A Gerbil Model of Cerebral Ischemia Suitable for Drug Evaluation

D. M. Jarrott, M.D. AND FLOYD R. DOMER, Ph.D.

SUMMARY Cerebral ischemia was produced in the Mongolian gerbil by bilateral occlusion of the carotid arteries. Although the cerebral ischemia so produced was not total, a mortality rate of 100% was obtained if the occlusion was maintained for 60 min in gerbils weighing 45-55 gm. Few deaths were observed after 50 min of bilateral carotid arterial occlusion. Test drugs were administered, after the removal of the arterial clips, to groups of gerbils to determine the mortality rate associated with each drug. Isoproterenol 50 mg/kg, amphetamine 5.0 mg/kg, and methylprednisolone 35 mg/kg improved survival after cerebral ischemia. Atropine 1 mg/kg, thiosemicarbamide 4 mg/kg, aminoxyacetic acid 100 mg/kg, theophylline 100 mg/kg, and phenytoin 50 mg/kg were associated with a reduced survival after cerebral ischemia. The known tendency of the gerbil to exhibit spontaneous seizures and the frequency and severity of the observed post-ischemic seizures suggest that the lethality of prolonged cerebral ischemia may be, in part, related to seizures triggered by the cerebral ischemia.

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THE EASE WITH WHICH strokes can be produced in the Mongolian gerbil undoubtedly accounts for its popularity as a model of focal cerebral ischemia. In those studies ligation of one artery was used to produce a severe neurological deficit and a high mortality rate which resembled the syndrome of middle cerebral arterial occlusion in humans. The neurological signs which develop in gerbils after ligation of one carotid artery have been described by numerous investigators. Head turning, splaying or rotation of the limbs, circling, unilateral ptosis and enophthalmos are signs attributed to focal cerebral damage ipsilateral to the occluded carotid artery. Coma, "rolling seizures" and generalized convulsions are signs which indicate more severe or generalized cerebral damage and lead to death in 40-60 percent of gerbils after unilateral carotid arterial ligation. These neurological signs and mortality rates are correlated with histopathological evidence of cerebral infarction.

Bilateral carotid arterial ligation in the gerbil is uniformly fatal because the collateral blood supply from the vertebral-basilar system is insufficient to prevent total cerebral infarction when the carotid flow is blocked. This susceptibility is not unique to the gerbil, since 100 percent mortality rates have been reported for other laboratory rodents subjected to bilateral carotid arterial ligation. It was the gerbil's special susceptibility to unilateral carotid arterial ligation, however, which led to its introduction as a useful model of cerebral ischemia. When one carotid artery is ligated, the ipsilateral cerebral hemisphere is dependent upon the anterior communicating artery for the critical blood supply which would prevent infarction and allow the animal to survive. The presence or absence of neurological signs after unilateral carotid ligation has been convincingly correlated with the anatomical variations in the anterior communicating arterial complex. From a practical standpoint, the blood flow available from the posterior circulation can be neglected, although microscopic collaterals are present and minimal residual blood flow during bilateral carotid arterial occlusion has been recorded.

The production of global cerebral ischemia by bilateral carotid arterial occlusion in the gerbil does not resemble a human illness as closely as does unilateral carotid arterial ligation. However, it is a technically easy method for the study of cerebral ischemia per se. A large volume of mammalian cerebral tissue can be made intensely ischemic for any desired period of time, up to and including the duration which results in 100 percent mortality. In such a model the end point is clear-cut and simply determined, i.e., survival vs death of the animal. The purpose of the present investigation was to establish an "expected" mortality curve during the duration of ischemia as the dependent variable. Single administrations of test drugs were then introduced as independent variables to determine possible changes in the mortality rate following identical periods of bilateral carotid arterial occlusion.

Methods

A total of 504 adult Mongolian gerbils of either sex weighing 30-60 gm were obtained from Tumblebrook Farm, anesthetized and placed in the supine position. The anesthetic agents which were employed were diethyl ether (open drop), ketamine hydrochloride 35 mg/kg i.p., alpha D-chloralose 100 mg/kg i.p., urethane 1.25 g/kg i.p. and pentobarbital sodium 60 mg/kg i.p. An anterior midline cervical incision was made and both carotid arteries were dissected free of accompanying structures using a 16x Sparta binocular operating microscope. Bilateral carotid arterial occlusion of 5-70 min duration was produced with Sparta aneurysm clips. At the end of the ischemic period, the clips were removed and the patency of the carotid arteries assured by direct visualization through the microscope. During the ischemic period, a note was made of any significant filling of the carotid artery distal to the occluding clip. The skin was sutured with

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4-0 silk, and the animals were returned, in groups of 3, to an 18 × 25 × 14 cm cage for recovery and observation. A neurological assessment was recorded at 6–8 hour intervals, using the method of McGraw. The neurological signs were assigned values as follows: hair roughed up, tremor, obtundation or paucity of movements-1; ptosis or convulsions-2; head cocked, eye fixed open, splayed hindlimb, rotation of hind limb, rolling seizure or circling behavior-3; coma-6. These scores were tabulated as the "stroke index" such that a higher score indicated a more severe post-ischemic neurological deficit. Pentobarbital was used as the anesthetic for the succeeding experiments in which test drugs were compared to saline. After closure of the cervical incision, approximately 3 minutes after removal of the carotid arterial clips, the gerbil/s received an intraperitoneal injection of saline or test drug. The volume of injection was 0.25–0.5 ml. The following drugs were tested: phenytoin sodium 50 mg/kg, atropine sulfate 1 mg/kg, amphetamine sulfate 5 mg/kg, uridine 100 mg/kg, chlorpromazine 5 mg/kg, methylprednisolone sodium succinate 35 mg/kg, physostigmine hydrochloride 0.5 mg/kg, aminooxyacetic acid (AOAA) 50 mg/kg, theophylline 50 mg/kg, thiosemicarbazide 4 mg/kg, and isoproterenol hydrochloride 50 mg/kg. The results were analyzed statistically using Fisher's Exact Test for small group analysis. Each drug and dose was administered to 6 gerbils anesthetized with pentobarbital 60 mg/kg without carotid occlusion to verify the absence of toxicity in the dosage selected.

The gerbils weighed at the time of purchase 30–35 gm. They were maintained in the vivarium, 3 or 4 to an 18 × 25 × 14 cm cage, and fed Purina rodent laboratory chow until they reached 45 gm. The drug evaluation experiments would then commence. A batch of 25–50 gerbils could be disposed of before they had grown to 55 gm in size. In this manner the weights of the gerbils at the time of the drug evaluation experiment, i.e. the carotid occlusion, was kept within the range of 45–55 gm. Some larger and some smaller gerbils were subjected to carotid occlusion before the time of the drug evaluation to verify the absence of toxicity in the dosage selected. The gerbils weighed at the time of purchase 30–35 gm. They were maintained in the vivarium, 3 or 4 to an 18 × 25 × 14 cm cage, and fed Purina rodent laboratory chow until they reached 45 gm. The drug evaluation experiments would then commence. A batch of 25–50 gerbils could be disposed of before they had grown to 55 gm in size. In this manner the weights of the gerbils at the time of the drug evaluation experiment, i.e. the carotid occlusion, was kept within the range of 45–55 gm. Some larger and some smaller gerbils were subjected to carotid occlusion before the time of weight was appreciated (see Results). These early experiments led to the selection of an anesthetic agent (pentobarbital 60 mg/kg i.p.) and intervals of ischemia (50 and 60 min) which were suitable for drug evaluation.

The results were examined for significance using a nonparametric method of comparison. In this method, the mortality data were used to construct a 2 × 2 contingency table of death versus survival for controls and gerbils given test drugs. This table was analyzed for significance using Fisher's Exact Test. For example, consider the 12 gerbils which received isoproterenol after 60 min of bilateral carotid occlusion (see Results). Only 3 of these 12 died as compared to 30 out of 30 deaths with gerbils receiving only saline. In this case the 2 × 2 table looks like this:

<table>
<thead>
<tr>
<th></th>
<th>Lived</th>
<th>Died</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>9</td>
<td>3</td>
</tr>
</tbody>
</table>

The probability of this distribution occurring by chance is calculated for Fisher's Test. If this probability is less than or equal to 0.05, i.e. p ≤ 0.05, the drug effect was taken as significant and one which merited further study. Since the purpose of the experiments with drugs was to identify rather than to prove protective (or deleterious) drug effects, the statistical method is helpful only in so far as it can suggest fruitful choices for further research. No statistical "proof" is claimed beyond this. The requirement that the p of an even more skewed result be calculated and added to the overall probability is obviated because one of the cells of the 2 × 2 table is a zero.

**Results**

The mortality rate of the saline-treated gerbils was compared with gerbils given the different anesthetic agents (fig. 1). Ketamine anesthesia was associated with a high mortality rate, especially during the period of occlusion. After 10 min of carotid arterial occlusion the gerbils would begin to struggle and develop gasping respirations. Several gerbils developed frothy pulmonary secretions before they expired. Similar problems were encountered with diethyl ether as the anesthetic agent. With both anesthetic agents frothy pulmonary secretions and intra-operative deaths occurred. Since a smooth level of anesthesia for up to 60 min duration was desired, further experimentation with ketamine and diethyl ether was abandoned. Pentobarbital, urethane and chloralose anesthesia resulted in similar mortality curves. Gerbils anesthetized with pentobarbital recovered within 2–3

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**Figure 1.** Mortality rate of gerbils subjected to bilateral carotid arterial occlusion using different anesthetic agents. NaCl (0.9%) was administered to some gerbils as the control for the drug evaluation experiments.
hours, gerbils anesthetized with chloralose recovered after 6-8 hours, and those anesthetized with urethane after 10-12 hours. Since most deaths occurred within 12 hours (fig. 2), it was likely that some gerbils anesthetized with chloralose or urethane died prior to recovery from anesthesia. The gerbils anesthetized with pentobarbital recovered a normal posture and righting reflex 1-3 hours after removal of the carotid arterial clips. The signs of cerebral damage began to appear at 3 hours after the ischemic period and became progressively more severe until the animal succumbed. In the first 12 hours after ischemia 88% of the deaths occurred (fig. 3).

Convulsions, tremors and rolling seizures were the most frequent and prominent neurological signs observed. In almost every instance the death of a gerbil occurred during the prolonged tonic contraction of a generalized convolution. After a brief but violent period of thrashing, the gerbils commonly displayed rapid running movements and opisthotonos. Respiratory movements were interrupted during this 15-30 sec extensor phase. Agonal respiratory gasps were followed by a sudden limpness as death occurred. Opening of the chest cavity at that point revealed the heart to be contracting rhythmically but not forcefully. The similarity to the deaths which occur after electroshock or pentylenetetrazol-induced seizures in mice was striking. Occasionally a gerbil remained comatose after the ischemic period and died without apparent recovery from the anesthesia. There were no intraoperative deaths in gerbils anesthetized with pentobarbital, urethane or chloralose. Carotid arterial filling distal to the occluding clips ("back flow") was observed in virtually every instance.

Gerbils which did not die after prolonged cerebral ischemia recovered neurological function, often within 12 hours. After 24 hours of recovery they appeared to be normal. A persisting neurological deficit was frequently limited to unilateral ptosis indicating damage of the cervical sympathetic chain. Three days after 50 min of cerebral ischemia, the survivors could not be readily distinguished from normal gerbils. The stroke index recorded 6 hours after completion of 50 or 60 min of bilateral arterial occlusion is shown in figure 3. There was very little overlap between the scores obtained for the surviving and dying gerbils. The high stroke indices assigned to the dying gerbils was due to the frequency of seizures and abnormal inter-ictal postures when scored with an index designed for the relatively stable neurological deficits that occur after unilateral carotid arterial ligation. The stroke index was not helpful in characterizing the neurological deficits after bilateral occlusion, but it did predict the all-or-none aspect of death versus survival. There were no sex differences in mortality rate after 50 or 60 min of ischemia.

The results of the experiments using drugs are summarized in the table. Gerbils anesthetized with pentobarbital but receiving no treatment, had a mortality rate identical to the gerbils which received saline injection. Sixty minutes of bilateral carotid arterial occlusion was always fatal in gerbils weighing over 45g, although a lower mortality rate was obtained in smaller (i.e. younger) gerbils (fig. 4). Fifty min of bilateral carotid arterial occlusion was nearly always survived by gerbils weighing 45-55 gm. Gerbils weighing less than 45 gm or more than 55 gm were not used in the experiments of drug evaluation.

Administration of isoproterenol hydrochloride, amphetamine sulfate and methylprednisolone sodium succinate resulted in a significant improvement in mortality rate \( p \leq 0.05 \). Administration of physostigmine following 50 min of cerebral ischemia resulted in a higher mortality rate than the saline-treated gerbils. It is possible, however, that a beneficial effect was masked by the marked peripheral effects of inhibition.
Table 4. Mortality Rates in Gerbils Anesthetized with Pentobarbital 60 mg/kg I.P. and Subjected to Bilateral Carotid Arterial Occlusion of 50 or 60 Min Duration

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mortality after 50 min of cerebral ischemia</th>
<th>Mortality at 60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (anesthesia only)</td>
<td>14% (14)</td>
<td>100% (14)</td>
</tr>
<tr>
<td>NaCl (45-55 gm gerbils)</td>
<td>7% (30)</td>
<td>100% (30)</td>
</tr>
<tr>
<td>Isoproterenol 50 ug/kg</td>
<td>0% (6)</td>
<td>25%* (12)</td>
</tr>
<tr>
<td>Amphetamine 5 mg/kg</td>
<td>16% (6)</td>
<td>50%* (6)</td>
</tr>
<tr>
<td>Methylprednisolone 35 mg/kg</td>
<td>33% (6)</td>
<td>58%* (12)</td>
</tr>
<tr>
<td>Physostigmine 0.25 mg/kg</td>
<td>75% (4)</td>
<td>38%* (8)</td>
</tr>
<tr>
<td>Chlorpromazine 5 mg/kg</td>
<td>50% (6)</td>
<td>19% (6)</td>
</tr>
<tr>
<td>NaCl (35-40 gm gerbil)</td>
<td>0% (8)</td>
<td>43% (10)</td>
</tr>
<tr>
<td>Uridine 100 mg/kg</td>
<td>50% (6)</td>
<td>67% (6)</td>
</tr>
<tr>
<td>Atropine 1 mg/kg</td>
<td>67%** (6)</td>
<td>100% (6)</td>
</tr>
<tr>
<td>Thiosemicarbazide 4 mg/kg</td>
<td>67%** (6)</td>
<td>100% (6)</td>
</tr>
<tr>
<td>Phenytoin 50 mg/kg</td>
<td>86%** (14)</td>
<td>100% (6)</td>
</tr>
<tr>
<td>AOOA 100 mg/kg</td>
<td>100%** (6)</td>
<td>100% (6)</td>
</tr>
<tr>
<td>Theophylline 50 mg/kg</td>
<td>100%** (12)</td>
<td>100% (6)</td>
</tr>
</tbody>
</table>

*p < 0.05 vs. NaCl group at same weight = protective effect. **p < 0.05 vs. NaCl group at same weight = adverse effect. *Number of animals.

Discussion

The absence of posterior communicating arteries is the most often quoted reason for the gerbil's unique susceptibility to carotid arterial ligation. Even with both carotid arteries occluded, however, some blood reaches the cerebral hemispheres. Arterial filling distal to the arterial clip was observed in almost every gerbil in the present experiments. This was attributed to continued collateral perfusion of the cerebral hemispheres during occlusion. Thus, it is more accurate to describe the cerebral ischemia produced by bilateral carotid arterial occlusion in the gerbil as severe, partial ischemia rather than total ischemia. This is significant because residual blood flow during severe ischemia has been associated with a higher mortality rate than total ischemia. However, a residual cerebral blood flow was found to increase survival in gerbils which had been subjected to 30-45 min of bilateral carotid arterial occlusion.
The 100% mortality rate in gerbils subjected to 60 min of bilateral carotid occlusion suggests that the residual flow, if present, was insufficient to prevent the development of lethal changes.

The steep slope of the ischemia-mortality curve (fig. 2) between 50–60 min of occlusion was striking. This abrupt increase in the mortality rate was repeatedly observed in gerbils weighing 45–55 gm. Smaller (i.e., younger) gerbils had an increased tolerance for 60 min of cerebral ischemia. Larger (i.e., older) gerbils had such a reduced tolerance for ischemia that a 75% mortality rate was observed after only 30 min of occlusion (fig. 4). This finding led us to employ gerbils weighing 45–55 gm in the evaluation of drugs. By restricting the gerbils to this narrow weight range, we hoped to minimize the variations in tolerance for ischemia that were related to the size or age of the animal. Increasing delays in the supply of male gerbils led to use of female gerbils despite the sex differences which had been reported for susceptibility to unilateral carotid arterial ligation. Sex-related differences have not been observed in the present experiments in mortality rates after bilateral carotid occlusion.

The role of seizures in the ischemia-related mortality rates deserves special mention. The fact that the gerbil is naturally prone to convulsions has not been stressed in previous reports of experimental cerebral ischemia in gerbils. Repeated, violent seizures are common in gerbils after prolonged bilateral carotid arterial occlusion. Frequently they result in death. The threshold for seizures gradually decreases with increasing age of the gerbil. The incidence of seizures stabilizes after 4–6 months of age or 60–70 gm of weight. It varies from 20% for spontaneous seizures to 97% for stress-provoked seizures in a genetic strain bred for study of the epilepsies. There is no sex difference in the frequency of spontaneous or stressed-provoked (xenogenic) seizures. These seizures have been prevented by phenobarbital and trimethadione, but not by phenytoin or pentobarbital. Seizures which occur after pinealectomy resulted in death during a long-lasting, tonic phase in 19 of 61 gerbils (31%). Spontaneous or stress-provoked convulsions, on the other hand, are seldom fatal. The ischemic pineal gland (or adjacent nervous tissue) may be involved in the seizures which occur after prolonged bilateral carotid arterial occlusion since this region is the watershed zone between the carotid and vertebo-basilar vascular beds.

The increasing sensitivity to prolonged carotid occlusion (fig. 4), parallels the age-related increase in seizure tendency. The 30–35 gm, 6-week-old gerbils had a low tendency to convulse and a low mortality rate after 60 min of carotid occlusion. A 12–16 week-old, 60 gm gerbil had a much greater tendency to develop tonic-clonic seizures and a higher mortality rate after only 30 min of occlusion. The 45–55 gm gerbil utilized in the evaluation of drugs in the present experiments were 8–12 weeks old. They had an intermediate threshold for seizures and displayed an intermediate, ischemia-related, mortality rate. It is possible that the mortality observed after 50–60 min of bilateral carotid occlusion was a consequence of ischemia-produced seizures rather than ischemic brain damage per se. This would explain the lack of a sex difference in the ischemia-related mortality rate of the present experiments compared to the reported increased incidence of fatalities in males subjected to unilateral carotid ligation.

The mortality rate following bilateral carotid occlusion was virtually identical for 3 of the general anesthetics employed. From a technical standpoint pentobarbital was the optimal anesthetic agent because the animals were quiet during the period of carotid occlusion and recovered soon after release of the arterial clips. Pentobarbital has been reported to protect against brain damage in animals subjected to cerebral hypoxia or ischemia. A contrary report has also appeared. In finding an identical mortality rate in gerbils anesthetized with pentobarbital, urethane and chloralose, the present experiments demonstrate that pentobarbital does not have an unique protective effect in gerbils. The question of overall protection conferred by general anesthesia is unresolved. The increased mortality which was associated with ketamine has been reported previously in the gerbils subjected to unilateral carotid ligation.

The usefulness of the model for evaluation of drugs is improved by the number of significant results which were obtained even with relatively small groups of animals. A goal of the proposed model was the detection of an altered post-ischemic mortality rate following administration of a drug. Further experiments would then be necessary to evaluate any observed beneficial or adverse drug effect. Isoproterenol and amphetamine have been reported to increase cerebral blood flow. It is possible that enhancing the critical initial reperfusion of the brain following removal of the carotid clips contributed to the increase survival observed with these 2 agents. Diminished reperfusion of the brain, sometimes referred to as the "no reflow phenomenon," may be a factor in the post-ischemic mortality and could possibly be prevented or ameliorated by these 2 drugs. Against this, however, prevention of post-ischemic hypotension in gerbils after 30 min of bilateral carotid occlusion with metaraminol, an alpha-sympathomimetic agent, did not affect the mortality rate despite prevention of the "no reflow phenomenon" as evaluated by perfusion with India ink. More research is required to resolve these conflicting results.

Administration of thiosemicarbazide and aminoxyacetic acid reduced survival after prolonged cerebral ischemia. Thiosemicarbazide is a convulsant which reduces the level of brain gamma aminobutyric acid (GABA) while aminoxyacetic acid (AOAA) inhibits the metabolism of GABA and results in an elevation of its concentration in the brain. Thiocarbamazide could be potentiating the spontaneous seizure tendency known to be present in gerbils and known to be aggravated following cerebral ischemia. Although an anticonvulsant effect has been attributed to the increase in concentration of GABA following AOAA, this effect was apparently not
sufficient to reduce the post-ischemic mortality. The prolonged elevation of GABA in the brain after administration of AOAA may or may not have contributed to the increased mortality rate after cerebral ischemia, since GABA is known to be elevated after ischemia but to return to normal levels within 3 hours.28

Physostigmine significantly reduced the mortality rate after 60 min of bilateral carotid arterial occlusion. An increased mortality after 50 min of carotid arterial occlusion was observed in gerbils which developed fasciculations and neuromuscular weakness. The early deaths may be explained by the peripheral toxicity and would account for this seeming contradiction. Using Fisher's Exact Test a p < 0.05 was obtained for the protective effect observed after 60 min of ischemia. Physostigmine is known to reverse the depression of the central nervous system associated with anticholinergic overdose caused by atropine or tricyclic antidepressants.40 In addition, it will reverse the sedation produced by the benzodiazepines and phenothiazines.41 This may be a consequence of activation of cholinergic synapses. Atropine, on the other hand, blocks central cholinergic activity. In toxic doses it produces a central excitation followed by coma.42 These 2 agents have opposite effects on the epileptiform activity of chronically isolated cerebral cortex, but only in doses which modify the electroencephalographic activity of normally-innervated brain regions.43

We have previously reported the adverse effect of phenytoin on the survival rate after cerebral ischemia in gerbils.44 While a lack of protection might be predicted on the basis of failure to control spontaneous seizures in gerbils,45 the result obtained is suggestive of drug toxicity. This is not surprising considering the dose employed (50 mg/kg) even though no outward signs of drug toxicity were observed in non-ischemic gerbils. Further experiments with phenytoin have supported this belief, and a post-ischemic mortality rate identical to that of saline controls was obtained with a dose of 10 mg/kg (unpublished observation). Finally, aminophylline, a mixture of theophylline and ethylenediamine, has been reported to have an adverse effect on the survival of gerbils subjected to unilateral carotid ligation.46 In addition, mice subjected to anoxia had a higher mortality rate after the administration of aminophylline due to an increased cerebral metabolic rate.47

In conclusion, the present method involves a surgical procedure which is simple and can be performed without respiratory supports or monitoring of vital signs inherent in other, more complicated models of cerebral ischemia. Observation of mortality rates in small groups of animals can discriminate between protective and adverse effects associated with administration of a test drug. Although numerous models of cerebral ischemia have been used for drug evaluation, none lends itself quite as effectively to the evaluation of many different drugs. A non-parametric statistical test enhances the utility of comparing the results obtained in relatively small groups of animals.
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