The Effect of Experimental Hematoma on Serum Levels of Fibrinogen-related Antigen

By John Code, Erwin Regoeczi, Jack Hirsh, and Michael Buchanan

The effect of experimental hematomas on serum levels of fibrinogen-related antigen (FRA) was studied in rabbits. Homologous blood used for production of hematomas was mixed with $^{131}$I-fibrinogen, and the measurement of FRA was made using a radioactive technique (representing FRA from hematoma) and immunologically (representing total FRA). Total body radiation of animals bearing hematomas containing $^{131}$I-fibrinogen decayed slowly (mean $\tau$ = 189 hr) over the first 2-3 days and then rapidly (mean $\tau$ = 43 hr) during the subsequent days. Serum FRA levels measured immunologically rose transiently from 4 to 11 $\mu$g/ml during the slow phase of hematoma resolution, and returned to baseline levels during the rapid phase of resolution. The appearance of a small amount of protein-bound radioactivity in the blood of animals with hematomas paralleled the time course of the changes in immunologically determined FRA, but, even at its peak, circulating levels only averaged 0.36% of the administered tracer. From these observations, it seemed unlikely that the dissolution of fibrin which may form as the result of bleeding into tissues would significantly contribute to levels of circulating FRA.

ELEVATED LEVELS OF fibrinogen-related antigen (FRA) in serum are a useful laboratory index of disseminated intravascular coagulation. Levels of FRA have also been reported to be elevated in localized intravascular thrombosis, particularly pulmonary embolism. In these situations, FRA is thought to be produced by the lysis of the intravascular fibrin.

The diagnostic specificity of this measurement would be considerably diminished if circulating FRA could also be derived from extravascular sources. Considerable bleeding into tissues can occur in postoperative and posttraumatic states in which disseminated intravascular coagulation or pulmonary embolism are commonly seen. Moreover, bleeding into tissues can also occur as a result of the hemorrhagic tendency associated with disseminated intravascular coagulation. In the present study, we have examined serum FRA levels in rabbits during the resolution of large hematomas.

MATERIALS AND METHODS

Production of Hematomas

New Zealand White rabbits of either sex and of approximately 2.5 kg body weight were used. Blood was taken from donor rabbits through a carotid artery cannula into a plastic syringe without an anticoagulant, and was injected immediately into recipient animals. Four animals received 15 ml blood/kg intramuscularly, equivalent to 20%–25% of their estimated blood volume. As the plasma of donor rabbits contained an average of 3.1 (SD ± 0.61) mg fibrinogen/ml at a hematocrit
value of 33.5\% (SD \pm 2.8\%), the amount of fibrinogen deposited in hematomas was estimated to have been approximately 30 mg/kg body weight. The total blood to be given intramuscularly was divided into four aliquots and injected into each shoulder and each thigh region. In four other animals, the blood was rapidly mixed with homologous $^{125}$I-fibrinogen before intramuscular injection. The labeled fibrinogen, 97\% clottable with thrombin, was prepared as previously described,\textsuperscript{11} 14-15 \mu Ci being used for each experiment. Less than 0.1\% of the injected radioactivity was not protein-bound.

The effect of extravascular blood deposition on the levels of circulating FRA was assessed by the immunologic and radioactive techniques described below.

Estimation of Circulating FRA Immunologically

Blood samples were collected from the central ear artery before inducing the hematoma, 4 hr afterward, and daily thereafter for 1 wk. FRA was measured in serum as fibrinogen/fibrin-related antigen by a tanned red cell hemagglutination inhibition assay, as previously described.\textsuperscript{12} This assay was sensitive to 2 \mu g/ml of rabbit fibrinogen.

Measuring Radioactive FRA

This test was done in the rabbits in which the hematomas contained labeled fibrinogen. Blood samples (1 ml) were collected into dry heparin according to the following schedule: three samples on day 1, two samples on days 2-3, and one sample daily on days 4-6. From each specimen, a plasma sample was prepared to assay total plasma radioactivity and radioactivity soluble in 10\% (w/v) trichloroacetic acid, the latter representing nonprotein radioactivity. Counting was done in a Packard model 5975 analyzer. Levels of protein-bound radioactivity per milliliter of plasma were expressed as percentages of the dose. To calculate total circulating radioactive FRA, values for protein-bound activity per milliliter of plasma were multiplied by the mean intravascular distribution volume for fibrinogen in rabbits.\textsuperscript{11}

Rate of Elimination of Labeled Fibrinogen Incorporated Into Hematomas

This was studied in four rabbits by serial measurements of the total body $^{131}$I radiation. The animal total body counter used for this purpose has been described in detail elsewhere.\textsuperscript{13} The schedule of total body radioactivity measurements was similar to that given above for the radioactive blood samples. Prior to each measurement, the bladder of the animals was emptied and washed out, using a 10 FG Jaques Ba 3 catheter.

RESULTS

Fate of the Experimental Hematomas

Autopsies performed in four preliminary studies showed that the blood introduced intramuscularly gradually clotted in situ. At 30 min after injection clotting was partial, and at 1 hr it was complete. Clots retrieved after 1 hr from the thighs were insoluble in 5 M urea.

The total body radioactivity of all rabbits bearing radioactive hematomas decayed in a biphasic manner (Fig. 1). During the first phase, which lasted for approximately 2-3 days, radioactivity in the body decreased slowly ($t_{1/2} = 189$ hr, SEM \pm 25 hr); subsequently the slope became markedly steeper ($t_{1/2} = 43$ hr, SEM \pm 10 hr), and remained so until the end of the experiment. Ninety percent of the radioactive material originally present was eliminated from the body during the first 6-10 days.

Levels of Serum FRA

Levels of serum FRA, as obtained by inhibition of tanned red cell hemagglutination, are summarized in Table 1. Following the intramuscular injection of blood, there was a small but statistically significant increase in serum FRA at 24 and 48 hr.
Circulating Radioactive Fibrin Breakdown Products From Hematomas

In all animals in which the hematomas contained 131I-fibrinogen, a small amount of radioactivity was detected in the plasma as early as 1 hr after blood was injected. Concentrations of the circulating radioactivity followed a characteristic time course, which is shown in relationship to the total body radioactivity curve in Fig. 1. The circulating protein-bound radioactivity reached the highest levels at 24-48 hr which, when expressed as a percentage of the dose, averaged 0.36% (range 0.26%-0.51%) for the four animals studied. Based on the calculation that an average of 98 mg of fibrinogen was deposited with the hematomas in each animal, and that the average circulating plasma volume was 120 ml, this value represents a concentration of circulating radioactive FRA of 2.9 μg/ml. Subsequently, concentrations of protein-bound radioactivity declined, and by the time the total body radioactivity curves reached their steep terminal slope, only small quantities of protein-bound radioactivity remained detectable in the circulation.

Table 1. Levels of Serum FRA in Rabbits Bearing Hematomas

<table>
<thead>
<tr>
<th></th>
<th>0 hr</th>
<th>4 hr</th>
<th>24 hr</th>
<th>48 hr</th>
<th>72 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematomas</td>
<td>3.9</td>
<td>3.9</td>
<td>8.8*</td>
<td>11.0*</td>
<td>6.6</td>
</tr>
<tr>
<td></td>
<td>(0.6)</td>
<td>(0.6)</td>
<td>(3.1)</td>
<td>(2.2)</td>
<td>(1.3)</td>
</tr>
</tbody>
</table>

Times are taken as from the injection. Results are mean (μg/ml) with standard error in parentheses.

*Indicates significant increase relative to the value at 0 hr (p < 0.05).
The change in the slope of the total body radioactivity curve was associated with a further rise in the level of circulating radioactivity. However, this rise was entirely due to nonprotein radioactivity.

**DISCUSSION**

The results of this study indicate that the elimination of fibrin in hematomas produced in rabbits by the intramuscular administration of blood containing radioactive fibrinogen follows a characteristic kinetic pattern which we have defined by parallel measurement of total body and plasma radioactivities.

Decrease in total body activity during the slow initial phase of resolution (first 2-3 days) corresponded to 17%-24% of the radioactivity in the hematomas. During this period, a small amount of protein-bound radioactivity, representing labeled FRA from the hematomas appeared in the circulation, and serum levels of nonradioactive FRA were also slightly elevated. Subsequent resolution of the bulk of the hematomas occurred at rates which were over four times faster than the initial rate. However, in striking contrast to the initial findings, there was no concomitant rise in serum FRA levels, whether measured immunologically or by radioactive counting.

There was a slight disparity between the peak FRA levels determined immunologically and the peak levels of radioactive FRA calculated from the radioactivity data. Thus, the net increase in FRA 48 hr after inducing the hematomas was approximately 9 µg/ml, while radioactive FRA concentration based on calculations of the estimated amount of fibrinogen deposited in the hematomas, and the estimated plasma volume was approximately 3 µg/ml. These differences suggested that some of the serum FRA measured immunologically may not have been derived from the hematomas but may have been generated intravascularly in response to the tissue injury.

The results of parallel measurements of total body and plasma radioactivity suggest that rabbits are capable of eliminating considerable quantities of fibrin from extravascular sites by mechanisms which result in the release of only minute quantities of the lysed protein into the circulation at any one time. How this process is achieved is not quite clear, but it is likely that fibrin is phagocytosed in situ, and then subjected to proteolysis by polymorphonuclear leukocytes and macrophages. Leukocytes have been shown to be capable of digesting fibrin and fibrin degradation products. Resolution of the hematoma may be initiated by the leukocytes entrapped in the hematoma (slow phase of resolution) and may continue more efficiently after the local inflammatory reaction develops (rapid phase of resolution).

If the mechanism for disposal of extravascular fibrin in humans is similar to that in rabbits, our results suggest that extravascular fibrin deposition is unlikely to contribute significantly to raised serum levels of FRA.

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**REFERENCES**


3. Kwaan HC: Disseminated intravascular
The effect of experimental hematoma on serum levels of fibrinogen-related antigen

J Cade, E Regoecki, J Hirsh and M Buchanan