Attenuation of Ischemic Brain Edema by Pentobarbital after Carotid Ligation in the Gerbil

Pablo Lawner, M.D., John Laurent, M.D., Frederick Simeone, M.D., Eugene Fink, M.S., and Elliot Rubin, Ph.D.

SUMMARY  The efficacy of pentobarbital in the treatment of ischemic cerebral edema was evaluated in 160 gerbils. Animals underwent carotid ligation under ether or pentobarbital (50 mg/kg) anesthesia. The pentobarbital anesthetized group received an additional dose of 30 mg/kg 4 h after ligation. Animals were evaluated for neurologic deficit at 4 and 8 h post-ligation, then sacrificed. Water content of each hemisphere and swelling percentage in animals anesthetized with ether was 6.374 ± 0.89 SE, whereas gerbils who underwent sham carotid ligation showed a negligible (0.491 ± 0.15) swelling percentage (p < 0.01). Pentobarbital animals had a swelling percentage of 3.359 ± 0.68. This represents a significant edema reduction compared to ether-anesthetized animals (p < 0.01). Neurologic deficit was decreased by 56.7% (17/60 vs 30/60) in pentobarbital animals compared with ether animals (p < 0.025). Mortality at 8 hours was reduced by 75% (2/60 vs 8/60) in pentobarbital animals (p < 0.05).

MANY INVESTIGATORS have shown that barbiturates provide a "protective" effect for the brain in regional as well as global ischemia. Recently, barbiturates have also been shown to inhibit the formation of cerebral edema produced by cryogenic injury. The development of edema on the ischemic brain plays a significant role in the severity and final outcome of an ischemic process.

Unilateral carotid artery ligation in the Mongolian gerbil results in fatal cerebral infarction in 40 to 60% of animals. The affected animals develop neurologic deficit, including seizures, and usually die shortly thereafter with evidence of complete ipsilateral hemispheric infarction.

This study was designed to evaluate the effect of pentobarbital on the development of ischemic brain edema 8 h after unilateral carotid artery ligation in the gerbil.

Materials and Methods

The experimental series consisted of 160 adult Mongolian gerbils divided into 3 groups. Forty animals underwent a sham operation, 20 of them receiving ether anesthesia and 20 pentobarbital 50 mg/kg i.p. Sixty untreated-ligated were anesthetized with ether, and 60 barbiturate-treated animals were anesthetized with pentobarbital 50 mg/kg i.p. Prior to surgery all animals received 1% trypan blue 1 cc. i.p. and either pentobarbital or an equivalent volume of normal saline.

The right common carotid artery was exposed through a 2 cm midline cervical incision, carefully isolated from adjacent structures, coagulated with bipolar current and divided. In the sham-operated animals the right carotid artery was exposed and isolated, but neither coagulated nor divided.
TABLE 1  Mortality and Neurologic Deficit 8 Hours After Carotid Ligation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Mortality</th>
<th>Neurologic deficit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated-Ligated</td>
<td>60</td>
<td>8</td>
<td>30</td>
</tr>
<tr>
<td>Pentobarbital-Treated</td>
<td>60</td>
<td>2</td>
<td>17</td>
</tr>
</tbody>
</table>

（Significance）$p < 0.05$ $p < 0.025$

The animals were returned to individual cages and observed at 4 and 8 h post-carotid ligation for the development of neurologic deficit in the form of decreased level of consciousness, head cocking, splaying hind limb, circling behavior and seizures. At 4 h, the pentobarbital-treated group was given an additional 30 mg/kg of pentobarbital i.p. Eight hours after carotid occlusion, all animals were sacrificed by decapitation, the brains were quickly removed, and the brain stem was sectioned at the cerebral peduncles. The cerebrum was divided through the midline and each hemisphere weighed with an Ainsworth Delta NV balance (sensitivity 0.01 mg), placed in a vacuum oven at 60°C, and 0.5 atmospheres for at least 72 h by which time no further change in weight occurred with further desiccation. The procedure from the time of sacrifice until the sections were placed in the oven took no longer than 5 min. From the wet and dry weights, the percent water content of each hemisphere was calculated. Swelling percentage was calculated using the adaptation of the method of Elliott and Jasper described by Plum, et al.

Animals that died prior to the eighth hour were not utilized for edema measurements, but these early mortalities were analyzed by treatment category.

Non-parametric statistical tests of significance were chosen because the distributions did not conform adequately to the assumption of the parametric analyses. The Kruskal-Wallis Anova test was conducted over all the groups for each of the dependent variables. If statistical significance was detected, Mann-Whitney U tests were conducted on each of the combinations of groups to be compared. Significance was assumed when $p < 0.05$.

Results

Mortality and Morbidity

None of the sham-operated animals died or developed evidence of neurologic deficit. In the untreated-ligated group 8 (13.3%) animals died prior to 8 h, and 30 (50.0%) showed evidence of neurologic deficit. On the other hand, in the pentobarbital-treated group 2 (3.3%) died, and 17 (28.3%) developed neurologic deficit. This represents a 75% decrease in mortality ($p < 0.05$), and a 56.7% decrease in neurologic deficit ($p < 0.025$) (table 1).

Trypan blue staining was not observed in any of the 160 brains studied.

Edema

Sham-operated

The percent water content of the right and left hemispheres were not significantly different ($p > 0.05$). Swelling percentage was negligible (0.419 ± 0.15).

There was no significant difference between ether and pentobarbital sham-operated animals in percent water contents and swelling percentage ($p > 0.05$ for all comparisons) (table 2).

Untreated-ligated

In this group there was a significant increase in water content in the right hemisphere compared to sham-operated animals ($p < 0.005$). Swelling percentage was 6.734 ± 0.89 which represents a significant increase over sham-operated animals ($p < 0.001$) (table 3).

In those animals without neurologic deficit (N = 30) there was a significant increase in both water content of the right hemisphere ($p < 0.025$) and swelling percentage ($p < 0.01$) (table 4). In the group that developed neurologic deficit but survived the 8 hours

TABLE 2  Mean Percent Water Content and Mean Swelling Percentage in Sham-Operated Animals

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>% Water content right hemisphere</th>
<th>% Water content left hemisphere</th>
<th>Swelling percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shams-Ether</td>
<td>20</td>
<td>78.752 ± 0.16</td>
<td>78.695 ± 0.15</td>
<td>0.516 = 0.20</td>
</tr>
<tr>
<td>Shams-Pentobarbital</td>
<td>20</td>
<td>78.818 ± 0.08</td>
<td>78.747 ± 0.09</td>
<td>0.322 = 0.22</td>
</tr>
<tr>
<td>All Shams</td>
<td>40</td>
<td>78.785 ± 0.09</td>
<td>78.721 ± 0.09</td>
<td>0.419 = 0.15</td>
</tr>
</tbody>
</table>

TABLE 3  Mean Percent Water Content of Right and Left Hemispheres and Mean Swelling Percentage in Untreated-Ligated and Pentobarbital-Treated Animals

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>% Water content right hemisphere</th>
<th>% Water content left hemisphere</th>
<th>Swelling percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated-Ligated</td>
<td>52</td>
<td>80.305 ± 0.20</td>
<td>78.960 ± 0.09</td>
<td>6.374 = 0.89</td>
</tr>
<tr>
<td>Pentobarbital-Treated</td>
<td>58</td>
<td>79.458 ± 0.19</td>
<td>78.849 ± 0.08</td>
<td>3.359 = 0.68</td>
</tr>
</tbody>
</table>

（Significance）$p < 0.005$ $p > 0.05$ $p < 0.01$
there was a significant increase in percent water content in the right hemisphere \((p < 0.001)\), but not in the left hemisphere \((p > 0.05)\) (table 3). In those animals without neurologic deficit \((N = 43)\) percent water content of both hemispheres and swelling percentage was not significantly different from sham-operated animals \((p > 0.01)\) (table 3).

### Barbiturate-treated

In this group, water content of the right hemisphere was increased over sham-operated animals \((p < 0.05)\), but it was significantly less than in untreated-ligated \((p < 0.005)\).

The swelling percentage was 3.359 ± 0.68 which was higher than in shams \((p < 0.005)\), but 47.3% lower than in untreated-ligated \((p < 0.01)\) (table 3). In those animals without neurologic deficit \((N = 43)\) percent water content of both hemispheres and swelling percentage was not significantly different from sham-operated animals \((p > 0.01)\) for all comparisons. Percent water content of the right hemisphere was significantly decreased from untreated-ligated \((p < 0.025)\) (table 4). In the animals with neurologic deficit that survived the 8 h \((N = 15)\) there was a significant increase in percent water content in the right hemisphere \((p < 0.001)\), but not in the left \((p > 0.1)\). Swelling percentage was also significantly increased over shams \((p < 0.001)\), and no different from untreated-ligated with neurologic deficit.

### Discussion

Our data show that pentobarbital significantly decreased the development of ischemic brain swelling. Untreated-ligated animals without neurologic deficit had significant brain swelling compared to sham-operated animals, but pentobarbital therapy completely prevented the development of edema among those animals without neurologic deficit. In those animals that developed neurologic deficit, edema was maximal and it was not reduced by barbiturate administration. The precise difference between stroke-prone and stroke-insensitive gerbils is not fully understood, but a likely explanation is a variation in the degree of collateral circulation available. These data suggest that barbiturates protect those animals with marginal collateral circulation by extending the ischemia tolerance time until collateral channels can dilate. This is further supported by reports that in some models even a single dose of pentobarbital is beneficial after a major cerebral arterial occlusion.

In this study pentobarbital also significantly decreased the mortality by 75% and incidence of neurologic deficit by 56.7% over the period of study. As expected, trypan blue staining was not observed in any of the brains. This supports the concept that in the gerbil vascular permeability to serum proteins remains normal up to 18 h after carotid ligation, and that the edema seen up to this time is intracellular cytotoxic in nature.

This study confirms previous observations that pre-treatment with barbiturates decreases acute ischemic cerebral edema.

### References

10. Yatsu F, Diamond I, Graziano C, Linquist P: Experimental

### Table 4: Mean Percent Water Content and Mean Swelling Percentage in Untreated-Ligated and Pentobarbital-Treated Animals Without Neurologic Deficit

<table>
<thead>
<tr>
<th>Treated</th>
<th>n</th>
<th>% Water content right hemisphere</th>
<th>% Water content left hemisphere</th>
<th>Swelling percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated-Ligated</td>
<td>30</td>
<td>79.158 ± 0.08</td>
<td>78.805 ± 0.11</td>
<td>1.614 ± 0.52</td>
</tr>
<tr>
<td>Pentobarbital-Treated</td>
<td>43</td>
<td>78.870 ± 0.09</td>
<td>78.731 ± 0.07</td>
<td>0.662 ± 0.20</td>
</tr>
</tbody>
</table>

(Significance) \( p < 0.025 \) \( p > 0.2 \) \( p > 0.1 \)

### Table 5: Mean Percent Water Content and Mean Swelling Percentage in Untreated-Ligated and Pentobarbital-Treated Animals With Neurologic Deficit

<table>
<thead>
<tr>
<th>Treated</th>
<th>n</th>
<th>% Water content right hemisphere</th>
<th>% Water content left hemisphere</th>
<th>Swelling percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated-Ligated</td>
<td>22</td>
<td>81.333 ± 0.12</td>
<td>79.166 ± 0.15</td>
<td>12.721 ± 0.67</td>
</tr>
<tr>
<td>Pentobarbital-Treated</td>
<td>15</td>
<td>81.533 ± 0.29</td>
<td>79.195 ± 0.22</td>
<td>11.258 ± 0.83</td>
</tr>
</tbody>
</table>

(Significance) \( p > 0.6 \) \( p > 0.7 \) \( p > 0.2 \)
Barbiturates in Focal Ischemia of Primate Cortex: Effects on Blood Flow Distribution, Evoked Potential and Extracellular Potassium


SUMMARY The effect of an ultra-short acting barbiturate, methohexital, on the distribution of blood flow in baboon cerebral cortex was studied following occlusion of the middle cerebral artery under conditions of constant blood pressure. Further experiments assessed the effects of methohexital and pentobarbital on the threshold relationships (established in earlier work) between flow, cortical evoked potential amplitude and extracellular potassium activity. Regional flow was measured by the hydrogen clearance technique and the initial anesthetic was chloralose in all experiments. If flow after occlusion was > 25 ml/100g/min, so that electrical activity was sustained, methohexital reduced flow in proportion to the flow (r = -0.84, p < 0.001), but if flow was < 20 ml/100g/min, and electrical activity was reduced or absent, a significant elevation in flow occurred averaging 3.4 ml/100g/min (p < 0.01), an appreciable fraction of ambient flow. This result may be attributable to an inverse steal, blood being diverted into ischemic regions from vasoconstriction induced in relatively well-perfused areas. No statistically significant change could be demonstrated either in the flow threshold for the abolition of the evoked potential or in that for the massive increase in potassium, although methohexital tended to decrease, and pentobarbital to increase, these thresholds. However, methohexital significantly reduced the rate of decrease of the evoked potential for a given flow below the threshold. These effects may be among factors underlying any protective effect of barbiturate in focal cerebral ischemia on the neurological and neuropathological levels.

THE POSSIBILITY that barbiturates may exert a protective effect in the ischemic brain has stimulated a number of investigations using several experimental models. Protection has been suggested in terms of reduced infarct size, reduced neurological deficit and increased survival time following stroke, with both pre- and post-insult administration of the drug. However, as Michenfelder has pointed out, extending these models to clinical trials in order to assess the protective effect of barbiturates for the treatment of acute stroke may be difficult, chiefly because of the hemodynamic and pulmonary complications accompanying the anesthetic effect of barbiturates given at the doses suggested as necessary by experiment. More refined therapeutic regimes might nevertheless be designed which would avoid these complications. If this is to be achieved, the effects of the drugs on factors such as cerebral blood flow and its distribution, local metabolism or osmotic forces, which might be presumed to underlie any protective effect, must be measured and understood.

The present study was designed to assess the effects of 2 intravenous barbiturates, pentobarbital and ultra-
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