The Effect of Ellagic Acid on the Thrombin-Induced Hemorrhagic Syndrome in the Rat

By E. E. CLIFFTON, A. GIROLAMI AND D. AGOSTINO

ELLAGIC ACID has been shown to cause a hypercoagulable state in several animal species, presumably by activation of Hageman factor (factor XII). In the course of experiments with this compound in dogs, rabbits, and cats, a decrease in oozing from venipuncture sites and raw surfaces was noted. A significant decrease in bleeding from the amputated tail of the normal rat was also shown.

The intraperitoneal or intravenous administration of thrombin causes a bleeding syndrome in the rat and in the dog. This hemorrhagic condition is associated with a reduced level of fibrinogen (factor I), proaccelerin (factor V) and antihemophilic globulin (factor VIII). A mild to moderate decrease in prothrombin (factor II) has also been reported. In the dog increased fibrinolysis probably secondary to intravascular clotting was also observed after an initial phase of inhibition of fibrinolysis.

The purpose of the present investigation was to study the influence of ellagic acid on the hemorrhagic syndrome induced by thrombin in the rat.

MATERIALS

One hundred and eighty female rats (Carworth Farms) weighing 200–230 Gm. were used. All animals were maintained under the same conditions of diet (Purina Chow) and environment.

Thrombin solution (Thrombin topical—Parke-Davis) 1000 N.I.H. units/ml., was used.

Ellagic acid (4,4', 5,5', 6,6' hexahydropyridine acid 2,6:2',6' di lactone) as supplied by K & K Laboratories, Plainview, New York, was dissolved in 0.025 M sodium barbital buffer in 0.125 M sodium chloride at pH 7.5. A solution with a concentration of $2 \times 10^{-4}$M was used.

Anesthesia was intraperitoneal sodium pentobarbital (15 mg./animal).

METHOD

The animals were divided into 4 groups of 45 animals each.

Group I (control): 2.0 to 2.3 ml. of buffer (1 ml./100 Gm.) per animal.

Group II (ellagic acid): 2.0 to 2.3 ml. of the $2 \times 10^{-4}$M ellagic acid solution (1 ml./100 Gm.) per animal.

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Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. Animals</th>
<th>Duration of Bleeding (min.)</th>
<th>Blood Loss in cc.</th>
<th>Mean</th>
<th>Range</th>
<th>Standard Deviation</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Controls</td>
<td>45</td>
<td>8–40</td>
<td>3.1</td>
<td>0.3–5.6</td>
<td>1.7</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>II Ellagic acid treated</td>
<td>45</td>
<td>0–11</td>
<td>0.2</td>
<td>0–3.5</td>
<td>0.5</td>
<td>11</td>
<td>&lt;0.001*</td>
<td></td>
</tr>
<tr>
<td>III Thrombin treated</td>
<td>45</td>
<td>13–55</td>
<td>4.6</td>
<td>0.6–7.6</td>
<td>1.5</td>
<td>4.4</td>
<td>&lt;0.01†</td>
<td></td>
</tr>
<tr>
<td>IV Thrombin and ellagic acid treated</td>
<td>45</td>
<td>0–11</td>
<td>0.6</td>
<td>0–3.8</td>
<td>0.75</td>
<td>9</td>
<td>&lt;0.001†</td>
<td></td>
</tr>
</tbody>
</table>

The statistical analysis of the results shows significant difference between the control and ellagic acid-treated (groups I and II),* control and thrombin-treated (group I and III),† and thrombin-treated compared with thrombin and ellagic acid (groups III and IV). The range of and mean blood loss and the duration of bleeding in the 4 groups of animals studied are recorded.

Group III (thrombin): Each animal received 3000 units of thrombin (intraperitoneal). Two hrs. later 2.0 to 2.3 ml. of buffer were infused.

Group IV (thrombin plus ellagic acid): Each animal was given 3000 units of thrombin as the animal of group III. Two hrs. later 2.0 to 2.3 ml. of the ellagic acid solution was administered.

The ellagic acid solution or the buffer was administered intravenously in 5 minutes via a surgically exposed femoral vein in all cases.

Immediately after the administration of ellagic acid or buffer the tail of the animal was amputated 5 cm. from the tip. The animals were then placed on a flat surface with the tails hanging down into a test tube containing 5 mg. of EDTA.

The blood loss was measured 30 minutes after the tail wound had stopped bleeding. All animals were then kept under observation for 2 days for possible recurrence of bleeding from the site of amputation.

Results

The range and the means of blood loss observed in the 4 groups of animals are recorded in Table 1, together with the statistical analysis of the results. The difference between the mean blood loss (3.1 ml.) observed in the control group and the mean blood loss observed in groups II and III has been found to be highly significant (p < 0.001 or p < 0.01). The difference in blood loss between thrombin-treated animals and thrombin plus ellagic acid-treated animals (groups II and IV) also has been found to be statistically significant (p < 0.001). The mean blood loss for the 4 groups is graphically illustrated in Figure 1.

There was a wide variation in the blood loss of individual animals (Table 1). As a result, large values for the standard deviations were obtained in all groups. This, however, was due to the abnormal behavior of only a few
Fig. 1.—Average blood loss in ml. in the different groups of animals.

animals in each group. In the control group all but 6 animals had a blood loss greater than 1 ml. In the ellagic acid-treated group only 3 animals bled more than 1 ml. In the thrombin-treated group 43 animals had a blood loss of 2 ml. or more and 38 had a blood loss of 3 ml. or more. In the thrombin and ellagic acid-treated group only 7 animals bled more than 1 ml.

The duration of the bleeding was 8 to 40 minutes in the control group (group I) and 0 to 11 minutes in the ellagic acid-treated group (group II). In the thrombin-treated animals (group III) the bleeding was prolonged to 13 to 55 minutes. When the ellagic acid was administered after the thrombin (group IV), the bleeding time was shortened to 0 to 11 minutes, as with ellagic acid alone (group II).

No untoward side effect attributable to the administration of ellagic acid was noted. No recurrence of the bleeding was observed during the 2-day observation period.

In 62 of the 90 thrombin-treated animals (groups III and IV) there were signs of spontaneous bleeding (hematuria and epistaxis). These bleeding manifestations have been described by others in thrombin-treated rats. In the animals given only thrombin (group III) 32 showed hemorrhagic manifestations and the bleeding ceased during a 30-minute period in only 3 of these animals (9.3 per cent). In group IV, 30 of the 45 animals suffered spontaneous bleeding. The administration of ellagic acid after the thrombin resulted in cessation of the abnormal bleeding in 22 of the 30 animals (76.6 per cent) within 30 minutes. The difference in the number of animals in which the bleeding tendency ceased in these 2 groups was statistically significant (p < 0.01).


DISCUSSION

Ellagic acid activates Hageman factor in vitro\(^8\) and in vivo.\(^1,2\) As a result of this activation a state of hypercoagulability supervenes which is characterized by a striking shortening of the silicone clotting time,\(^1,2\) increased prothrombin consumption,\(^1\) shortening of partial thromboplastin time,\(^1\) and acceleration of thromboplastin generation.\(^1,2\)

In previously reported experiments we observed that ellagic acid, in vivo, diminishes the volume of blood loss and shortens the time of bleeding from the amputated rats’ tails.\(^3\) The present experimental results indicate that ellagic acid is effective in preventing the excessive bleeding caused by intraperitoneal injection of thrombin. It not only limited the amount of bleeding from the cut tail, but also the spontaneous bleeding (usually nasal or urinary) induced by thrombin.

The hemorrhagic syndrome secondary to intravenous or intraperitoneal thrombin is characterized in the rat and in the dog by low fibrinogen and low proaccelerin and antihemophilic globulin.\(^4,6\) Defective platelet plug formation after incision of mesentric vessels has also been reported in the rat.\(^9\) PTC (factor IX) does not seem to be affected.\(^4,5\) No studies are available concerning the behavior of the Hageman factor-PTA (factor XI) system in this condition. Apparently the activation of Hageman factor caused by the administration of ellagic acid in thrombin-injected rats is sufficient, at least with the dosage of thrombin used by us, to accelerate the coagulation process. This occurs despite the low levels of fibrinogen, proaccelerin, and antihemophilic factor caused by thrombin. It seems that in the presence of activated procoagulants the blood is able to supply an effective hemostatic mechanism, even with low levels of coagulation factors.

Other mechanisms of action cannot be excluded, even though ellagic acid has not been shown to have any other recognized activity besides the capacity to activate Hageman factor and therefore stimulate the first stage of blood coagulation\(^1,2,8\) and cause a state of hypercoagulability.\(^1,2\)

The possibility that the changes observed are due to impurities contained in the commercial ellagic acid used (K & K Laboratories, Plainview, New York) may be safely ruled out. Preliminary studies with purified ellagic acid have shown that this material has the same activity as the less pure preparation.

The results of this experiment are not without some clinical implication. Several bleeding conditions observed in humans have been attributed to thromboplastin release with consequent thrombin formation and with or without intravascular clotting and hyperfibrinolysis.\(^10-13\)

SUMMARY

The intraperitoneal administration of thrombin increased the bleeding due to the amputation of the tail in the rat (average blood loss 4.6 ml.). The intravenous administration of ellagic acid in thrombin-injected rats reduced the average blood loss to 0.6 ml. This value was slightly more than the average blood loss noted in animals given only ellagic acid (0.2 ml.). Both these figures
were much less than the average blood loss observed in control animals (3.2 ml.).

**Summario in Interlingua**

Le administration intraperitonee de thrombina augmentava le sanguination resultante del amputacion del cauda in le ratto. (Le perdita medie de sanguine eseva 4,6 ml.) Le administration intravenose de acido ellagic in rattos pretrcatate con injectiones de thrombina reduceva le perdita medie de sanguine a 0,6 ml. Iste valor eseva levemente plus alte que illo del perdita medie de sanguine notate in animales tractate exclusivamente con acido ellagic (0.2 ml). Ambe iste valores eseva marcatamente inferior a illo del perdita medie de sanguine observate in animales de controlo. (Isto eseva 3,2 ml).

**REFERENCES**

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