Effect of Epsilonaminocaproic Acid on the Turnover of Labelled Fibrinogen in Rabbits

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The concept that coagulation proceeds constantly in vivo with fibrinolytic removal of the end product, fibrin, is still to be validated. This hypothesis, underlying the v. Rokitansky-Duguid-Astrup theory of the pathogenesis of atherosclerosis, postulates that fibrin polymers are constantly deposited on the vascular wall as a consequence of clotting, and then promptly lysed by a "preventive" fibrinolytic enzyme system. The disturbance in the normal physiology of the coagulation balance could be an important factor in hemorrhagic phenomena on the one hand, or in the pathogenesis of atherosclerosis or its complications on the other.

The present communication describes an attempt to validate this concept by experimentally tipping the postulated balance by means of an agent, epsilonaminocaproic acid (EACA) which is known to block the fibrinolytic as well as the fibrinolytic system. Under these conditions one would expect retarded turnover of fibrinogen that was suitably labelled and administered to animals that had also received the fibrinolytic blocking agent.

**Methods**

**I\(^{131}\) LABELLED BOVINE FIBRINOGEN**

Armour Fraction I from bovine plasma (60% of protein clottable with thrombin, as estimated by Kjeldahl method) was labelled with I\(^{131}\) according to the technique adapted from the method of Eisen and Keston. The final solution contained about 1% fibrinogen in 0.1 M phosphate buffer, pH 7.8, and approximately 50 μCi of 1\(^{131}\)/ml. About 95% of this radioactivity was precipitable with 10% trichloroacetic acid, and approximately 50% was clottable with thrombin. No attempt was made to purify the preparation further since it was assumed that the impurity would not affect the turnover rate of fibrinogen.

0.5 ml of this material was injected intravenously into 11 healthy rabbits (New Zealand Albino), weighing 2 kg to 3 kg. After 20 hours, during which time the administered fibrinogen was presumably distributed throughout the body, 3 ml of blood were withdrawn by cardiac puncture ("O" sample), and mixed with 0.1 M sodium oxalate (1:10 volume proportion). This was followed by intravenous injection of 4.0 ml of a 25% sterile solution of EACA (1.0 g) or of 0.9% of NaCl to the control group. At selected intervals over a period of 33 to 48 hours thereafter, blood was obtained by cardiac puncture, followed each time by EACA or saline infusions. Each animal received a total of 3 g to 4 g of EACA.

In order to estimate the specific radioactivity of fibrinogen, 0.5 ml (in duplicate) of each plasma sample was diluted with 0.05 M phosphate buffer containing CaCl\(_2\), pH 6.4, and the resulting mixture was clotted by addition of 25 units of bovine thrombin (Upjohn). After 30 minutes the clots were transferred to filter paper, thoroughly washed (for about one hour), first by several rinses with physiological saline, and then 20 to 40 times with distilled water in order to remove all detectable nonclottable radioactivity. The clots were then transferred to suitable tubes for counting in a well-type scintillation detector equipped with a pulse height analyzer. Radioactive emission (0.365 Mev peak) was determined by means of a 2 v window. Under such circumstances overall radiation detection efficiency was 50%, and background was consistently 4 to 5 counts/min. All samples from a particular animal were counted concurrently to obviate differences due to decay of radioactive iodine itself.

After counting, the amount of fibrin was estimated (in duplicate) in each clot by the Folin-Ciocalteau method as described by Niewiarowski, permitting calculation of the specific clot radioactivity. The specific activity of the "O" sample,
TURNover OF LABELLED FIBRINOGEN

I\textsubscript{131} FIBRINOGEN TURNOVER

A

CONTROL

B

EACA

S.A. HALF-LIFE

X-X 20.5 Hours

0-0 18.0 Hours

G-G 15.0 Hours

G-G 12.5 Hours

G-G 11.5 Hours

MEAN=15.5

S.A. HALF-LIFE

0-0 14.5 Hours

X-X 12.0 Hours

G-G 16.0 Hours

G-G 13.0 Hours

G-G 20.5 Hours

MEAN=16.1

INTERVAL — HOURS

FIGURE 1

Disappearance in vivo of \( \text{I}_{131} \) labelled bovine fibrinogen. Abscissa indicates intervals beginning 20 hours after administration of the fibrinogen. Epsilonaminocaproic acid (EACA), or saline in the controls, was infused after each blood sampling. Ordinate indicates specific radioactivity (S.A.), in counts per minute, of the recoverable fibrin clot.

containing approximately 1.5 mg to 3.0 mg of protein, was 200 to 500 counts/min per mg.

Euglobulin lysis times were determined 30 minutes after the injection of EACA or saline in some animals by the method employed by Kowalski et al.\textsuperscript{11}

Results

The disappearance of the tagged fibrinogen from the blood is presented in figure 1. It is noteworthy that the individual curves reflecting disappearance rates are remarkably parallel, and in the EACA-treated rabbits did not differ significantly from the saline-treated group despite inhibition of fibrinolysis by the drug as judged from retarded euglobulin lysis (table 1). The relationship between the logarithm of the specific clot radioactivity and the interval following tagged fibrinogen administration was not rectilinear, at least for 60 hours following injection, contrary to what was theoretically expected.

Discussion

EACA (10\textsuperscript{-4}M) is a potent inhibitor of plasminogen activator, and at a higher concentration (10\textsuperscript{-3}M) it is said to inhibit plasmin as well.\textsuperscript{12} Our experiments were so designed as to give an expected concentration of EACA in the blood that would markedly exceed these levels, at least immediately after each injection. Since, however, the drug is excreted very rapidly\textsuperscript{13,14} the required level
may not have been maintained during the entire experimental period. Nevertheless, on the basis of available data,\textsuperscript{12-14} we can assume that under our conditions the levels of circulating EACA were substantial, and that the plasminogen activator was blocked completely for almost the whole experimental period, while the optimal concentration required to stop plasmin action was presumably maintained only for about an hour after each injection. The marked elevations in the euglobulin lysis time, 30 minutes after the EACA was injected, certainly indicates block of the fibrinolytic mechanism, at least to considerable extent. On the basis of experience of Fletcher et al.\textsuperscript{15} and unpublished observations of our own, fibrinogenolysis in vitro is also inhibited by EACA.

We have postulated that if under normal circumstances fibrinogenolysis as well as fibrinolysis proceeds constantly in vivo, acting upon fibrinogen, and fibrin that is constantly evolving in vivo, and that if this process affects the turnover rate of fibrinogen, inhibition of the lytic mechanism should theoretically result in retarded fibrinogen turnover. Since this was not found, it appears that the inherent fibrinolytic mechanism was not operative in the removal of the labelled fibrinogen, at least to an extent sufficient to affect the obviously gross and progressive changes observed. One may further deduce that even if it does occur, constant in vivo fibrinogenolysis, and perhaps fibrinolysis, does not significantly influence the maintenance of a dynamic coagulation balance. To be sure, due allowance must be made for other variables, i.e., heterogeneity of the fibrinogen used in the study, and the possibility that in labelling with I\textsuperscript{131} the bovine fibrinogen was so altered as to influence its removal from the rabbits' circulation by mechanisms other than fibrinolytic.

These data are in agreement with recent work of Lewis et al.\textsuperscript{16} who showed that inhibition of coagulation with heparin, which functions via a totally different mechanism, does not alter fibrinogen turnover. Accordingly, question can be raised regarding the validity of the concept of constant in vivo coagulation.

As already mentioned, a plot of the logarithm of the specific activity against time was curvilinear instead of rectilinear. This observation, also in accordance with the data of Lewis et al.,\textsuperscript{16} indicates that removal of labelled bovine fibrinogen within the first sixty hours following administration does not follow first order kinetics.

**Summary**

Bovine fibrinogen labelled with radioactive iodine was injected intravenously into eleven healthy rabbits. Twenty hours later and at selected intervals thereafter, blood was withdrawn, followed each time by 1.0 g of EACA or saline. The disappearance of the radioactive fibrinogen from the blood did not appear
significantly altered by the drug despite the induced retardation of fibrinolysis. This suggests that the inherent fibrinolytic mechanism did not play a significant role in fibrinogen turnover. The relevance of the data to the postulated in vivo coagulation process and clotting balance is discussed.

References

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