the findings of earlier reports of 14 to 15 minute "thresholds" for severe permanent brain damage after GBI without systemic circulatory arrest in rhesus monkeys.

Earlier investigators reported ischemic "thresholds" of 6, 8, 11 and 8 minutes. The importance of maintaining normal PI cerebral perfusion pressures was probably not appreciated in these studies, and the limiting factor in determining the ischemic threshold or tolerance of the brain appeared to be the ability to provide adequate life support. As life support techniques improved the "threshold" of severe brain injury increased. Cantu and Ames reported complete recovery without ND after 14 minutes GBI in dogs, emphasizing the importance of maintaining adequate cerebral perfusion pressure PI.

After 16 minutes GBI in 10 of 19 monkeys studied, criteria of complete ischemia were obtained with survival until sacrifice at 7 days PI. These 10 monkeys had a mean total ND score of 50% between 24 hours and 7 days PI. Unsuccessful studies with renal "shutdown" may have been related to excessive amounts of norepinephrine required to maintain normal MAP or from inappropriate antidiuretic hormone secretion. Throughout these studies we have
learned to appreciate the precarious state of the brain which can be easily "pushed" to brain death by mild hypoxia or hypotension as a result of ICU accidents. These include "bucking" the ventilator; inadequately controlled convulsions; and pulmonary atelectasis or edema resulting from long term spontaneous ventilation while intubated without PEEP or sighing. If immobile, it is also important that the monkeys be regularly rotated from one lateral position to the other.

Monkeys subjected to 20 minutes GBI developed a gradual increase in ICP paralleled by an increase in MAP; secondarily leading to hypotension unresponsive to vasoressors and, eventually, cardiac arrest. The rise in ICP suggested that after 20 minutes of GBI the volume of edematous brain tissue had exceeded the capacity of cerebrospinal fluid to compensate for the increase in brain tissue H$_2$O. The "critical mass" of edematous brain tissue resulting in a generalized increase in ICP can be roughly calculated using data on the percent increase in brain tissue H$_2$O in severe brain edema. The increase in brain tissue volume in edematous brain ranges from 10% to as high as 18%. Löfgren, et al. found relatively little change in ICP with an increase in brain H$_2$O of up to 5% but a rapid and marked rise in ICP (up to 100 torr) if brain H$_2$O increased by 10%. These results suggest that the critical mass of edematous brain tissue should be approximately 25% or 20 g in the 80 g monkey brain or 300 g in the 1200 g human brain.

On the basis of our results we speculate that the relationship between duration of GBI and neurologic deficit is as shown in figure 6. However, our neurologic examination is heavily biased for gross motor function and although cranial nerve and sensory tests are conducted, they are by no means comprehensive. Based on our ND scoring system our 16 minute model is situated at the midpoint of the steep portion of the S-shaped curve. The area between the interrupted lines delineates ± two standard deviations. Between 10 and 15 minutes GBI there is little change in ND and 0% falls within one standard deviation of the mean. Assuming that the relationship between ND and duration of ischemia is symmetrical on both sides of the midpoint of the steep portion of the curve, after 17 minutes GBI 100% ND is included within one standard deviation. Beyond 17 minutes, ND increases to 100%. After 19 and 20 minutes of ischemia the duration of PI survival is expected to decrease.

Our speculation on the relationship between duration of GBI and total ND score obviously ignores earlier reports suggesting that the tolerance of the brain to ischemic-anoxic damage is between 4 to 8 minutes of GBI. Brierley concluded that the threshold of ischemic brain damage histologically, is between 4 to 8 minutes of GBI. In 10 patients who suffered circulatory arrest for 2-15 minutes, neurologic recovery indicated lower ischemic tolerance of the human brain compared to monkeys. However, neurologic recovery after total system circulatory arrest (as in patients) is slower than with arrest of only brain circulation (as in our monkey model). In most cases of cardiac arrest in patients neither the exact duration of ischemia nor
the quality of resuscitation and PI intensive care are well documented.

Experimental models of GBI vary in their simulation of that found in people under diverse clinical conditions. The brain lesions in this model have regularly included lesions in the midbrain, particularly oculomotor nuclei and adjacent regions as reported in the model described by Miller and Myers. In our study, other cranial nerves and brain stem nuclei were less regularly affected. The distribution of cortical lesions in our model was more caudal than

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**Figure 5.** Infarction and neuronal necrosis scores in the rhesus monkey brain at seven days postischemia after 16 minutes global brain ischemia. NC = neocortex, H = hippocampus, TH = thalamus, CS = corpus striatum, BS = brain stem, CE = cerebellum.

**Figure 6.** Hypothetical relationship between percent neurologic deficit (maximum deficit = 100%) and duration of global brain ischemia, based upon our quantitated neurologic deficit scoring system in monkeys subjected to global brain ischemia without systemic circulatory arrest. The area between the interrupted lines represents plus or minus two standard deviations according to the variability observed after 16 minutes of global brain ischemia with seven days postischemic survival.
that reported by Miller and Myers with maximum severity in the medial occipital cortex (calcarine) and parietal lobes. Major or predominant localization of lesions to the arterial border zones as described with hypotension in the model of Brierley, et al. could not be confirmed in our model but differences in experimental methodology may be responsible.

In summary, we have developed a reproducible model in the rhesus monkey of global brain ischemia resulting in severe permanent brain damage but with survival until sacrifice at 7 days PI. We have also developed standardized PI monitoring, intensive care techniques and quantitated neurologic deficit and brain histopathological scoring systems. With this model we now have the means to evaluate definitively the efficacy of a number of therapeutic procedures which may be of benefit in ameliorating PI brain damage. It is also useful to investigate pathophysiologic and biochemical mechanisms of postischemic encephalopathy.

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