Pentobarbital Protection from Cerebral Infarction Without Suppression of Edema

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SUMMARY We studied the mechanism of barbiturate protection from focal cerebral infarction in cats by examining in detail edema formation 72 hours after acute, permanent occlusion of the left middle cerebral artery (LMCA). Neurological function, gas exchange, vital signs, and intracranial pressure (ICP) were observed during the post-occlusion period, and infarct size and cerebral edema were measured after sacrifice.

Infarct size was reduced only when pentobarbital was given before occlusion and continued for 24 hours. Edema formation was not suppressed even though the extent of infarction was. Clinical evidence of stroke developed and ICP rose in most cats after occlusion despite the presence of pentobarbital sufficient to reduce infarct size.

Elevated ICP accounted for most premature deaths despite intensive cardiopulmonary support. Water and electrolyte changes in the ischemic hemisphere continued to develop throughout the 72 hour post-occlusion period in pentobarbital-treated cats, suggesting that resolution of edema was delayed by the drug. We conclude that pentobarbital reduces infarct size and attenuates the expected time course of ischemic edema in cats, but that the drug has little effect on the severity of edema that develops after arterial occlusion.

METHODS

Successful experiments were performed in 37 cats (3.1 kg ± 0.1 se) that were anesthetized with halothane (4% in oxygen), intubated, paralyzed (succinylcholine, 2 mg/kg IM), and ventilated with a small animal respirator connected to an Ohio anesthesia machine. Halothane concentration was then reduced to 0.8-2.0% to maintain surgical anesthesia. A femoral artery was cannulated to monitor systemic arterial pressure (SAP) and to sample blood intermittently for measurement of arterial oxygen (PaO₂), carbon dioxide (PaCO₂), pH, and hematocrit. A second femoral vein was cannulated to infuse fluids and drugs as necessary. Body temperature was measured by a rectal probe and maintained within a physiological range (36-38°C) by a heating pad. End tidal carbon dioxide (PET CO₂) was measured from expired air by an infrared CO₂ analyzer.

Cats were positioned prone in a stereotaxic frame, and a multiperforated polyethylene catheter was placed in the parietal subdural space to measure intracranial pressure (ICP). The catheter was sealed in place with dental acrylic. EEG electrodes were attached to skull screws overlying the two cerebral hemispheres with the indifferent electrode at the glabella. SAP, PET CO₂, ICP and EEG were displayed continuously on a Gilson ink recorder.

The left middle cerebral artery (LMCA) was exposed transorbitally through an enlarged optic canal. Halothane was discontinued in all cats when the MCA was exposed. The LMCA was either preserved intact (Group I) or occluded by bipolar coagulation (Groups II, III, and IV). The dural opening was sealed with a fat pledget to prevent CSF leak and the palpebral fissure was closed with clips. Antibiotics were administered before each experiment and every six hours thereafter. All surgical maneuvers were performed with aseptic technique.

Cats were divided into four groups: Group I, sham-operated controls (n = 6); Group II, MCA occlusion (n = 12); Group III, MCA occlusion 30 minutes after 30 mg kg IV pentobarbital, given over 5 minutes (n = 10); and Group IV, MCA occlusion 30 minutes after 30 mg/kg IV pentobarbital, given over 5 minutes and 5 mg/kg IV given every 4 hours for 24 hours (n = 9).

Cats were returned to their cages when they were alert with adequate spontaneous ventilation and cough-gag reflexes. Continuous intensive care during the...
postocclusion period included pulmonary toilet, chest physical therapy, and intermittent positive pressure ventilation. Arterial blood gases were measured intermittently to assure adequate respiration (PaCO₂ < 40 mm Hg, PaO₂ > 70 mm Hg). Body temperature was controlled with heat or cold to approximate normothermia (36–38°C). Samples for serum pentobarbital levels were taken 1 hour after MCA occlusion, at the resumption of spontaneous EEG bursts, and at endotracheal extubation. If SAP fell below 70 mm Hg during the 72-hour postocclusive period, phenylephrine was given by IV drip to assure adequate systemic arterial perfusion. In Group IV, serum samples for pentobarbital levels were obtained at 24, 48, and 72 hours after LMCA occlusion. Intravenous Ringer’s lactate or soft food and water were provided to maintain normal hydration as indicated by body temperature, SAP, and hematocrit determinations. Neurological function was assessed daily.

On the third postoperative day, cats were anesthetized with 30 mg/kg IV pentobarbital. 2% Evans blue (10 cc, pH 7.35) was administered intravenously over 30 minutes. Two hours later cats were sacrificed with an administration of pentobarbital (Euthanal, 60 mg). Each brain was removed, weighed, and chilled. A 4.0-mm thick coronal section was taken at the level of the optic chiasm. Tissue was sampled from the basal ganglia, subcortical white matter, and cortex bilaterally for water and electrolyte (Na⁺, K⁺) analysis. Water content was assessed from specimens both by wet-dry analysis and by specific gravity computation after suspension of the wet specimens in a bromobenzene-kerosene column. Dried samples were analyzed for Na⁺ and K⁺ by either flame photometry or atomic absorption spectrophotometry.

Each brain was inspected grossly to confirm occlusion of the left MCA at its origin, and then fixed in 10% buffered formalin. Cerebral hemispheres were weighed separately. Infarction size was determined by weight. Infarcted tissue was grossly obvious and was separated readily from normal tissue by blunt dissection with the aid of an operating microscope. Dissection of infaracts was done by the same observer (MN) when several specimens had accumulated. Individual brains and treatment protocols were blinded at the time of dissection. The infarct size was determined by comparing necrotic tissue weight with non-necrotic tissue weight of the same hemisphere and was expressed as percent hemisphere infarcted. Student’s unpaired t-test was used to analyze the data.

Results

Baseline SAP, which was similar in all four groups before pentobarbital administration, fell transiently after the initial dose, then returned to baseline spontaneously before MCA occlusion. MCA occlusion caused SAP to fall slightly. An hour after occlusion SAP had returned either to baseline or exceeded it slightly. Blood pressure then remained stable until sacrifice 72 hours after occlusion. SAP rose preterminally in some cats that died prematurely despite supportive care. No attempt was made to control arterial hypertension when it developed.

Halothane anesthesia was maintained for 1.5 to 3.5 hours during which time the left MCA was exposed and/or occluded. Thereafter cats were allowed to awaken promptly (Groups I: 0.2 hours; II: 0.4 hours) or anesthesia was continued with pentobarbital infusion. Ventilation time for cats in Group III was 2.3 ± 0.4 hours and in Group IV was 7.7 ± 1.8 hours. Some cats in Groups III and IV required intermittent ventilation thereafter to maintain adequate gas exchange.

Volume of infused fluids, body temperature, and hematocrit did not vary significantly among the experimental groups.

ICP was measured only in cats treated with pentobarbital. Successful chronic recordings were obtained from 8 to 10 cats treated with pentobarbital before occlusion only and from 9 of 9 treated with pentobarbital for 24 hours after occlusion. ICP exceeded 65 mm Hg in 5 cats (63%) within 48 hours of occlusion in Group III. All five died prematurely, presumably from intracranial hypertension, despite adequate ventilation. ICP exceeded 65 mm Hg in two cats (22%) from Group IV, both of which died within 48 hours of occlusion. Of the remaining 7 cats in Group IV, three developed intracranial hypertension up to 40 mm Hg; one of the seven died prematurely.

Neurological deficits corresponded to the experimental groupings. None of the sham-operated cats was neurologically impaired. All untreated cats with MCA occlusion (Group II) had mild to severe right hemiparesis when they awakened within one half hour of occlusion. Six of the ten cats in Group III never regained consciousness. The remaining four had right hemiparesis within 24 hours of occlusion, a deficit that gradually improved over the next 48 hours. Five cats in Group IV remained stuporous or comatose for 72 hours; one awakened at 48 hours postocclusion with a right hemiparesis, and three died prematurely without regaining consciousness.

Early deaths were attributed most often to raised ICP. Cardiovascular collapse, refractory to phenylephrine and fluid infusion, occurred in two cats and neurogenic pulmonary edema developed in one. Premature death (i.e. < 72 hrs after occlusion) in the individual groups was: I, 0%; II, 50%; III, 60%; IV, 33%. Serum pentobarbital levels were measured periodically as shown in the table.

After removal, gross inspection of brains showed occlusion of the left MCA between its origin and the lateral lenticulostriate artery in all cats except those that underwent sham operations. Areas commonly affected by ischemia-infarction were the left basal ganglia and the internal capsule. Subcortical white matter and cortex were less predictably involved. The left hemisphere was generally swollen in all cats except those undergoing sham operations. Evans blue staining was grossly evident but was usually spotty in distribution, confined to the basal ganglia, internal capsule, and medial temporal lobe. The intensity of staining varied from brain-to-brain without isolation to grossly...
TABLE

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<tr>
<th>Pentobarbital Blood Levels After Occlusion</th>
<th>Pentobarbital mcg/ml</th>
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<tr>
<td></td>
<td>Group III</td>
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<tr>
<td>1 hr after occlusion</td>
<td>22 ± 5</td>
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<tr>
<td>Extubation</td>
<td>16 ± 2</td>
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<tr>
<td>EEG bursts</td>
<td>16 ± 2</td>
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<tr>
<td>24 hours</td>
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<td>48 hours</td>
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Infarcted areas. The right cerebral hemisphere was unaffected except for distortion by the swollen left hemisphere. Infarction size was calculated in all cats in Groups II–IV whether or not they survived for 72 hours. Infarction size differed among the groups as follows: I, 0%; II, 8.3 ± 1.5%; III, 9.0 ± 2.4%; IV, 4.9 ± 1.1% (fig. 1).

Water content in both cerebral hemispheres from sham-operated control cats (Group I) were identical. Water content from the right hemisphere in cats with LMCA occlusion (Groups II, III, and IV) were similar to those of control cats. Thus, LMCA occlusion did not cause water content to rise in the right hemisphere in any of the three groups with occlusion during the 72 hour period of observation (fig. 1).

Water contents in the left hemisphere were significantly higher in basal ganglia, subcortical white matter, and cortex than in the right hemisphere of cats in each of the experimental groups (II, III, IV) 72 hours after occlusion.

Levels of Na⁺ rose in the left hemisphere of all cats with MCA occlusion whether pentobarbital had been given or not. Differences from controls were apparent within 24 hours and persisted for 72 hours after occlusion (fig. 3). Levels of Na⁺ rose in the contralateral hemisphere as early as 24 hours after occlusion in Group IV cats even though water content did not. Levels of K⁺ fell as Na⁺ rose in all specimens with increased water content.

**Discussion**

In this study, the extent of infarction was reduced in cats that underwent permanent occlusion of the MCA when a dose of pentobarbital was given before the insult and supplemented for 24 hours with additional small doses. Pentobarbital blood levels achieved by that regimen were substantially lower than those required in our earlier studies in which a single dose of pentobarbital was administered before occlusion. The expected extent of infarction was only reduced significantly when supplemental doses of pentobarbital were given to maintain blood levels above 18 μg/ml for 24 or more hours. On the other hand, edema formation was not suppressed at the 72-hour end point even though the extent of infarction was reduced. Water and electrolyte content in the ischemic zone appeared to be reduced at earlier intervals (24,48 hours), but our data at those intervals were insufficient to prove an early edema suppression effect. Intracranial pressure rose within the first 24 hours in most cats after MCA occlusion despite the presence of pentobarbital in the blood; pressure elevation preceded most premature deaths despite intensive cardiopulmonary support. Our mortality in untreated and single-dose barbiturate cats with MCA occlusion was high, consistent with observations by others who have employed proximal MCA occlusion in their studies. However, mortality in cats treated with barbiturates for 24 hours was significantly lower.

We did not find the pentobarbital suppression of edema observed by Smith and Marque 24 hours after cold injury to the canine cortex, studied by Simeone and colleagues eleven hours after MCA occlusion in monkeys, and described by Lawner and coworkers.
eight hours after unilateral carotid occlusion in gerbils. Cats that succumbed within 48 hours in our study may have had less edema if they had continued to receive pentobarbital for longer than the 24 hours. The suppressive effect was lost by the third day after occlusion with the amounts given.

Even though we provided cardiopulmonary support for 72 hours after occlusion, 15 of the 31 cats with MCA occlusion died prematurely. This mortality rate (48%) may be attributed not only to proximal occlusion of the MCA, but also to untreated intracranial hypertension, often accompanied preterminally by elevated arterial blood pressure. We did not encounter evidence of cardiorespiratory failure or adrenal insufficiency described by others in animals with MCA occlusion that did not receive intensive care. Late arterial hypertension may have had a significant effect on the ischemic, swollen hemisphere after occlusion and upon eventual infarct size and edema analyzed after death.

Even though the volume of necrotic, infarcted tissue was usually less than 9% of the affected hemisphere, edema formation was marked in all cats with LMCA occlusion whether or not pentobarbital had been given. Plum and colleagues showed earlier that the amount of edema formed after arterial occlusion is directly related to the extent of ischemic necrosis. We failed to confirm their observations in this study because tissue water content was elevated similarly in the ischemic hemisphere regardless of the extent of tissue necrosis. Furthermore, the zone of increased permeability of the blood-brain barrier shown by Evans blue extravasation should have approximated either the extent of necrosis or the severity of edema around it. In fact, the distribution of Evans blue dye brains studied 72 hours after MCA occlusion was variable and did not correlate with administration of pentobarbital.

Water content did not increase in the contralateral hemisphere in any of our cats after LMCA occlusion even though Na⁺ increased in cats that received pentobarbital for 24 hours (Group IV). This apparent inconsistency of water and electrolyte change and the lack of edema bulk flow from the ischemic hemisphere to the opposite side may be related to compartmental shifts of ions before an increase in water content. The normal time course for ischemic edema formation has been defined by other workers: Water begins to accumulate in brain within a few minutes of the ischemic event and is accompanied by an increase in tissue Na⁺ levels, osmolality, and lactate levels. Tissue pH and K⁺ levels fall simultaneously, and the extracellular space shrinks.

**Figure 2.** Water content (specific gravity method) in tissue samples from the left basal ganglia, subcortical white matter, and cortex.
As cell bulk expands. These water, electrolyte, metabolic, and compartmental disturbances comprise the cytotoxic phase of ischemic edema. Subsequently changes in water and electrolytes occur within the extracellular compartment, probably from extravasation of serum through and between capillary endothelium. This vasogenic phase of ischemic edema develops within hours of ischemia, peaking both clinically and experimentally within 2-5 days after the insult. Edema resolves several days to weeks later.

Because we studied edema formation within three days of occlusion, we expected to find the peak of edema formation during its vasogenic phase, when infarction had occurred and necrosis was grossly obvious. This normal consequence of MCA occlusion was observed in cats not treated with pentobarbital (Group II). Changes in water, Na+, and K+ were maximal in that group within 48 hours of occlusion and began to return toward control values by the third day.

In cats treated with pentobarbital, however, water and electrolyte changes in the ischemic hemisphere continued to occur throughout the period of observation, suggesting that edema resolution had not begun even 72 hours after occlusion. 24. 20

We believe this study shows that pentobarbital suppresses infarction, but does not control edema. Furthermore, it seems likely that pentobarbital attenuates the normal formation and eventual resolution of ischemic edema, yet it has little effect on its severity after arterial occlusion. If this latter supposition is correct, the resolution phase predicted for ischemic edema would be blunted by pentobarbital and would not begin until well after the 72-hour end point used in this study.

Acknowledgments

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References

3. Corkill G, Sivalingam S, Reitan JA: Dose dependency of the post-


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