(S)-Emopamil Reduces Brain Edema From Collagenase-Induced Hemorrhage in Rats

Gary A. Rosenberg, MD; Milo J. Navratil, BS

**Background and Purpose**
Calcium channel blockers reduce edema due to cerebral ischemia, but little is known about their usefulness in hemorrhage. Therefore, we studied the effect of the calcium channel blocker (S)-emopamil in collagenase-induced hemorrhage.

**Methods**
Adult rats had hemorrhagic necrosis induced by the intracerebral injection of 0.4 U of bacterial collagenase. Six groups of rats were given either 10 or 20 mg/kg (S)-emopamil at different times after induction of the lesion. Brain water and electrolyte levels in these rats were measured 24 hours after collagenase injection. Also, lesion volume in other rats was measured either 4 or 24 hours after formation of the lesion with the drug given at 1 hour or both 1 and 5 hours, respectively.

**Results**
Administration of 20 mg/kg (S)-emopamil 1 hour after lesion induction significantly decreased water and electrolyte content in both posterior regions (P<.05). This beneficial effect was lost when a second 20-mg/kg dose was given at 5 hours. A single 20-mg/kg injection at 1 hour had no effect on lesion volume at 4 hours. Two doses significantly increased volume at 24 hours (P<.05).

**Conclusions**
Early administration of (S)-emopamil is beneficial in hemorrhagic lesions, but a subsequent delayed injection may be deleterious. Knowledge of the time of hemorrhage will be important in use of these agents in treating hemorrhage.

**Key Words**
- bacterial collagenase
- calcium channel blockers
- brain edema
- intracerebral hemorrhage
- (S)-emopamil

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**Materials and Methods**
Sixty male Sprague-Dawley rats weighing 250 to 300 g were used in the study, which was approved by the animal research committee at the University of New Mexico School of Medicine and the Veterans Affairs Medical Center and conformed to guidelines established by the National Institutes of Health. The method of induction of a collagenase-induced hemorrhage has been described. Briefly, the rats were anesthetized with a mixture of 1.75% halothane and 70% nitrous oxide/30% oxygen. A burr hole was placed 3 mm lateral to midline at the bregma, and a 23-gauge infusion needle was lowered 5 mm below the dura. Saline (2 μL) containing 0.4 U of bacterial collagenase (Type VII-S, Sigma Chemical Co) was infused over 9 minutes into the left caudate/putamen with a microinfusion pump (Harvard Apparatus). The animals were killed either 4 or 24 hours after the collagenase infusion with an overdose of sodium pentobarbital.

Immediately after death, the brains were removed and sectioned for measurements of water and electrolyte content. Four regions were measured: the left caudate/putamen containing the hemorrhage (L1), the right uninjected caudate/putamen (R1), and the parietal and hippocampal regions of both hemispheres (L2 and R2). Water content was determined by weighing tissue wet and after drying for 24 hours in a 100°C oven, using the following formula: ([wet weight−dry weight]/wet weight)×100. Sodium and potassium tissue con-
tent was measured in the samples after extraction with nitric acid using a flame photometer (Corning Medical).

Six groups of rats with 0.4-U lesions had intraperitoneal injections of (S)-emopamil ([25]-2-isopropyl-5-[methylphenethylamino]-2-phenylvaleronitrile hydrochloride). The first group was a control group with collagenase lesions and no drug treatment (n=13). Group 2 (n=6) received a single 10-mg/kg injection of the drug 1 hour after induction of the lesion. Group 3 (n=5) received a single 20-mg/kg injection 1 hour after the lesion. Group 4 (n=6) had a 20-mg/kg injection 5 hours after the lesion. Group 5 (n=6) had two 20-mg/kg injections, the first at 1 hour and the second 5 hours after the lesion. Finally, group 6 (n=4) received two 20-mg/kg injections, one at 1 hour and the second 8 hours after the lesion. All of these rats were killed at 24 hours.

The size of the hemorrhagic mass was measured in another group of rats that had 0.4-U collagenase lesions, using a computer image analysis system. Untreated lesioned rats were studied at 4 (n=4) and 24 hours (n=8). One group (n=4), treated with 20 mg/kg IP (S)-emopamil 1 hour after collagenase infusion, was killed at 4 hours. A second group (n=4) was given 20 mg/kg (S)-emopamil at 1 and 5 hours after collagenase infusion and was killed at 24 hours. The brains were removed, frozen with 2-methylbutane, and stored at -80°C. Sections of 20 μm were cut with a cryostat, beginning 0.5 mm from the tip of the frontal pole and continuing to sample every 10th section to the sylvian aqueduct. Images of the unstained tissue sections were entered into a computer with a video camera (Fig 1). The digital image clearly shows the hemorrhagic region against adjacent normal tissue. Area of necrosis was calculated with a threshold recognition program. Total brain area was calculated by manual tracing of the tissue section. Necrosis volumes were calculated by summing for all calculated tissue sections and multiplying by the distance between sampled tissue sections. The image analysis system consists of a frame grabber in a PC computer, a Chromapro 45 light table with Dumas configuration, a Cohu charge-coupled device camera, and a Digital Equipment VAX II workstation. The DEC workstation has two custom image analysis software programs for the calculation of lesion and brain area.

All values are given as mean±SD. The percent water and electrolyte data were analyzed with ANOVA with the Fisher least significant difference test for multiple comparisons. The volume data was analyzed by Student’s t test. All statistical analyses were performed with the SAS system (SAS). Significance was taken as P<.05.

**Results**

There were no significant differences for water, potassium, or sodium content at the lesion site (L1) for any of the experimental conditions. However, there were significant changes in both posterior regions for both water (Table 1) and sodium content (Table 2).

There was a significant decrease in water content in the left and right posterior regions when 20 mg/kg

<table>
<thead>
<tr>
<th>Drug Time and Dose</th>
<th>L1</th>
<th>L2</th>
<th>R1</th>
<th>R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (0.4 U collagenase)</td>
<td>81.69±0.24*</td>
<td>80.82±0.49</td>
<td>79.93±0.27</td>
<td>80.18±0.33</td>
</tr>
<tr>
<td>1 Hour, 10 mg/kg</td>
<td>81.64±0.29</td>
<td>80.62±0.34</td>
<td>79.70±0.33</td>
<td>79.83±0.39</td>
</tr>
<tr>
<td>1 Hour, 20 mg/kg</td>
<td>81.71±0.25</td>
<td>80.17±0.57†</td>
<td>79.66±0.28</td>
<td>79.49±0.54†</td>
</tr>
<tr>
<td>5 Hours, 20 mg/kg</td>
<td>81.91±0.55</td>
<td>81.01±0.26</td>
<td>80.05±0.31</td>
<td>80.30±0.28</td>
</tr>
<tr>
<td>1 and 5 Hours, 20 mg/kg</td>
<td>81.65±0.10</td>
<td>81.14±0.48</td>
<td>80.06±0.21</td>
<td>80.28±0.33</td>
</tr>
<tr>
<td>1 and 8 Hours, 20 mg/kg</td>
<td>81.94±0.27</td>
<td>80.70±0.46</td>
<td>79.88±0.44</td>
<td>79.69±0.41</td>
</tr>
</tbody>
</table>

L1 indicates left caudate/putamen containing hemorrhage; L2, left parietal/hippocampal region; R1, right uninjected caudate/putamen; and R2, right parietal/hippocampal region. Samples were taken 24 hours after a 0.4-U bacterial collagenase-induced hemorrhage.

*Mean±SD.
†Significantly less than control by Fisher least significant difference test (P<.05).
(S)-emopamil was given at 1 hour (Table 1). Both regions had corresponding statistically significant decreases for sodium content, which was also decreased at site R1 (Table 2); potassium content was significantly increased for site L2 at that dose and time (Table 3).

Rats treated with one dose of 20 mg/kg (S)-emopamil at 5 hours failed to show a response to the drug for either water or sodium content but had a significant increase in potassium content at sites L2 and R1. More importantly, when a second dose of drug was given to rats treated initially at 1 hour, the beneficial effect noted in the single 20-mg/kg dose group was lost (Tables 1 and 2).

Rats given one injection 1 hour after lesion induction showed no differences in lesion volumes between control and drug at 4 hours. Untreated collagenase-injected rats killed 24 hours after the lesion had a lesion volume of 63 ± 3 mm³ (Fig 2). Rats treated at 1 and 5 hours with 20 mg/kg (S)-emopamil had a significantly increased lesion volume of 84 ± 2.7 mm³ compared with untreated controls (*P < .001; Student’s t test).

**Discussion**

The drug reduced water and sodium content in both posterior brain regions in rats with collagenase-induced hemorrhage but did not affect edema at the hemorrhage site. A statistically significant reduction was seen with one 20-mg/kg dose given at 1 hour. This beneficial effect was lost when an additional dose was given at 5 hours. A single 20-mg/kg injection at 1 hour did not increase lesion volume by 4 hours. When the 20-mg/kg dose was given at 1 and 5 hours, however, the volume of the lesion was significantly increased when measured at 24 hours.

Collagenase causes damage to the basal lamina around blood vessels and opens the blood-brain barrier by 20 minutes after injection.6 The hemorrhagic region grows in size for several hours, coalescing into a mass lesion around 4 hours. Edema at the injection site in the caudate reaches a maximum by 24 hours and remains elevated for 48 to 72 hours before beginning to resolve, while the vasogenic edema in the posterior regions is maximal at 24 hours and resolves by 48 hours.3 Historically, the evolving lesion resembles a rapidly progressing hemorrhagic infarction. Cerebral blood flow is reduced in the region of the mass but is unaffected by the edema in the posterior region.7 The mass lesion does not affect physiological parameters including blood pressure, pH, blood gases, serum electrolytes, or temperature.7

The pathophysiology of intracerebral hemorrhage has generated considerable controversy (see Reference 25 for discussion). The two main theories are that (1) a vessel ruptures, releasing a large collection of blood, and (2) tissue breaks down into a necrotic hemorrhagic mass after an infarct. The inability of early investigators to visualize the onset of the hemorrhage had made this an unresolvable argument. Collagenase-induced bleeding is an artificial method to induce hemorrhage. There is a rapid conversion of multiple hemorrhagic sites into a necrotic mass. Other models of intracranial bleeding have used injection of autologous blood.15,26 Neither of the models fits the original pathological descriptions of

**Table 3. Effect of (S)-Emopamil on Potassium Content (mEq/mg dry wt) for Different Sites**

<table>
<thead>
<tr>
<th>Drug Time and Dose</th>
<th>L1</th>
<th>L2</th>
<th>R1</th>
<th>R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (0.4 U collagenase)</td>
<td>415 ± 22.4*</td>
<td>471 ± 22.0</td>
<td>510 ± 16.8</td>
<td>501 ± 14.4</td>
</tr>
<tr>
<td>1 Hour, 10 mg/kg</td>
<td>412 ± 17.7</td>
<td>472 ± 17.7</td>
<td>512 ± 10.7</td>
<td>500 ± 8.2</td>
</tr>
<tr>
<td>1 Hour, 20 mg/kg</td>
<td>438 ± 23.2</td>
<td>502 ± 13.3†</td>
<td>526 ± 20.6†</td>
<td>504 ± 12.0</td>
</tr>
<tr>
<td>5 Hours, 20 mg/kg</td>
<td>418 ± 34.4</td>
<td>493 ± 9.4†</td>
<td>532 ± 3.7†</td>
<td>512 ± 6.9</td>
</tr>
<tr>
<td>1 and 5 Hours, 20 mg/kg</td>
<td>417 ± 23.6</td>
<td>473 ± 19.7</td>
<td>513 ± 9.5</td>
<td>495 ± 9.6</td>
</tr>
<tr>
<td>1 and 8 Hours, 20 mg/kg</td>
<td>403 ± 14.8</td>
<td>463 ± 14.8</td>
<td>523 ± 4.3</td>
<td>510 ± 0.0</td>
</tr>
</tbody>
</table>

L1 indicates left caudate/putamen containing hemorrhage; L2, left parietal/hippocampal region; R1, right uninjected caudate/putamen; and R2, right parietal/hippocampal region. Samples were taken 24 hours after a 0.4-U bacterial collagenase-induced hemorrhage.

*Mean ± SD.
†Significantly greater than control by Fisher least significant difference test (P < .05).
intracranial bleeding. Recent reports, however, using serial computed tomographic (CT) scans have revealed a group of patients with rapid conversion of an infarct into what appears on CT scan to be a primary cerebral hemorrhage. This is closer to the situation that occurs in collagenase-induced bleeding.

(S)-Emopamil, a calcium channel blocker, rapidly crosses the blood-brain barrier and increases cerebral blood flow. In focal cerebral ischemia in rats it reduces the size of the infarct at a dose of 20 mg/kg given 1 hour after the lesion with a repeat dose at 2.5 hours. Brain edema is reduced in rats with a fluid percussion injury at a dose of 10 or 20 mg/kg given 15 minutes after injury with a repeat dose at 2.5 hours. A recent study showed that (S)-emopamil attenuates the increase in extracellular glutamate in global cerebral ischemia in rabbits. There are several possible explanations for the action of a calcium channel blocker in the collagenase-induced hemorrhage model. It could prevent collagenase from proteolytically attacking the capillary. This is unlikely because of the lack of an effect on lesion size by 4 hours with the single drug dose. The single early dose probably affected edema in the posterior regions by either reducing formation and movement of edema from the lesion site to the posterior region or by facilitating the removal of edema from the posterior regions. Intracellular calcium is influenced by a calcium-sodium exchange and by a reversible, energy-dependent Ca\(^{2+}\)/Na\(^{+}\) adenosine-triphosphatase pump. Early administration of a calcium channel blocker could reduce calcium entry and sodium exchange, which would interfere with development of brain edema. A single dose of the calcium channel blocker 5 hours after the injury was ineffective, which could be because a critical time period is necessary for calcium exchange blockade to be effective. The adverse effect, on the other hand, of the second dose may be due to an increase in blood flow, thus enhancing the formation of edema.

Because the collagenase lesion is well formed by 4 hours, the second injection at 5 hours is the most likely cause of the observed increase in lesion volume. There may be critical periods during the formation of the lesion when the administration of a calcium channel blocker may be deleterious. The necessity for early administration of the calcium channel blocker is consistent with ischemia studies. They differ, however, in that subsequent doses produce an adverse effect in our hemorrhagic model but do not interfere with the beneficial effects in ischemic or traumatic models.

In the collagenase-induced hemorrhage model, the calcium channel blocker (S)-emopamil significantly reduces water and sodium content when given at 1 hour. A subsequent dose at 5 hours reverses this beneficial effect on edema and significantly increases hemorrhagic mass size. These findings emphasize the problems of extrapolating results obtained in ischemic models to those in hemorrhagic models. Because of the possible loss of benefit and increase in lesion size with subsequent doses, use of these agents in hemorrhage will require further study to determine optimal times for intervention.

Acknowledgments

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References


16. Kauppinen RA, McMahon HT, Nicholls DG. Ca\(^{2+}\)-dependent and Ca\(^{2+}\)-independent glutamate release, energy status and cytosolic free Ca\(^{2+}\) concentration in isolated nerve terminals fol-
In the accompanying article, Rosenberg and Navratil showed that the calcium channel blocker (S)-emopamil significantly decreased water and electrolyte content in a rat model of collagenase-induced hemorrhage. Administration of (S)-emopamil 1 hour after lesion induction significantly decreased water and electrolyte content. However, this beneficial effect was lost when a second dose was given at 5 hours. In addition, two doses of (S)-emopamil significantly increased lesion volume at 24 hours. Thus, while early administration of (S)-emopamil is beneficial in this model, a subsequent delayed injection may be deleterious.

(S)-Emopamil, a novel calcium channel blocker and serotonin S2 antagonist, has previously been shown to reduce ischemic damage after both focal and global ischemia. In the present study, the beneficial effects of this agent may be due to its effect on calcium-mediated processes and/or serotonin-mediated events. In this regard, serotonin receptor blockage has been shown to be neuroprotective in models of brain ischemia. Thus, it would be interesting to determine whether a pure serotonin S2 receptor blocker would also decrease water content in this model of collagenase-induced hemorrhage.

An important conclusion of the study is that in contrast to the beneficial effects of early (S)-emopamil treatment, a subsequent delayed injection actually worsened outcome. This detrimental effect was attributed to increased blood flow and enhanced edema formation. In the clinical setting, the therapeutic window for (S)-emopamil may therefore be highly restricted and may vary between hemorrhagic and nonhemorrhagic brain injuries.

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(S)-emopamil reduces brain edema from collagenase-induced hemorrhage in rats.
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