Experimental epsilon-aminocaproic acid (EACA) administration in the presence of subarachnoid blood

TOM EWALD, M.S., II, STEVE MAHALEY, JR., M.D., PH.D., JACK GOODRICH, M.D., ROBERT WILKINSON, M.D., AND DON SILVER, M.D.
Divisions of Neurosurgery, General Surgery, and Nuclear Medicine, Duke University Medical Center, Durham, North Carolina

Epsilon-aminocaproic acid (EACA) was administered to dogs intrathecally, orally, or intravenously with blood present in the subarachnoid space. These animals were compared with appropriate controls with regard to the subsequent transport of intrathecally injected radioactive albumin from cerebrospinal fluid (CSF) to the blood and the presence of hydrocephalus or adhesive arachnoiditis at the various times of sacrifice. An adhesive arachnoiditis sufficient to produce hydrocephalus or a delay in CSF protein effluence was not observed. The administration of EACA to dogs in the presence of subarachnoid blood did not appear to be associated with any deleterious effects.

Key Words - epsilon-aminocaproic acid (EACA) - subarachnoid blood - adhesive arachnoiditis - intrathecal radioactive albumin

The use of epsilon-aminocaproic acid (EACA) has been postulated as an adjunctive measure in the treatment of patients with subarachnoid hemorrhage from aneurysms. This drug is an antifibrinolytic agent and, by preventing perianeurysmal clot lysis, might prevent rebleeding. Norlén and Thulin have reported using EACA for this purpose with apparent success. They reported no rebleeding during treatment for up to 16 days prior to surgery. Two postoperative deaths occurred, one of which was due to bilateral anterior cerebral artery thrombosis 10 days following surgery. While considering the consequences of antifibrinolytic activity in the cerebrospinal fluid (CSF), it must be remembered that subarachnoid hemorrhage alone can produce adhesive arachnoiditis of the basal leptomeninges in 2 to 12 weeks after bleeding and may result in hydrocephalus. The effective plugging of arachnoid villi by red blood cells introduced into the subarachnoid space has been shown by Shabo and Maxwell. Thus, the concern has been expressed that the administration of EACA in the presence of blood in the subarachnoid space might compound the dangers of arachnoiditis by preventing blood lysis and perhaps impeding removal of blood products from the CSF.

In this study, autogenous blood was injected into the subarachnoid space of dogs, and EACA was administered into the cisterna magna, intravenously, or orally. The animals were observed for any functional changes, the transport of protein from the CSF to blood was studied, the fibrinolytic activity of the blood, the arachnoid, and the dura was studied, and autopsy examinations were done at 1/2, 1, 2, or 4 months.
Materials and Methods
Mongrel male and female dogs weighing from 9 to 15 kg were used. Five groups were established, as follows:

**Group 1.** In five dogs, 2 ml of autogenous blood were injected intracisternally.

**Group 2.** In five dogs, 2 ml of EACA (5 mg) were injected intracisternally.

**Group 3.** In five dogs, 2 ml of autogenous blood containing 5 mg of EACA were injected intracisternally.

**Group 4.** In five dogs, 2 ml of autogenous blood were injected intracisternally and 300 mg/kg/day of EACA given intravenously for 10 days.

**Group 5.** In five dogs, 2 ml autogenous blood were injected intracisternally and 300 mg/kg/day of EACA given orally for 10 days.

The cisternal injections were done under light sodium pentobarbital anesthesia with spontaneous respiration. Each animal was placed in the lateral position, and a short 20-gauge lumbar puncture (LP) needle was used for cisternal puncture. Beginning on the day following cisternal blood introduction, intravenous EACA injections were given at about the same time each day without anesthesia in Group 4. Orally administered EACA was mixed daily with each dog's food in Group 5. Observations were made daily for any evidence of illness or neurological symptoms. Blood samples were obtained for fibrinolytic assays before and at 30-min intervals after the injections for 4 hours in Groups 1, 2, and 3. Blood samples for fibrinolytic activity were obtained daily, prior to the administration of EACA, in Groups 4 and 5.

There were five dogs in each group. The animals were sacrificed at \( \frac{1}{3} \), 1, 2, or 4 months following initial cisternal injection. Twenty-four hours prior to sacrifice, each dog was anesthetized with sodium pentobarbital, and a repeat cisternal puncture was carried out. Following a "clear" (not blood-tinged) tap, 100 microcuries (\( \mu \)Ci) of high specific activity I-131 labeled human serum albumin (RISA-131-H)* in a volume of 0.2 ml were injected with multiple barbotage. A brain scan was done 3 hours after injection to ascertain whether the isotope had obtained a good subarachnoid distribution or was in part or totally located epidurally (Fig. 1). Blood samples drawn from an indwelling venous catheter hourly for 8 hours and at 24 and up hours were counted with a scintillation well counter to determine the presence of the I-131 tracer. The counts of aliquots of each blood sample were compared with those of a 1:1000 dilution of RISA-131-H standard. The blood counts were expressed as \% injected dose \( \times 10^{-3} \)/ml/kg.

Each dog was then sacrificed and perfused intravascularly with Ringer's solution. The brain and spinal cord were exposed and photographed in situ and after removal. Any ev-

---

* Supplied as RISA 131-H by Abbott Laboratories, North Chicago, Illinois.

---

**FIG. 1.** Control brain scans performed 3 hours after deliberate injection of RISA-131-H into the subarachnoid space (upper), epidural space (lower), and part subarachnoid, part epidural spaces (middle). A scan on each experimental animal was checked to be sure that it had a "subarachnoid" pattern.
EACA in the presence of subarachnoid blood

Evidence of gross abnormality was noted, and histological sections of the cerebri, cerebellum, brain stem, and cervical cord were made in the coronal plane. Sections were stained with hematoxylin and eosin, Luxol-fast-blue, periodic acid Schiff, Masson’s trichrome, and Turnbull’s blue (for iron). Frozen sections (8 μ) of arachnoid and dura from the cervical cord and convexity of the brain were assayed for fibrinolytic activity by the fibrin slide technique. 8

Results

It was considered important to know how extensively the 2 ml of autogenous blood would spread in the subarachnoid space following injection into the cisterna magna. Therefore, several control dogs were so injected and sacrificed immediately. Gross examination of these dogs (Fig. 2) revealed a distribution of the blood about the brain stem, cervical cord, basal cisterns, interhemispheric subarachnoid space, and minimally over the convexities of the cerebral hemispheres. Although there was a relative lack of exposure of the convexities of the cerebral hemispheres to blood, it was not felt advisable to inject larger volumes of blood into the subarachnoid space, since the normal total volume of cerebrospinal fluid in the dog is only 8.5 to 10 ml. 1

In the study groups, dogs that were injected with blood were occasionally somewhat unsteady in gait for 24 to 48 hours but thereafter showed no functional changes during the various study periods.

The RISA-131-H transport data in control dogs revealed a very slow rise in blood radioisotope counts for 6 to 8 hours, followed by a peak of blood levels at 24 to 48 hours. The RISA-131-H data from dogs in the study groups showed similar gradual increases in blood counts, with 24-hour values equal to or slightly greater than those seen in the controls (Table 1). In no case was the 24-hour blood RISA-131-H value less than normal, as would have been expected to occur if there had existed a blockage of protein transport from the CSF to blood. Additionally, 24-hour RISA-131-H blood values were never as low as that reported by Abbott and Alksne 1 for hydrocephalic dogs (Table 2).

In the RISA-131-H study, it was considered important to know whether the I-131 counted in the blood was still protein-bound. Therefore, dialysis of each blood sample, as well as the original RISA-131-H used for subarachnoid injection, was carried out, using the micro technique of Fritz, et al. 9 It

TABLE 1

Percent of the injected dose of intrathecal RISA \(^ {131} \) (×10^-3/ml/kg) circulating in the blood at 24 hours after injection (measured before sacrifice at \(\frac{1}{2}, \frac{1}{2}, \frac{3}{2}, \frac{3}{4}, \text{and 4 mos} \))

<table>
<thead>
<tr>
<th>Dog Group</th>
<th>% of RISA (^ {131} ) Dose Circulating at</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\frac{1}{2}) mo</td>
</tr>
<tr>
<td>Control</td>
<td>3.28</td>
</tr>
<tr>
<td>1</td>
<td>4.85</td>
</tr>
<tr>
<td>2</td>
<td>2.85</td>
</tr>
<tr>
<td>3</td>
<td>3.28</td>
</tr>
<tr>
<td>4</td>
<td>3.08</td>
</tr>
<tr>
<td>5</td>
<td>4.27</td>
</tr>
</tbody>
</table>

Fig. 2. Ventral surface of the brain and cervical spinal cord of a control animal injected with blood intracisternally, showing location of blood (dark areas) about the brain stem, cord, and base of brain.
TABLE 2
Percent of injected dose of intrathecal RISA\(_{131}\)
circulating in the blood at 24 hours after injection in our series as compared to Abbott and Alksne's series

<table>
<thead>
<tr>
<th>Series</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ewald, et al. (1971)</td>
<td>36</td>
</tr>
<tr>
<td>Abbott and Alksne (1968)*</td>
<td></td>
</tr>
<tr>
<td>normal dogs</td>
<td>46</td>
</tr>
<tr>
<td>hydrocephalic dogs</td>
<td>16</td>
</tr>
<tr>
<td>hydrocephalic patients</td>
<td>24</td>
</tr>
</tbody>
</table>


was somewhat surprising to find that the percent of unbound I-131 in the stock RISA-131-H varied considerably from shipment to shipment, ranging from 0.2% to 65%. Dialysis of the serial blood samples from the experimental groups revealed that the dialyzable (unbound) I-131 rapidly appeared in the blood during the first hour following injection into the CSF and actually accounted for only a negligible percentage of the blood count at 24 hours (Fig. 3). Therefore, although it was of considerable interest that the commercial RISA-131-H varied so much in the percent protein-bound and that the unbound I-131 was transported from the CSF to blood very rapidly, it appeared that the blood radioactivity count from 2 to 24 hours did essentially indicate protein-bound I-131 transport from the CSF to blood.

On gross examination of the brain and cervical cord of the dogs in each group, the only abnormality noted was a brownish pigment about the meninges in the area of the cisterna magna and cervical cord in dogs from each group that received blood injection and one case of slight ventricular dilatation in an animal in Group 1 (blood injection only).

Histological examination of the brain and cervical cord of each animal showed a mild thickening of the arachnoid and a moderate number of macrophages in the subarachnoid space of most dogs in each group that received cisternal blood injection (Fig. 4). Many of the macrophages contained iron pigment. These changes were noted in the subarachnoid spaces about the cervical cord, brain stem, base of the brain, and interhemispheric surfaces. Animals in Group 2 that received an intracisternal EACA injection alone showed no histological changes. The combination of cisternal blood injection with EACA administration by any route employed did not produce any recognizable increase or decrease in meningeal findings, over and above those seen with blood injection only.

![Fig. 3. Graph analyzing blood samples following intracisternal injection of RISA-131-H.](image-url)

Very little of the radioactivity in each blood sample was dialyzable (non-albumin-bound) after the first hour. The RISA used for injection contained 2.4% dialyzable I\(_{131}\).
The fibrinolytic activity of the blood samples was assayed by the rapid clot lysis test. Marked variations of lytic activity occurred among the samples. There appeared to be no significant effect of the injected blood and/or EACA on the circulating fibrinolytic activity.

The sections of arachnoid and dura were assayed for fibrinolytic activity by the fibrin slide technique. The specimens obtained from all of the dogs contained approximately the same amount of fibrinolytic activity as did sections of arachnoid and dura removed from normal dogs (Fig. 5).

**Discussion**

The administration of EACA directly into the cisterna magna, intravenously, or orally in dogs with blood in the subarachnoid space was not accompanied by any observable clinical change in these animals over a period extending to 4 months. The dose of EACA selected for administration is comparable to that found in the past to enter the CSF of dogs in quantities (10 mg/100 ml) sufficient to produce effective prolongation of rapid clot lysis.

The use of RISA-131-H CSF-to-blood transport data as a diagnostic test for impairment of protein absorption from the CSF was suggested in 1954 by Sweet, et al. Dupont, et al., reported that 50% of the maximum blood level of RISA-131 was reached 7 to 8 hours following intraventricular injection in dogs. Tator, et al., have reported that radioisotope blood levels for patients given intrathecal RISA were not appreciably altered in conditions affecting CSF dynam-
FIG. 5. Photomicrographs showing comparable areas of clot lysis (clear zones) in sections of the dura. **Upper Left:** Dura from control animal. ×115 (inset ×285). **Lower Left:** Dura from an animal that had received an intracisternal injection of EACA 1/2 mo prior to autopsy. ×115 (inset ×285). **Upper Right:** Dura from an animal that had received an intracisternal injection of EACA and blood 2 mos prior to autopsy. ×115 (inset ×285).

ics, although actual count data were not reported. However, Abbott and Alksne did report a correlation between radionuclide blood levels and hydrocephalus, with significantly lower blood level being present at around 24 hours after cisternal injection of RISA-131 in hydrocephalic animals. In our study the rates of protein transport during the first 24 hours after cisternal injection were found to be similar in the animals from each group, suggesting no impairment of CSF-to-blood protein transport associated with the administration of EACA under the protocol of this study.

An excellent description of histological findings in the brains of dogs following injection of blood into the subarachnoid space was made by Bagley in 1928. While some animals showed meningeal reaction, others appeared completely normal histologically. Slight to marked ventricular dilatation was found in one of 14 adult dogs that had received six injections of blood, whereas ventricular enlargement was a more common finding in puppies. The presence of cells containing pigment was also noted. Similar histological changes were described by Kibler, et al., in a review of the problem of hydrocephalus following subarachnoid hemorrhage in humans. Although inflammatory cells with iron pigment and a degree of thickening of the arachnoid were present in our experimental animals, the changes were neither more frequent nor more extensive in the groups treated with EACA, nor was there any associated hydrocephalus.

The fibrinolytic activity of the arachnoid and dura resides primarily in the vasa vaso- rum and small vessels of these structures and is primarily an activator activity (i.e., converts plasminogen to plasmin). At the time of sampling the meninges (1/2, 1, 2, and 4 months after the administration of blood and/or EACA), both arachnoid and dura contained similar amounts of fibrinolytic activity as did the meninges from untreated dogs. This indicates that the fibrinolytic inhibitory effect of EACA does not persist after the EACA is discontinued and suggests that the meninges should be able to cope with fibrin deposits in their usual manner after cessation of EACA.

One of the potential dangers of the administration of EACA is that it might totally inhibit fibrinolysis and contribute to the production of disseminated intravascular coagulation. The fibrinolytic activity of the blood samples did not demonstrate an inhibition of
blood fibrinolytic activity in the dose schedules outlined above, although the amount of EACA given to Groups 4 and 5 has been shown to inhibit the fibrinolytic activity of the CSF.\(^9\) Thus, at least in dogs, it appears that EACA can be given in the amounts listed above without completely inhibiting fibrinolytic activity. However, the administration of a fibrinolytic inhibitor must upset the homeostatic balance between coagulation and fibrinolysis. Therefore, patients must be monitored carefully for evidence of in vivo thromboses, and the simultaneous use of low doses of heparin must be given serious consideration.

Conclusions

The administration of EACA to dogs in the presence of subarachnoid blood did not appear to be associated with any deleterious effects within the limits of this protocol. Specifically, an adhesive arachnoiditis sufficient to produce hydrocephalus or a delay in CSF protein effluence was not observed. These data, although not directly transferable to the human analogy, should be of interest to those who anticipate clinical trial of this drug in patients with subarachnoid hemorrhage. The question of vascular thrombosis associated with the use of an antifibrinolytic agent has yet to be clarified and may lead to a consideration of combined anticoagulation and EACA therapy. Finally, it is felt that the use of 24-hour blood levels of intrathecally injected RISA should be re-emphasized as a means for evaluation of possible transport delays in the CSF of both high and normal pressure hydrocephalic patients.

References


Received for publication July 27, 1970.
This paper was presented in part at the Southern Neurosurgical Society meeting in Durham, North Carolina, on March 6, 1970.
This work was supported by U.S. Public Health Service Grants 2 R01 NS04368–08, HE–11309, and HE–08929.
Address reprint requests to: M. S. Mahaley, Jr., M.D., Division of Neurosurgery, Duke University Medical Center, Durham, North Carolina 27706.