The effect of epsilon aminocaproic acid and tranexamic acid on thrombus size and strength in a simulated arterial aneurysm

Russel H. Patterson, Jr., M.D., and Peter Harpel, M.D.
Departments of Surgery (Neurosurgery) and Medicine (Hematology), Cornell University Medical Center, New York, New York

An arterial sac was created in rats by ligating the abdominal aorta, and the size and strength of the thrombus that formed in the sac were studied in the rats whose drinking water contained one of two antifibrolytic agents, 5% epsilon aminocaproic acid (EACA), or 1%, 2.5%, or 5% tranexamic acid (trans-AMCHA). The thrombus in the rats treated with 5% EACA, although no larger, was able to resist an intra-aortic pressure of 80 mm Hg, which was 2.5 times as much as in untreated animals. The weight of the thrombus was the same in rats that received 1% trans-AMCHA as in controls, slightly more in those receiving 2.5% trans-AMCHA, and 2.5 times greater with 5% trans-AMCHA. In the latter group the thrombus could resist an intra-aortic pressure eight times greater than that withstood by the thrombus in control animals. This evidence suggests that treatment with antifibrinolytic drugs may preserve the size and strength of the thrombus in a saccular aneurysm which has recently hemorrhaged.

Key Words: fibrinolytic inhibitors · subarachnoid hemorrhage · rat · intracranial aneurysm

A number of studies have shown that the risk of surgical repair of an intracranial aneurysm is greatest soon after rupture when vascular spasm, cerebral swelling, and neurological deficits are prominent. The immediate danger of recurrent hemorrhage has prompted a search for nonsurgical methods to tide the patient over until the arterial defect can be repaired with greater safety. Anti-hypertensive agents have gained favor for this purpose, and Mullan and Dawley have suggested that a drug which stabilized the thrombus in the aneurysmal sac might effectively prevent recurrent hemorrhage. The present report describes the evolution in strength and size of the thrombus that forms in a blind arterial sac created in the rat and how the administration of the antifibrinolytic agents, epsilon aminocaproic acid (EACA) and tranexamic acid (trans-AMCHA; trans-4 amino-methylcyclohexane-1-carboxylic acid) modify the process.

Material and Methods

Pairs of rats of Sprague-Dawley stock were matched for weight within 10 gm over a range of 230 to 290 gm. One of each pair received drinking water containing either 5% EACA, 1% trans-AMCHA, 2.5% trans-AMCHA, or 5% trans-AMCHA, and the other served as a control. The treated rats drank the water at a rate of 40 cc/24 hours. In proportion to body weight this was equivalent to a daily dose of EACA in an adult human of 50 gm, or twice the dose usually necessary to maintain therapeutic blood levels. Control and experimental animals were not distinguished throughout the
experiment in order to avoid inadvertent bias.

Twenty-four hours after the first administration of fibrinolytic inhibitor, an arterial sac was created in both rats. The operation was performed under ether anesthesia using clean but not sterile technique. The aorta was separated from the inferior vena cava for 7 mm proximal to the aortic bifurcation, and any arterial branches from the isolated segment coagulated and divided. The aorta was then ligated at its bifurcation, which created a blind arterial sac 7 mm long (Fig. 1). Control and experimental animals were sacrificed at intervals during the next 2 weeks, and either the thrombus in the arterial sac weighed or its strength tested.

The thrombi which were to be weighed were removed from the aorta after the animals were anesthetized with ether, anticoagulated with heparin, and exsanguinated. The mean, standard deviation and standard error of the weights of the thrombi obtained from treated and control animals were compared using Student's t-test.

Other pairs of animals were also anesthetized, anticoagulated, and exsanguinated, but then the blind sac of the aorta was freed from surrounding adhesions, and a blunt 20-gauge needle 2 mm long was fixed in the aortic lumen proximal to the thrombus. The distal aorta was divided just before the ligature placed at the first operation, which exposed the distal end of the thrombus and permitted the cannulated aorta to be removed from the abdomen. Pressure from a reservoir of tinted saline then was applied to the thrombus through the cannula. The pressure was raised in steps of 25 mm Hg every 5 sec until 300 mm Hg was reached and thereafter in steps of 100 mm Hg until fluid first leaked and finally spurted by the thrombus and out the end of the aorta (Fig. 1).

The LT_{50}, or pressure at which the arterial stump leaked in 50% of the animals in a group, was determined from a plot of the cumulative percentage of animals which leaked against pressure on a logarithmic scale (Fig. 2). These plots resembled characteristic dose-response curves. A statistical comparison between treated and untreated groups was made by the Mann-Whitney U test.

Plasma from rats receiving 5% EACA and their controls was stored by freezing, and the plasma concentrations of EACA in each animal quantitated by thin layer chromatography on silica sheets* using a solvent

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* ChromAR Sheet 500, supplied by Mallinckrodt Chemical Works, Laboratory Products, New York, N.Y.
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Fig. 2. The LTₐ in 15 rats receiving 5% EACA and 13 controls calculated from a graph that depicts what percentage of aortas in a group leaked at each increment of intra-aortic pressure.

mixture of n-butanol, glacial acetic acid, distilled water (4:1:3). One-tenth milliliter of rat plasma was added to 0.4 ml acetone, and the precipitate removed by centrifugation. Fifty microliters of supernatant were applied to the chromatogram. The EACA by this method was found to have an Rf of 0.62, and was separated from other amino acids in rat plasma. This technique proved unsatisfactory for trans-AMCHA, as this substance moved in similar location as a ninhydrin positive spot found in normal rat plasma.

Results

Rats in both the control and experimental groups lost body weight following ligation of the aorta. The amount ranged around 20 gm in each group except those receiving 5% trans-AMCHA, which sustained a mean weight loss of 87 gm or 30% of their body weight (Fig. 3). The size of the thrombus in untreated animals increased for the first 3 days and then decreased (Fig. 4).

Fig. 3. Mean body weight loss 9 days after aortic ligation in treated and control animals.

Fig. 4. Mean weight of the intra-aortic thrombus in untreated animals at intervals following aortic ligation. The number of animals in each group and standard deviation are represented.
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Results with EACA

EACA and Thrombus Weight. Animals receiving 5% EACA were sacrificed 3, 9, and 14 days after aortic ligation. The mean weight of the thrombus did not differ statistically from that in paired controls (Fig. 5).

EACA and Thrombus Strength. The ability of the thrombus to seal the end of the aorta against pressure followed much the same pattern. The mean pressure at which the aorta leaked (LT}_{50} is shown after 3 days in treated and untreated animals in Fig. 2. Although 305 mm Hg were required in the treated animals and only 205 mm Hg in the untreated, this difference was significant only to the 0.1 level in a 2-tailed test.

The LT}_{50} for treated and untreated groups at 3, 9, and 14 days after operation is shown on Fig. 6. Nine days after operation the LT}_{50} was 80 mm Hg in the group treated with EACA and 33 mm Hg in their controls. This difference was significant to the 0.05 level. No significant difference existed between the two groups at 14 days.

Plasma Levels of EACA. Plasma levels of EACA were determined in 83 rats. Four of 42 rats receiving the drug had no measurable plasma levels, and three of 41 control rats did (Table 1). The concentration of drug in the treated group exceeded 1.5 mg% in only 10% of the animals.

Results with Trans-AMCHA

Trans-AMCHA and Thrombus Weight. Trans-AMCHA administered in a concentration of 1% did not affect the weight of the thrombus at 9 days, but in rats receiving 2.5% trans-AMCHA the thrombus was slightly larger than in the control group (p < .05). However, those rats given 5% trans-AMCHA had thrombi 2.5 times as large as in the control group, the difference being significant to the 0.015 level (Fig. 7).

Trans-AMCHA and Thrombus Strength. The resistance of a 9-day-old thrombus to being pushed out of the aortic sac by progressive increments of intra-aortic pressure was increased markedly in the group of rats that received 5% trans-AMCHA. A mean of 200 mm Hg was required in the treated group whereas 25 mm Hg were sufficient in the controls. This eightfold difference was significant to the .02 level. The resistance of the thrombus to elevated intra-aortic pres-

![Graph](image1)

**Fig. 5.** Mean weight of the intra-aortic thrombus at 3, 9, and 14 days following aortic ligation in rats receiving 5% EACA, and in controls.

![Graph](image2)

**Fig. 6.** The LT}_{50}, 3, 9, and 14 days following aortic ligation in rats receiving 5% EACA, and in controls.

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma EACA (mg per 100 cc)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>5% EACA (42 rats) controls</td>
<td>4</td>
</tr>
<tr>
<td>(41 rats)</td>
<td>38</td>
</tr>
</tbody>
</table>

**TABLE 1**

The number of rats in each of six ranges of plasma concentration of EACA.
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TABLE 2
Pressure at which leakage occurred 9 days following aortic ligation in 50% of the rats receiving 2.5% or 5% trans-AMCHA, and controls

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Rats</th>
<th>Aortic Leak Pressure (LTso)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>10</td>
<td>22 mm Hg</td>
</tr>
<tr>
<td>2.5% AMCHA</td>
<td>12</td>
<td>105 mm Hg (p &lt; .05)</td>
</tr>
<tr>
<td>control</td>
<td>10</td>
<td>25 mm Hg</td>
</tr>
<tr>
<td>5% AMCHA</td>
<td>11</td>
<td>212 mm Hg (p &lt; .02)</td>
</tr>
</tbody>
</table>

sure also was increased in the group of animals that received 2.5% trans-AMCHA. A leak did not occur in 50% of the animals in this group until a pressure of 105 mm Hg was reached, whereas 22 mm Hg was sufficient in 50% of the control group. This difference was significant to the .05 level in a 2-tailed test (Table 2).

Discussion

The blind stump of the rat aorta used in this experiment differs in many ways from an intracranial aneurysm in humans. The thin wall of a saccular aneurysm is inadequate to resist arterial blood pressure and gradually stretches and dilates, which is not the case in a ligated aorta. The flow of blood is tangential to the mouth of the saccular aneurysm whereas in the rat model the arterial sac receives the full force of the aortic blood flow.

Nevertheless, similarities exist between a ruptured aneurysm and the ligated aorta. A thrombus forms in the blind arterial sac of the rat, but our observations show that after 3 days it begins to decrease in size and strength. Presumably the same sequence of thrombosis and thrombolysis after rupture of an intracranial aneurysm first arrests subarachnoid hemorrhage and later accounts for rebleeding. The mechanisms that serve in time to reduce the strength and size of a thrombus in rats or humans are incompletely understood. Activation of the plasma fibrinolytic system as well as the release of proteases from polymorphonuclear leukocytes that infiltrate the thrombus are among the current speculations.

Mullan and Dawley proposed that EACA acid might inhibit the activation of the fibrinolytic enzyme system and thereby stabilize the thrombus, which would reduce the incidence of recurrent hemorrhage. They administered the drug to 35 patients and identified a recurrent hemorrhage in just two, which suggests that the effects of the drug were beneficial. Norlén and Thulin have reported similar results in patients receiving either EACA or trans-AMCHA. However, the experience of the Cooperative Study of Intracranial Aneurysms has demonstrated the difficulties of evaluating treatment in subarachnoid hemorrhage and the need for stringent controls. A proper clinical study has proven lengthy and expensive, and requires a volume of patients that few individual centers can produce.

![Fig. 7. Weight of the intra-aortic thrombus 9 days after aortic ligation in rats receiving 1%, 2.5%, and 5% trans-AMCHA, and in controls.](image)

![Fig. 8. Bond structure of EACA and AMCHA.](image)
A laboratory model for screening purposes would be helpful, but saccular aneurysms of uniform size and clotting characteristics are not constructed easily in animals, and an experimental aneurysm that ruptures spontaneously is unknown.2,3 The rat model presented here is easily prepared, and within it thrombosis and thrombolysis follow a predictable pattern.

EACA is a potent inhibitor of fibrinolysis. As it is rapidly absorbed from the gastrointestinal tract, peak blood levels following a single oral dose are reached within an hour. Thereafter the plasma concentration falls steadily over the subsequent 2 or 3 hours at a rate somewhat slower than after intravenous administration because of continued absorption from the gut.3 In these experiments, administration of the antifibrinolytic drugs was begun 24 hours prior to the time the arterial sac and its clot were created. This was done to afford maximum protection to the clot and thereby disclose, under optimal circumstances, whether the drug had any beneficial effect.

The work of others6 has shown that EACA inhibits fibrinolysis at concentrations in plasma of greater than about 10 mg%. In the present experiments, the plasma levels of EACA were generally less than 1.5 mg%, which raises the question of whether the dosage of the drug was adequate. The rats drank the 5% solution of EACA at a rate sufficient to maintain blood levels greater than 10 mg% in a human. Possibly the move from the animal room to the laboratory prior to sacrifice of the rats inhibited their drinking with a resultant fall in plasma levels of drug by the time of exsanguination.

Under the conditions of our experiments, EACA did not preserve the size of the thrombus but strengthened it temporarily. Nine days after ligation of the aorta, a pressure of 33 mm Hg was sufficient to force fluid by the thrombus in one half of the control animals, whereas 80 mm Hg were required in those treated with EACA.

Because we were uncertain if an adequate concentration of EACA in the plasma had been obtained, the effect of trans-AMCHA on the thrombus in the arterial sac was studied. Trans-AMCHA is an anti-fibrinolytic drug closely related to EACA (Fig. 8). Its absorption and mode of action are similar to EACA, but trans-AMCHA inhibits fibrinolysis as effectively as EACA at 1/10 the concentration in human plasma and is more slowly excreted.4

The effect of trans-AMCHA on the size and strength of the thrombus was studied in rats 9 days after ligation of the aorta. This interval was chosen because earlier studies showed that at 9 days the size and strength of the thrombus were at a minimum. The thrombus was somewhat larger and stronger in rats receiving 2.5% trans-AMCHA than in control animals, whereas no difference was observed in those receiving 1% trans-AMCHA. The thrombus in rats receiving 5% trans-AMCHA was 2.5 times as large as in a control group, and a mean intra-aortic pressure of 200 mm Hg was required to expel it from the lumen of the aorta, which was eight times the pressure required in untreated animals.

The 5% AMCHA appeared poorly tolerated by the rats, for the mean weight loss of animals who received it for 9 days was 87 gm. Weight loss in the control groups and in those that received the drug in 1% and 2.5% concentration was about 21 gm during the same period. Whether anorexia, adypsia, diarrhea, or something else accounted for the weight loss in animals receiving 5% trans-AMCHA was not determined.

Drugs that stabilize or strengthen the clot in an aneurysm deserve continued study because removing the threat of recurrent hemorrhage reduces the need for an early operation with its attendant risk and might conceivably obviate the need for surgery in some cases. One can speculate that anticoagulants might be employed to reduce the incidence of ischemic cerebral infraction secondary to vascular spasm if recurrent hemorrhage were no longer an important consideration.

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Address reprint requests to: Russel H. Patterson, Jr., M.D., Associate Professor of Surgery (Neurosurgery), The New York Hospital—Cornell Medical Center, 525 East 68th Street, New York, New York 10021.