A Gerbil Model of Cerebral Ischemia Suitable for Drug Evaluation

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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, thiosemicarbazide 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.

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 was confirmed 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
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 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 x 25 x 14 cm cage, and fed Purina rodent laboratory chow until they reached 45 gm. The drug evaluation experiments would then commence. A 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.

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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 opisthotonus. 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 pentylentetrazol-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 ≤ 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
Carotid arterial occlusion in gerbils weighing 35-60 gm survived 60 min of bilateral carotid arterial occlusion. With these possible drug-related deaths, 5 of 8 gerbils died within one hour of drug administration. These were the only deaths which occurred prior to 3 hours after carotid occlusion in the entire series. Even of acetylcholinesterase. Fasciculation, salivation and neuromuscular weakness were observed in post-ischemic gerbils receiving physostigmine. Three of the gerbils died within one hour of drug administration. These were the only deaths which occurred prior to 3 hours after carotid occlusion in the entire series. Even with these possible drug-related deaths, 5 of 8 gerbils survived 60 min of bilateral carotid arterial occlusion. This improved mortality rate was significant at p ≤ 0.05 level. The apparently adverse effect at 50 min of ischemia was not significant owing to a small N.

Gerbils given chlorpromazine after cerebral ischemia had a prolonged arousal time. This dosage was sufficient to sedate unanesthetized non-ischemic gerbils markedly for 3–4 hours. The mortality rate was not different from the saline-treated gerbils of the same weight range. The suggestive beneficial effect on survival after 60 min of ischemia must be discounted owing to the small size of the gerbils used to test this particular drug. Uridine produced no apparent effect when administered to non-ischemic gerbils. The mortality rate after cerebral ischemia in gerbils given uridine was not significantly different from that of saline-treated gerbils.

Administration of atropine sulfate and thiosemicarbazide resulted in a significant adverse effect on post-ischemic survival (p ≤ 0.05). The gerbils that had received atropine did not appear qualitatively different from the saline-treated gerbils despite the increased mortality rate which was observed. The gerbils that had received the convulsant thiosemicarbazide, however, were noticeably different from the saline controls. They died after a single, brief seizure which had mainly a tonic extensor component. The violent repeated convulsions and rolling seizures seen in the saline-treated gerbils were not observed.

Neither phenytoin nor theophylline had a demonstrable effect when given to awake or anesthetized non-ischemic gerbils; the effect on post-ischemic mortality was significantly adverse (p ≤ 0.05). AOAA administration to unanesthetized gerbils resulted in moderate sedation for 3–4 hours; the righting reflex was maintained but spontaneous activity ceased during that interval. The gerbils which had received AOAA after 50 minutes of carotid arterial occlusion were more likely to die than were saline-treated animals. The 100% mortality rate after 50 minutes of cerebral ischemia was significantly different from that of the saline-treated gerbils (p ≤ 0.05).

Discussion

The absence of posterior communicating arteries is the most often quoted reason for the gerbil's unique susceptibility to carotid arterial ligation.1,2,6 Even with both carotid arteries occluded, however, some blood reaches the cerebral hemispheres.12,13 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.16,17 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.12,20

Table: 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 µg/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 (35-40 gm gerbil)</td>
<td>50% (6)</td>
<td>19% (6)</td>
</tr>
<tr>
<td>NaCl (35-40 gm gerbil)</td>
<td>0% (8)</td>
<td>43% (12)</td>
</tr>
<tr>
<td>Uridine 100 mg/kg</td>
<td>50% (6)</td>
<td>67% (8)</td>
</tr>
<tr>
<td>Atropine 1 mg/kg</td>
<td>67%** (6)</td>
<td>67% (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>AOAA 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.

FIGURE 4. Mortality rate after 30 and 60 min of bilateral carotid arterial occlusion in gerbils weighing 35-60 gm (6-15 weeks of age).
The 100% mortality rate in gerbils subjected to 60 min of bilateral 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 stress-provoked 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 vertebral-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 amino-oxyacetic acid reduced survival after prolonged cerebral ischemia. Thiosemicarbazide is a convulsant which reduces the level of brain gamma aminobutyric acid (GABA) while amino-oxyacetic acid (AOAA) inhibits the metabolism of GABA and results in an elevation of its concentration in the brain. This decrease in GABA could be potentiating the spontaneous seizure tendency known to be present in gerbils and known to be aggravated following cerebral ischemia.
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.

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. In addition, it will reverse the sedation produced by the benzodiazepines and phenothiazines. 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. 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.

We have previously reported the adverse effect of phenytoin on the survival rate after cerebral ischemia in gerbils. While a lack of protection might be predicted on the basis of failure to control spontaneous seizures in gerbils, 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. In addition, mice subjected to anoxia had a higher mortality rate after the administration of aminophylline due to an increased cerebral metabolic rate.

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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