Survival of Rabbits After Prolonged Cerebral Ischemia

RONALD J. KOLATA, DVM

SUMMARY  Cerebral ischemia was produced by a combination of vascular occlusion and mild systemic hypotension in 2 groups of rabbits. Arterial blood pressure, arterial pH, arterial blood gases, blood glucose and PCV were monitored and recorded before, during and for 3 hours after reperfusion. Return of EEG activity, vasomotor control, spontaneous ventilation and corneal reflex were also recorded. At 4, 8, 12, 24 and 48 hours after reperfusion, the rabbits' neurologic status was assessed according to an arbitrary scale based on motor function. The 2 groups differed in return of reflexes and motor function. Eighty percent of the rabbits ischemic for 20 minutes and 75% of the rabbits ischemic for 30 minutes survived. The graduated response of motor function to cerebral ischemia is attributed to the ventilatory and circulatory support given the rabbits for the first 3 hours after reperfusion. The graduated response of motor function to ischemia supports the suggestion that motor function can be used as an index of neurologic damage.

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with tantalum clips. Next the carotid artery is isolated from the nerve trunks that are adjacent to it within the carotid sheath, and a ligature is placed loosely around it so that it can be immobilized for clamping. Two-thirds of a 12-inch length of umbilical tape is placed into a space created between the cervical vertebrae dorsally and the trachea ventrally. The remaining 1/3 is left protruding from the incision. The wound is covered with a sterile gauze pad, the rabbit rolled over, and the surgical procedure repeated on the previously unoperated side. Once the vertebral arteries are ligated and the carotid arteries isolated, the previously buried length of umbilical tape is brought out of the skin incision.

After control measurements are made, anesthesia is stopped, and trimetephan camphor sulfonate is infused intravenously until peak systolic pressure is < 80 mm Hg. Cerebral ischemia is immediately induced by clamping the common carotid arteries with non-crushing vascular clamps and by firmly tightening the umbilical tape ligature by tying it around the perivertebral muscles at the level of the third cervical vertebra, thereby occluding muscular branches anastomosing with the occipital branch of the vertebral artery. During CI, the peak systolic pressure is kept between 70–80 mm Hg by infusion of trimetephan or norepinephrine as necessary.

**Method of Procedure**

To investigate the efficacy of this method of producing reversible cerebral ischemia, New Zealand white rabbits of either sex and weighing between 2–2.5 kg were used. Twenty-four rabbits were surgically prepared as described. Five were used to determine the completeness of the cerebral ischemia achieved by this method. Five were allowed to recover from surgery without undergoing a period of ischemia to assess whether or not grossly detectable neurological signs were caused by the surgical procedure. Fourteen rabbits had selected physiological and neurological parameters monitored before, during, and after CI to determine the degree of neurological impairment caused by prolonged ischemia.

**Physiological Measurements**

The skin overlying the cranium was incised on the dorsal midline and reflected laterally. Stainless steel screws, to serve as dural electrodes, were threaded into the skull. A screw was positioned 1 cm to either side of the midline of the skull at the frontoparietal suture. A third screw, to serve as a reference electrode, was threaded into the sagittal crest. The skin incision was sutured closed leaving the screws protruding.

A catheter was inserted into the femoral artery to enable blood pressure recording and for obtaining blood samples. Catheters were also inserted percutaneously into both saphenous veins to facilitate injection of drugs. Five hundred units of heparin per kg were administered. The rabbits were placed in sternal recumbency, and needle electrodes were placed into contralateral sides of the chest to allow recording of the electrocardiogram.

The endotracheal tube was connected to a Harvard Model 601 ventilator. Anesthesia was maintained by connecting a Drager halothane vaporizer to the inlet of the ventilator so that room air was drawn through the vaporizer. The ventilator was adjusted to provide an arterial oxygen tension (PaO₂) of at least 80 torr and an arterial carbon dioxide tension (PaCO₂) of 28–34 torr. Control readings of EEG and ECG activity and control values for blood glucose and packed cell volume (PCV) were obtained.

Arterial pressure was monitored with an E and M P1000 pressure transducer and ECG activity with an E and M cardiac preamplifier MK IV. Both variables were recorded using a Physiograph Four recorder (E and M Instrument Co.). Arterial pressure was monitored and recorded continuously during the 3 hour observation period. ECG activity was also monitored and recorded in conjunction with blood pressure to identify any persistent arrhythmia. EEG activity was monitored and recorded using a Grass model III D Electroencephalograph (Grass Instrument Co.). EEG activity was recorded during the control period, during the onset of CI until a silent recording was obtained, and then intermittently during and after the ischemia period to detect the reappearance of EEG activity. The sensitivity of the machine was set to record a 1 cm deflection for each 50 microvolt change in potential. A silent EEG recording was considered to be activity 5 μV peak to peak or less. ECG activity was monitored using the ECG preamplifier of the encephalograph so that ECG artifacts appearing in the EEG tracing could be identified.

Arterial gases and pH were determined using an IL 213 Digital Blood Gas Analyzer (Instrumentation Laboratory, Inc.). All measurements were made in duplicate. Blood gases and pH were monitored intermittently after the ventilator was adjusted for each rabbit. Values of samples taken at the midpoint of the ischemia period, at 10 minutes after reperfusion, and at 1, 2, and 3 hours after reperfusion were recorded. When spontaneous respiratory activity became strong and regular, the rabbit was disconnected from the ventilator and its blood gases and pH monitored. When it could maintain a PaCO₂ < 40 torr and a PaO₂ of at least 80 torr, mechanical ventilatory assistance was discontinued. During the post-ischemia period, rabbits not maintaining a PaO₂ of at least 80 torr were considered to have abnormal pulmonary function and were eliminated from the study. Blood glucose determinations were made using a YSI model 23A Glucose Analyzer (Yellow Springs Instrument Co.). PCV was determined using an Adams Autocrit Centrifuge (Clay-Adams, Inc.).

Once the control samples were obtained, anesthesia was stopped and trimetaphan was infused until peak systolic pressure was < 80 mm Hg. Cerebral ischemia was immediately induced by clamping the common...
Table 1  Effect of 20 Minutes of Cerebral Ischemia on Some Blood Parameters in 10 Rabbits

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Mid isch.</th>
<th>End isch.</th>
<th>1 Hr.</th>
<th>2 Hr.</th>
<th>3 Hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PaO2 (torr)</td>
<td>96 ± 11</td>
<td>95 ± 8</td>
<td>105 ± 8</td>
<td>105 ± 13</td>
<td>106 ± 11</td>
<td>105 ± 8</td>
</tr>
<tr>
<td>PacO2 (torr)</td>
<td>32 ± 3</td>
<td>29 ± 4</td>
<td>30 ± 4</td>
<td>28 ± 2</td>
<td>30 ± 3</td>
<td>30 ± 5</td>
</tr>
<tr>
<td>ApH</td>
<td>7.45 ± .03</td>
<td>7.46 ± .03</td>
<td>7.46 ± .03</td>
<td>7.47 ± .04</td>
<td>7.45 ± .05</td>
<td>7.41 ± .06</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>106 ± 20</td>
<td>104 ± 23</td>
<td>96 ± 31</td>
<td>104 ± 46</td>
<td>107 ± 68</td>
<td>110 ± 57</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>37 ± 2</td>
<td>34 ± 2</td>
<td>38 ± 2</td>
<td>38 ± 2</td>
<td>38 ± 2</td>
<td>38 ± 2</td>
</tr>
</tbody>
</table>

carotid arteries with noncrushing vascular clamps and by firmly tightening the umbilical tape ligature. During CI, the peak systolic pressure was kept between 70–80 mm Hg by infusion of Arfonad or norepinephrine.

In a group of 10 rabbits (Group I), after 20 minutes, the carotid arteries were unclamped and the cervical umbilical tape ligature was removed. In a group of 4 rabbits (Group II), cerebral ischemia was maintained for 30 minutes and was terminated as in Group I.

As soon as ischemia was ended, peak systolic pressure was raised to 100 mm Hg by infusion of norepinephrine until the rabbit gained vasomotor control and could maintain that pressure unassisted. After removal of the umbilical tape ligature, the wound was sutured.

At the time of return of vasomotor control, respiratory activity, corneal reflex, and EEG activity were recorded. Three hours post ischemia, the catheters and recording electrodes were removed and each rabbit was moved to an individual cage.

In this study, neurological recovery was defined as return of gross motor functions, i.e., ability to assume and maintain normal posture and ability to coordinate movement and survival during the 48 hours observation period. Each rabbit’s functional status was assessed and given a numerical value according to a scale (table 5). Each rabbit was evaluated at 4, 8, 24, and 48 hours post-ischemia. After the final evaluation, each rabbit was killed with an overdose of barbiturate.

Results

Completeness of Interruption of Cerebral Blood Flow

The completeness of the interruption of cerebral blood flow was investigated in 5 rabbits. One rabbit was decapitated at the atlantooccipital joint after induction of cerebral ischemia and the disappearance of EEG activity. No arterial blood flow was seen while the rabbit was being ventilated and its heart was beating. One rabbit was killed and barium suspension was injected into the left ventricle at a pressure of 150–200 mm Hg while all clamps and ligatures were in place. No barium was seen in the vessels of the brain by radiographic or direct visual examination. In the remaining 3 rabbits, cerebral blood flow was measured by autoradiographic technique using ^14C labelled antipyrine. No flow was detected.

Neurological Changes Due to Surgical Procedure

An additional five rabbits, allowed to recover from surgery without undergoing a period of CI, were observed intermittently for 48 hours. No difference in behavior or motor abilities was seen between the surgically prepared rabbits or normal rabbits housed in the same environment.

Physiological Data

The control values of blood gases, pH, blood glucose, and PCV were similar in Group I and Group II.
II (tables 1 & 2). The duration from arterial clamping to
the onset of EEG silence was between 35 and 70 sec
in both groups. Times to recovery of reflexes are seen
in table 3. There were significant differences in
recovery of corneal reflex and EEG activity between
the groups, and the times at which ventilatory
assistance could be discontinued was also different
between groups. Respiratory movements (gasping)
were noted at about the same time as vasomotor con-
trol returned.

In both groups the mean arterial pressure rose
steadily once vasomotor control returned. Arterial
pressure reached its peak 17-20 minutes post-ischemia
and then returned to a stable but lower pressure by
30-44 minutes post-ischemia (table 4). It remained at
this level to the end of the monitoring period.

Paco₂ decreased in both groups during the period of
ischemia. It remained at less than the control value
during the recovery period because the rabbits tended
to hyperventilate spontaneously. Changes in PCV and
blood glucose concentration were insignificant.

Neurological Status

At 4 hours post-ischemia the rabbits of both Group
I and Group II were recumbent and unresponsive un-
less stimulated vigorously. The rabbits ischemic for 30
minutes were initially more depressed and less responsive
than the rabbits ischemic for 20 minutes (table 5). At 48 hours, the rabbits having a score of 1 were up-
right and would eat and drink, but were not as responsive
as the normal rabbits living in the same environment.
They would remain sitting for long periods without moving, and tremors of the head and limbs were present when they moved about. Rabbits having a score of 2 would move only when vigorously stimulated. They did not eat or drink spontaneously. Those with a score of 3 were recumbent, appeared blind, and could not control their rear limbs.

The 3 rabbits that died did so within 18 hours post-
ischemia. At 8-10 hours after ischemia the following
signs appeared in these rabbits: lethargy, progressive
extensor rigidity, irregular panting respiration, opis-
phantous, and periodic running movements.

Although there was no statistically significant
difference in neurological scores between the rabbits
ischemic for 20 to 30 minutes, the mean scores of
Group I were consistently higher than the scores of
Group II (table 5).

Histopathology

Subdural hemorrhages were found on the brains of
all rabbits ischemic for 30 minutes. No hemorrhages
were found within the substance of these brains. The histopathological lesions consisted of loss of neurons, ischemic cell changes, increased vascularity, and status spongiosis. These lesions were found in the hippocampus and the cerebellum. Many dark neurons were seen throughout the cortex and brain stem. There was no clear distinction in the severity of the lesions between the two groups.

Discussion

The cerebral blood supply of the rabbit flows
through the internal carotid and vertebral arteries.
There is a small collateral supply which consists of
anastomoses between arteries supplying the muscles
of the neck and the carotid and vertebral arteries.
These vessels were controlled by the surgical prepara-
tion used. Although the surgical preparation does not
preclude blood flow through the ventral spinal artery,
flow does not occur probably because of the
trimethaphan-induced hypotension. However, Sainio²
found that blood flow to the brain of rabbits, in which
he inflated a cervical pressure cuff to induce cerebral
ischemia, had no collateral flow through the ventral
spinal artery, and Hossmann¹⁰ has shown that

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I (10 rabbits)</th>
<th>Group II (4 rabbits)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Return of vasomotor control</td>
<td>8 ± 2 Min</td>
<td>7 ± 1 Min</td>
</tr>
<tr>
<td>Return of resp. activity</td>
<td>8 ± 4 Min</td>
<td>7 ± 1 Min</td>
</tr>
<tr>
<td>Return of corneal reflex</td>
<td>26 ± 3 Min</td>
<td>37 ± 6 Min</td>
</tr>
<tr>
<td>Return of EEG activity</td>
<td>26 ± 3 Min</td>
<td>34 ± 7 Min</td>
</tr>
<tr>
<td>Ventilatory support discontinued</td>
<td>23 ± 7 Min</td>
<td>39 ± 7 Min</td>
</tr>
</tbody>
</table>

* (t = 3.502, p = < .005)
† (t = 2.377, p = < .05)
‡ (t = 2.570, p = < .025)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I (10 rabbits)</th>
<th>Group II (4 rabbits)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak mean pressure</td>
<td>124 ± 2 mm Hg</td>
<td>123 ± 5</td>
</tr>
<tr>
<td>Interval from end of ischemia</td>
<td>17 ± 3 Min</td>
<td>18 ± 7</td>
</tr>
<tr>
<td>Stable mean pressure</td>
<td>95 ± 10 mm Hg</td>
<td>101 ± 2</td>
</tr>
<tr>
<td>Interval from end of ischemia</td>
<td>34 ± 5 Min</td>
<td>34 ± 6</td>
</tr>
</tbody>
</table>

II (10 rabbits) (4 rabbits)

Table 4  Arterial Pressure Changes During Post-Ischemic Period

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I (10 rabbits)</th>
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<td>101 ± 2</td>
</tr>
<tr>
<td>Interval from end of ischemia</td>
<td>34 ± 5 Min</td>
<td>34 ± 6</td>
</tr>
</tbody>
</table>

II (10 rabbits) (4 rabbits)

Table 5  Mean Neurologic Scores* of Rabbits Subjected to 20 and 30 Minutes of Cerebral Ischemia

<table>
<thead>
<tr>
<th>Hours post ischemia</th>
<th>4</th>
<th>8</th>
<th>24</th>
<th>48</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (10 rabbits)</td>
<td>3.0 ± 0</td>
<td>2.5 ± 0.71</td>
<td>2.6 ± 1.17</td>
<td>2.5 ± 1.27</td>
</tr>
<tr>
<td>Group II (4 rabbits)</td>
<td>3.0 ± 0</td>
<td>3.0 ± 0</td>
<td>3.0 ± 0.82</td>
<td>2.7 ± 0.96</td>
</tr>
</tbody>
</table>

*Criteria for scoring: 1 = Alert, normal upright posture. Minor motor dysfunction. 2 = Poorly responsive to stimuli, can attain normal posture but has severe incoordination. 3 = Responsive only to vigorous stimulation, cannot maintain normal posture 4 = Dead.
collateral flow through the anterior spinal artery does not occur in monkeys unless systemic pressure is elevated (150 mm Hg or greater). A significant feature of the method of achieving cerebral ischemia described herein is that it does not prevent venous drainage from the head, which makes it more like circulatory arrest than some methods.8

Ganglionic blockade is an important facet of this model. It is used to produce controlled moderate hypotension to limit collateral circulation to the brain but, more importantly, to blunt the massive outburst of vasomotor activity which accompanies clamping the carotid arteries and the onset of cerebral ischemia. This vasomotor activity is a diphasic blood pressure change characterized by an initial hypotensive period lasting 3–5 seconds, followed by a hypertensive period lasting 5–8 minutes. Peak pressure is reached within the first minute after the onset of cerebral ischemia. During the period of peak pressure, premature ventricular contractions were seen to occur. During pilot experiments, this vasomotor response was followed by the sudden onset of pulmonary edema in many of the rabbits. These vasomotor events were probably due to baroreceptor and carotid sinus reflexes and, in part, by central reflexes.11 A diphasic hemodynamic response, similar to that in the rabbits, has been seen in dogs subjected to transcranial electrical stimulation.12 In the dogs, this was found to be a central response mediated by the vagus and sympathetic nerves which could be eliminated by vagotomy and sympathectomy. Routine use of atropine and trimethaphan prior to induction of cerebral ischemia abolished these vasomotor events in the rabbits and prevented the problem of pulmonary edema. This observation may explain the occurrence of pulmonary edema noted in other such studies and may bear some relation to the pulmonary damage seen with hypoxemia.5, 9, 15

An additional vasomotor change that was seen in this study was a transient but pronounced rise in mean arterial blood pressure after cerebral ischemia. The same sort of change has been seen in dogs.12 Systemic pressure rose, once spontaneous vasomotor control was regained. The rise was slow, reaching its peak 15–20 minutes after reperfusion, and then slowly declining to a stable pressure 30–40 minutes after reperfusion (table 4). This response was probably a manifestation of cerebral hyperperfusion that is known to follow prolonged cerebral ischemia.8 This hyperperfusion is recognized to cause cerebral edema. The rabbits that died in this study had signs of increasing intracranial pressure (vide supra) most likely caused by cerebral edema.

Over 70% of the rabbits (11/14) survived through the 48 hour observation period. This is a greater number than would be expected to survive such prolonged cerebral ischemia on the basis of historically accepted limits.14 However, it has long been known that the vegetative functions of the brain will return after prolonged ischemia, and recent evidence suggests that extracranial factors are of great importance in limiting post-ischemia recovery.15, 16, 20 Monkeys have survived and recovered from 20 minutes of cerebral ischemia, and evidence for return of neuronal activity has been obtained after 30 and 60 minutes of cerebral ischemia.1, 10, 17–20 It is believed, on the basis of these findings, that adequate support of cardiovascular and respiratory functions in the post-ischemia period is critical to survival.16, 21, 22 This evidence encourages the belief that the cardiopulmonary support afforded these rabbits allowed a prompt return of adequate cerebral blood flow and that this accounts for the greater than expected survival rate.

Results of the tests for completeness of cerebral ischemia indicate that complete ischemia was achieved. The physiological data obtained from, and the neurological scores assigned to, the rabbits indicate a reproducible degree of cerebral ischemic injury was achieved. The difference in the results between the 2 groups indicates that the severity of the insult varied with duration. Return of corneal reflex, EEG activity, and adequate spontaneous ventilation were significantly prolonged in the 30 minute ischemia group and motor deficits were more profound. This finding supports the suggestion that motor disability can be used as an index of neuronal damage and be a more useful end point for the evaluation of treatment regimens than survival alone.7 These findings, combined with the fact that this method of producing cerebral ischemia in rabbits does not have the disadvantage of causing ischemia or hypoxia of other vital organs, and the fact that the ischemia is reversible and that animals can be recovered for prolonged study, seems to make this model suitable for use in investigation of cerebral ischemia in lower animals.

Acknowledgments

The author wishes to acknowledge the dedicated technical assistance of Mrs. M.E. Kolata, the encouragement and support of the late Dr. D.B. Polis of the Naval Air Development Center, Warminster, PA, and the help of Drs. M. Reivich and J. Greenberg of the Cerebral-Vascular studies unit of the University of Pennsylvania Medical School.

References

models of brain ischemia. Stroke 7: 14-17, 1976

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**Occlusion of the Vertebral or Basilar Artery**

**Follow Up Analysis of Some Patients with Benign Outcome**

**LOUIS R. CAPLAN, M.D.**

**SUMMARY** Ten patients with angiographically verified occlusion of the basilar or vertebral artery have been followed for an average of 2.75 years. None has developed further ischemia after the initial stroke, and 4 patients survived without any clinical deficit. In occlusive disease of the posterior circulation, the critical period for deficit acquisition is at the time of occlusion. Extent of the deficit depends on the rapidity of development of adequate collateral circulation, and the presence of distal embolization at the time of occlusion. Some patients survive basilar occlusion without permanent deficit.

**OCCLUSION of the basilar artery is generally considered a very serious event incompatible with normal survival. Kubik and Adams1 described 18 patients with brainstem infarction due to occlusion of the basilar artery discovered at postmortem examination and emphasized the abrupt onset and frequent fatal outcome. Marshall2 subsequently pointed out that many untreated patients with the clinical picture of posterior circulation vascular disease do not develop serious deficits; however, the underlying vascular pathology in this group of clinical patients was unknown. Occlusion of the vertebral artery, the most frequent cause of lateral medullary infarction identified at postmortem examination,3 has usually been associated with a relatively benign clinical course.4 In frequent studies have attempted to correlate the severity of the neurological deficit with angiographically verified vascular pathology in the posterior circulation.4-4 No reference could be found on follow up of patients with angiographically documented vertebrobasilar occlusive disease.

The author's files, and those of the Harvard Stroke Registry,9 were searched for patients meeting the following criteria: 1) patients who were examined and followed carefully by the author during hospitalization for acute stroke; 2) technically satisfactory angiograms taken during the stroke which documented an occlusion of either a vertebral or the basilar artery; 3) patients who survived the acute stroke and had been followed by the author more than 6 months. The temporal profile of illness and clinical outcome in this group of patients gave insights into the pathogenesis of the clinical deficit and mechanisms of compensation.

**Results**

**Case Material**

Ten patients (8 male, 2 female) from 26-71 years of age (average 52.2 years) were studied clinically and angiographically. Six had occlusion of the basilar artery (2 proximal and 4 midportion beyond the
Survival of rabbits after prolonged cerebral ischemia.
R J Kolata

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