Dose Dependency of the Post-Insult Protective Effect of Pentobarbital in the Canine Experimental Stroke Model


SUMMARY In a canine stroke model, dose dependent protection by postocclusion pentobarbital was suggested from 10-40 mg/kg. In 28 dogs investigated (10 from a previous study) a distinct, significant reduction in right cerebral hemisphere infarction occurred in animals given 15-20 mg/kg pentobarbital intramuscularly 1 hour postocclusion. Increased dosages did not alter statistically the infarct size and 2 dogs at the 50 mg/kg and 80 mg/kg levels died of barbiturate-induced respiratory failure.

STUDIES BY Yatsu, Smith, and Hoff have demonstrated that pre-insult administration of pentobarbital is protective in the experimental stroke model, significantly reducing the degree of infarction and/or the eventual neurological deficit. Some studies show that the protective effect can be conferred by one injection of pentobarbital or intraperitoneal pentobarbital, others indicate that continuous barbiturate administration produces this effect. Shapiro has shown recently that with patients there can be a useful clinical effect gained by the administration of pentobarbital to head injuries and this effect is associated with and presumably at least in part due to the lowering of intercranial pressure. The pressure reduction is more marked when the intracranial pressure is raised then when it is normal.

That administration after stroke onset could have a protective effect was suggested by a previous study. Dose dependency of a suspected pharmacological effect is a criterion for its acceptance as such. The current study was undertaken to examine the possibility of dose dependency of this post-insult protective effect.

Methods

Eighteen mongrel dogs weighing between 15-22 kg received preoperative atropine sulfate 0.6 mg intramuscularly. Anesthesia was induced by mask with nitrous oxide-oxygen-halothane sufficient to place an orotracheal cannula and maintained similarly at inspired concentration of 70%-28.5%-1.5% respectively. Ventilation was controlled by a piston respirator set at a calculated minute volume of 250 ml/kg. In a majority of the dogs a direct mean femoral arterial pressure was measured and varied between 75-90 torr. A peripheral venous line infused normal saline at 5 ml/kg/hr.

Under sterile conditions a right temporal craniectomy was performed wide enough to allow easy elevation of the temporal lobe exposing the carotid bifurcation when the dura was opened. The right internal carotid and middle cerebral arteries were clipped in a tandem fashion by a clip and skin, the anesthetic was discontinued and the dogs regained consciousness promptly. One hour after the clipping, varying doses of pentobarbital (10-80 mg/kg) were administered intramuscularly. The dosage given was selected at random from a schedule.

The dogs were kept under normal kennel conditions for 1 week and then sacrificed and the brains removed and fixed in formalin solution for another 7 days. The percentage of infarction of the right cerebral hemisphere was calculated by techniques described previously. Ten dogs from a previous study were added to the data generated in this investigation to alleviate duplication of zero pentobarbital control points and the 40 mg/kg level. One dog at the 25 mg/kg pentobarbital level had an abnormally massive infarct of the right cerebral hemisphere and was excluded. Analysis of variance showed he was markedly different from other dogs at this dose level as well as those in surrounding groups.

Statistical significance between each dosage level was determined by the Student’s and Dunnett’s t-test using a standard subroutine on a Tektronix Model 31 calculator.

Results

Table 1 lists the percent infarct observed in the right cerebral hemisphere of 25 dogs studied in our laboratory. On the bottom line the average values at each dose level suggest a progressive decrease in infarct size with a plateau at the 15-20 mg/kg dosages.

Figure 1 illustrates the diminution in percent right cerebral hemisphere infarct with the dose related "break point" at the 15-20 mg/kg level. Two dogs administered 50 mg/kg and 80 mg/kg of pentobarbital respectively recovered from the operation, but lapsed into unconsciousness following the administration of the drug and died of respiratory failure reminiscent of barbiturate overdose.

Discussion

This study confirms previous work that postocclusion administration of barbiturate is effective in attenuating cerebral infarct in the canine stroke model. Moreover, this protection is apparently dose dependent over the range of 10-40 mg/kg intramuscular pentobarbital with significant infarct size reduction occurring by 15-20 mg/kg levels. At dosages greater than 20 mg/kg maximal protection continued, until death ensued in the 50 and 80 mg/kg dogs from respiratory failure. None of our dogs was given any...
TABLE 1.

## Infarct Size (%) in the Right Cerebral Hemisphere of Individual Dogs With Graded Pentobarbital Doses Given 1 Hour Post-insult and the Average ± 1 Standard Error of the Mean at Each Dose Level. Significant differences Between Treated Dogs and the 0 Dose Group are Denoted by Asterisks: * = p <0.05, **p <0.01, ***p <0.001

<table>
<thead>
<tr>
<th>Dog</th>
<th>Dose of pentobarbital administered (mg/kg)</th>
<th>0</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>22.3</td>
<td>12.5</td>
<td>6.5</td>
<td>9.0</td>
<td>6.3</td>
<td>4.0</td>
<td>4.6</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>12.0</td>
<td>20.0</td>
<td>10.0</td>
<td>3.0</td>
<td>4.8</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>28.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>22.5</td>
<td>15.8</td>
<td>4.0</td>
<td>11.0</td>
<td>9.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>23.0</td>
<td>25.0</td>
<td>5.4**</td>
<td>5.6*</td>
<td>4.0</td>
<td>5.1***</td>
<td></td>
</tr>
<tr>
<td>AVE</td>
<td></td>
<td>21.6</td>
<td>14.9</td>
<td>8.3*</td>
<td>5.4**</td>
<td>5.6*</td>
<td>4.0</td>
<td>5.1***</td>
</tr>
<tr>
<td>± 1 SEM</td>
<td></td>
<td>±2.6</td>
<td>±4.3</td>
<td>±1.8</td>
<td>±2.0</td>
<td>±0.8</td>
<td>±1.1</td>
<td></td>
</tr>
</tbody>
</table>

Our animals were anesthetized with halothane at constant concentrations intermediate between their animals. Our dogs untreated with barbiturates demonstrated an average infarct of 21.6% which is similarly situated between their halothane results. Apparently halothane itself has some dose dependent effect on cerebral ischemia in the canine stroke model by a mechanism as yet unknown.

It has been suggested that the deleterious effects of halothane in focal brain ischemia could be due to increased intracranial pressure (ICP) resulting from raised cerebral blood flow with halothane. If so, the lowering of ICP with post-insult barbiturates would help explain their protective nature in our stroke model. However, that this is the sole respiratory or cardiovascular support after the barbiturate injection and yet all survived except the two high dose animals.

The fact that our dogs were not supported physiologically after receiving barbiturates may introduce extraneous variables into the experimental design. First, pentobarbital causes respiratory depression and a rise in PaCO₂ and with increasing drug dosage there would be a presumed increasing hypercarbia. It is doubtful if this disturbance would be beneficial to ischemic brain since the potential for an "intracerebral steal" would exist with obvious detrimental effects on the peri-infarctional ischemic areas. Hypotension evolving from larger barbiturate dosages would adversely influence the ischemic areas of the cerebrum as well. While core temperature was not monitored in the animals after treatment, it is doubtful if any possible short term hypothermia would have reduced the measured stroke area. In general, the lack of intensive care for the dogs after pentobarbital treatment should have adversely affected our results and the persistence of increasing protection from brain ischemia by pentobarbital in these animals strengthens the dose-dependent relationship.

The application of these data to the human clinical situation requires that particular note be paid to the dangers of respiratory inadequacy at higher dosages. In this respect it appears that the protection conferred by 20 mg/kg was as effective at the 40 mg/kg level reported in our previous study. This might facilitate the human clinical situation in that the risk of overdose and respiratory failure need not be approached so nearly.

The exact dose equivalency for pentobarbital between man and dog is speculative since differences certainly exist in receptor mass, tissue reservoirs, redistribution binding, and other factors affecting the drug response. Yatsu reported recently on the complete resolution of symptoms in an acute stroke victim with treatment by phenobarbital. The dosages used were approximately 5 times the usual hypnotic levels in man, while the lowest effective point in our canine study was about 14 times the recommended human hypnotic dose of pentobarbital. Obviously considerable work needs be done to quantify dose efficacy in humans for post-insult barbiturate protection.

The effect of halothane on the infarct size is variable. Smith, et al. found that "light" halothane used during the arterial clipping produced infarcts occupying 10% of the cerebral hemisphere whereas "deep" halothane with or without hypotension increased infarct size to about 30%.

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Dose Dependent Reduction of Glucose Utilization by Pentobarbital in Rat Brain

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SUMMARY A new method of determining the rate of glucose utilization in brain regions of individual rats has been used to measure the dose dependency of the reduction of the metabolic activity of the cerebral cortex by pentobarbital. Cerebral cortical glucose utilization is depressed to a basal level of 44% of the control rate when cerebral pentobarbital levels exceed 50 μg per g of tissue. The major portion of this effect occurs between the cerebral pentobarbital range of 10-20 μg per g, which can be achieved by 1/5 to 1/10 the normal anesthetic intraperitoneal dosage. If a depression of brain metabolism is responsible for the previously reported protection of the brain from ischemic damage, these data suggest a substantial reduction of brain metabolic rate is achieved in the rat at a barbiturate dosage which may be therapeutically relevant in the human after acute brain ischemia.

CEREBRAL ISCHEMIA leads to deprivation of substrates (glucose and oxygen) and excessive accumulation of metabolic intermediates (e.g. lactate) in the brain.1 Most protective mechanism seems unlikely in that the intracranial pressure in all these experimental animals would be expected to have been in the normal range since they had had wide temporal craniectomies and therefore subtemporal decompression over the area of swollen brain. It seems more likely that the reduction of intracranial pressure, though beneficial to the survival of ischemic brain, is not a primary phenomenon but is a secondary manifestation of it. It is possibly related to interaction with the metabolic mechanism for the control of cerebral blood flow and therefore of cerebral blood volume. It might be commented that the procedure itself induces the infarction, if this were so then results suggest barbiturate protection from this insult at least. However, tandem occlusion of major vessels is a well established technique for the production of experimental ischemia induced stroke.1,2

While useful intraoperative reduction of ICP by barbiturates has been shown in man,11-12 only isolated attempts have been made to apply this potential protection to the acute stroke victim. Certainly the elderly, generally debilitated patient would withstand substantial barbiturate treatment poorly at best, but more fit persons with isolated cerebral lesions many well benefit from barbiturate intervention. Our study suggests that post-insult therapy is both efficacious and significantly dose dependent allowing for lower dosages to affect a maximal response.

References
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