Detection of Preformed Venous Thrombi in Dogs by Means of I\(^{131}\)-Labeled Antibodies to Dog Fibrinogen

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Diagnostic measures to determine the existence and exact location of established or propagating thrombi would be of obvious clinical significance. The use of a gamma-emitting isotope attached to a compound which would concentrate in such lesions could provide a diagnostic technique with the advantages of rapidity, portability, safety, and simplicity. Several investigators have studied the fate of I\(^{131}\)-labeled fibrinogen or plasminogen in animals in which thrombi were actively propagating or later induced.\(^1,3\)

It seemed possible that antibody to fibrinogen, labeled with I\(^{131}\), could also localize and concentrate in preformed thrombi. Previous studies have indicated that such antibody preparations combined with circulating fibrinogen are carried along with the fibrinogen as it is converted into fibrin.\(^4,5\)

Since early sequelae to blood vessel damage include inflammation and fibrin deposition, such isotopically labeled antibodies should accumulate along with the fibrin. These preparations are also amenable to removal from circulation by immunological techniques.

The application of a scintillation scanner to detect the radioactive thrombus is appropriate since this technique provides a graphic representation of the radioactivity in the suspected and normal limbs and demonstrates an area of isotope concentration. However, such a system requires a high ratio of radioactive isotope in the lesion compared to that in the rest of the limb because of the method of detection. The major proportion of the general radioactive background comes from the I\(^{131}\)-labeled rabbit antibody in the circulation. It was, therefore, decided to reduce this by administration of a goat antiserum to rabbit gamma globulin. Previous studies have shown that such a procedure substantially reduces the blood-borne radioactivity in rats at the same time that the radioactivity localized in tumors remained stable.\(^6\)

Methods

PREPARATION AND I\(^{131}\)-LABELING OF ANTI-DOG-FIBRINOGEN

The method of preparing fibrinogen and the techniques used for immunization of rabbits has been described previously.\(^7\) Separation of antibody reacting with dog fibrinogen from other components of rabbit serum was accomplished by an absorption and elution technique. Twenty milliliters of rabbit antiserum to dog fibrinogen were mixed with 80 ml of dog plasma. Calcium chloride and an amount of thrombin sufficient to cause clotting of the plasma were added to this mixture. The resultant clot was stirred with a glass rod to achieve fragmentation and was centrifuged for 15 minutes at 13,000 rpm. The clot was then homogenized with 30 ml of saline by means of a motor-driven glass homogenizer and then centrifuged again for 15 minutes at 13,000 rpm. The supernatant fluid was discarded and the residue, which contained dog fibrin to which was bound the specific antibody, was then eluted for five minutes with 10 ml of pH 11.6 phosphate buffer. This preparation was centrifuged at 13,000 rpm for 15 minutes. The resultant supernatant, which contained the purified antibody to dog fibrinogen, was removed and dialyzed overnight.

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against pH 8 borate buffer. Aliquots consisting of 4 mg of this one-time purified antibody to dog fibrinogen were iodinated using a modification of MacFarlane's technique. With each preparation, in vitro tests were performed with dog and rabbit plasma as a measure of the fraction of the I\(^{131}\) in the antibody preparation which would bind to freshly formed fibrin. The test was done by mixing dilutions of I\(^{131}\)-labeled antibody with calcium chloride, thrombin, and citrated dog blood or plasma. The clot was stirred with a glass rod, centrifuged, and washed with saline. I\(^{131}\) content was measured and the percentage of the initial radioactivity that was present in the clot was calculated. In the preparation used for these studies, the amount of initial radioactivity bound by the dog plasma clot varied from 50 to 55% and about 5% was bound nonspecifically in the rabbit plasma clot.

**EXPERIMENTAL PROCEDURE**

Under pentobarbital sodium anesthesia, the upper femoral veins of adult mongrel dogs, weighing 9.5 to 23.6 kg, were exposed bilaterally. The average weight of these animals was 17.8 kg. Three experimental procedures were established. In one group of 12 dogs (nos. 1-10, 12, 18) a thrombus was induced by injection of 5000 units of thrombin directly into the proximally ligated femoral vein. No incision or manipulation was performed on the contralateral side. In another group of 6 dogs (nos. 11, 13-17) after induction of a thrombus by thrombin, the opposite femoral vein was exposed and simply ligated. In the third group (nos. 19-21) one vein was ligated and the contralateral femoral was surgically exposed and the incision then closed. One to three days after the surgical induction of thrombosis, the animals received an intravenous infusion of the I\(^{131}\) antibody preparation and at the same time were given 325 mg of potassium iodide to block accumulation of released radioactive I\(^{131}\) in the thyroid. The amount of I\(^{131}\)-labeled purified antibody which was injected varied from 100 to 1500 \(\mu\text{g}\) of I\(^{131}\) in a volume of 1 to 5 ml. The highest amounts of radioactivity were given when clots were to be used for radioautography or when rapid scanning was desired.

Twenty-four hours after the injection of I\(^{131}\) anti-dog fibrinogen, i.e., two to four days after the surgical procedures described above, the dogs were again anesthetized, a blood sample taken, and the animals were scanned from the pelvis posteriorly using a Picker magnascanner. The scanning time was 10 to 30 minutes depending on the I\(^{131}\) dose injected. Following this procedure the animals received an intravenous injection of goat antiserum to rabbit gamma globulin. One hour after the administration of this preparation a blood sample was obtained and a second scanning performed. During the interval between the two scans, the scanner was left on and no electronic changes were made thus providing a more objective estimate of the effect of the goat antiserum on the clarity of the scan.

After this second scan, the incisions were opened. The vessels and thrombi, when present, were removed as well as the contralateral normal or ligated blood vessel. I\(^{131}\) determinations were performed on these tissues as well as the blood samples and all data are expressed as per cent of the injected dose found per unit weight, the "unit" being 1% of the animal’s weight. In several instances radioautographs were made from the sectioned blood vessel containing thrombi or ligated blood vessels.

**Results**

1. **IMMUNOLOGIC REMOVAL OF I\(^{131}\) ANTI-DOG-FIBRINOGEN USING ANTISERUM TO RABBIT GAMMA GLOBULIN**

Previous studies with rats had shown that immunological removal of 75% of circulating I\(^{131}\) rabbit gamma globulin was accomplished when goat antibody protein was injected in doses equaling twice that of the original labeled protein. When larger amounts of anti-rabbit gamma globulin were infused, there was essentially the same degree of complexing with the I\(^{131}\) rabbit gamma globulin. However, when the goat antiserum was decreased to an amount equivalent to or less than the original injection of rabbit gamma globulin, the removal of the labeled protein was less efficient. When goat antibody was given in an amount equivalent to one-quarter of the original rabbit gamma globulin, there was no decrease in the circulating radioactivity.

Because of the use of larger sized animals in this investigation, i.e., dogs rather than rats, and the variable length of time between the injection of the I\(^{131}\) preparation and antiserum to it, a study was made of the efficiency of removal from circulation of various amounts of I\(^{131}\) anti-dog fibrinogen using four and one-half times as much antibody protein given one and three days later. In this experiment a series of 10 dogs was injected with 0.1 to 0.8 mg I\(^{131}\) rabbit antibody to dog fibrinogen. One to three days later the animals were infused with about four and one-half times as much goat
antibody to the rabbit gamma globulin. Blood samples were taken at various times before and after injection of the goat antiserum, counted on a NaI well-type scintillation counter, and the percentage of the injected dose found in an amount of blood equivalent to 1% of the body weight was determined. The average of results is plotted in figure 1.

In all the animals, circulating $^{131}$I decreased substantially and rapidly after the injection of goat antiserum. In the animals injected one day after the labeled antifibrinogen, as well as those injected three days after receiving the labeled antifibrinogen, there was a decrease in blood-borne $^{131}$I to about one-third of the pre-goat values. The actual $^{131}$I radioactivity found in blood samples obtained before and after injections of goat antiserum form part of tables 2 and 3.

2. DETECTION OF ONE-DAY-OLD VENOUS THROMBI

A total of 18 dogs was studied. Thirteen of these animals were injected with the isotopically labeled preparation 20 to 24 hours after the thrombus induction. Table 1 shows the $^{131}$I activities found in the blood and blood vessel containing a thrombus, expressed as a percentage of the injected $^{131}$I found in a weight of organ equivalent to 1% of the animal's body weight. Within one to two hours after the injection of the goat antiserum, the blood activities were reduced to an average of 26% of their earlier levels. The concentration of radioisotope found in the blood vessel and thrombus was always higher, ranging from 1.3 to 5.6 times, than the $^{131}$I found in blood before the goat serum was given. Subsequent to the administration of goat serum, the ratios increased to between 7.0 and 33.3.

The effect of reducing the circulating $^{131}$I activity can be noted in the photoscans of figures 2 and 3. Two incisions were made, one on each lower extremity. In one extremity a thrombus was induced and in the other extremity the incision was simply closed. The upper half of the figure demonstrates the lower abdomen and proximal portions of the hind limbs and one can detect some increase in radioactivity in the area of each incision. This is somewhat accentuated at the site of the thrombus. The bladder contained considerable radioactivity due to excretion of $^{131}$I. The lower half of these figures shows the effect of administering goat serum. The amount of radioactivity in the region of the thrombus has been sustained but the general radioactivity has been decreased. Localization of the $^{131}$I at the site of thrombosis is now more apparent. One-minute counts, using a scaler attached to the probe of the magnascanner, over the incision on the side on which no thrombus was present demonstrated a 50% decrease in net radioactivity within 15 minutes after injection of the goat antiserum to rabbit gamma globulin. Samples of the liver taken two and one-half hours after immunological removal of the $^{131}$I anti-dog fibrinogen demonstrated that 9% of the injected $^{131}$I dose was found in the organ. The spleen contained 2.9% and the lungs 0.9%.

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Tables 1 and 2 provide % activity found in blood and vessels with thrombus. The % activity in blood before and after goat serum administration varies between animals, with some showing a decrease and others an increase. The weight of blood vessel plus thrombus and the ratios of thrombus to blood vessel weight also show variability. The ratios of thrombus to blood vessel weight before and after goat serum are calculated.

Figure 4 shows a radioautograph together with a matching hematoxylin and eosin-stained section of each of three blood vessels and thrombi. The radioactivity in the blood vessel wall corresponds to areas of acute inflammation in the media and adventitia. In the clot itself, the greatest concentration of isotope is in the periphery, where there is some acute inflammation and signs of beginning organization of the clot. There is some relationship between I$^{131}$ accumulation and the amount of acidophilic material. In one instance, figure 4B, there was suggestion of beginning formation of endothelial nuclei, canalization by capillaries and ingrowth of a fresh blood supply.

3. EVALUATION OF OLDER THROMBI
A series of animals was studied two and three days following thrombus induction. The results obtained are shown in table 2. There is essentially no difference between the amount of I$^{131}$ found in the thrombus and the blood vessel in animals in which the thrombus had been established two to three days before isotope injection. In addition, there is no

Circulation Research, Vol. XVII, October 1965
TABLE 3

$I^{131}$ Activity in Normal, Ligated, and Thrombus Containing Vessels

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Duration of thrombus before $I^{131}$ injection</th>
<th>Per cent of injected dose found in tissue equivalent to 1% of dog's body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>days</td>
<td>Blood vessel plus thrombus</td>
</tr>
<tr>
<td>11</td>
<td>1</td>
<td>22.68</td>
</tr>
<tr>
<td>13</td>
<td>1</td>
<td>9.64</td>
</tr>
<tr>
<td>14</td>
<td>2</td>
<td>14.00</td>
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<td>15</td>
<td>2</td>
<td>9.21</td>
</tr>
<tr>
<td>16</td>
<td>2</td>
<td>25.39</td>
</tr>
<tr>
<td>17</td>
<td>3</td>
<td>10.27</td>
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<tr>
<td>18</td>
<td>3</td>
<td>101.57</td>
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<tr>
<td>19</td>
<td>1</td>
<td>---</td>
</tr>
<tr>
<td>20</td>
<td>1</td>
<td>---</td>
</tr>
<tr>
<td>21</td>
<td>3</td>
<td>---</td>
</tr>
</tbody>
</table>

* $I^{131}$ antibody to dog fibrinogen had been injected one day earlier.
† Tie not present at end of experiment; probably slipped off.

FIGURE 2

Photoscans of a dog (no. 1) that had had a thrombus induced two days earlier. Upper scan was made just before, and the lower scan one hour after, intravenous administration of goat antiserum to rabbit gamma globulin. One day before the scanning, animal had been given 270 µc of $I^{131}$ rabbit antibody to dog fibrinogen.

obvious divergence from the results obtained in animals in which the thrombus had been induced only one day before the antibody injection. The radioautographs demonstrated the

same pattern in that there were discrete, intense deposits of $I^{131}$ in the media and adventitia of the blood vessels as well as in the periphery of the clot.
Results from control studies with ligated veins appear in table 3. Specific activities of the ligated blood vessels were comparable to activities found in those occluded by thrombi. The total amount of radioactivity is somewhat lower due to the absence of a thrombus. Radioautographs made from the samples showed distributions of radioactivity similar to those noted in the thrombosed vessels (fig. 5). Some areas of increased radioactivity correspond to zones of acutely inflamed tissue in the periphery of the blood vessel wall showing infiltration of polymorphonuclear cells and some hemorrhage. Normal blood vessels did not exhibit specific $^{131}$I accumulation in any areas.

**Discussion**

Detection of thrombi in blood vessels by a scintillation scanning system has been accomplished in dogs after intravenous injection of $^{131}$I-labeled anti-dog fibrinogen. This method deposits radioactivity in the thrombus and surrounding blood vessel. In all instances the $^{131}$I in the lesion was greater than that in a similar weight of circulating blood, indicating true concentration of the isotopically labeled protein. Radioautographs and stained sections indicate that the zones of highest radioactivity coincide with the areas in which active inflammation, hemorrhage, necrosis, and recanalization are occurring. These, in turn, all represent areas of deposition of fibrin. Similar results obtained in one- and three-day experiments demonstrate that localized concentration of anti-dog fibrinogen occurs during the period of active propagation and
also in the initial phases of organization. This has been verified in experiments involving simple ligation of the vein. It would seem that localized concentration of I\textsuperscript{125} anti-dog fibrinogen is a sequela of occlusion of the blood vessel and not only of clot propagation.

The use of a scanning technique to locate a thrombus in a limb shows a distinct advantage over a system that utilizes a stationary detector. In the latter case, one generally needs some prior knowledge of the clot site to expedite localization. The disadvantage of the moving detector is that the amount of time spent counting at a particular site is significantly less than that with a fixed probe. Therefore, one needs a ratio as large as possible between the concentration of isotope in the region of thrombus and that present in the circulating blood.

A rapid method of reducing the circulating (and not lesion-bound) I\textsuperscript{125} rabbit antibody to dog fibrinogen has been accomplished by the injection of goat antiserum to the rabbit gamma globulin. Circulating radioactivity was reduced within 30 minutes to between one-third and one-fourth of previous levels. In cases in which the thrombi are small, or when the quantity of concentrated I\textsuperscript{125} is low, it is probable that the immunologic removal of a major portion of the circulating radioactivity will result in successful detection of lesions. The isotopic demonstration of sites of thrombosis by means of I\textsuperscript{125} anti-fibrinogen, coupled with immunologic removal of the antibody from the circulating blood, will be applied on a clinical basis.

**Summary**

Dogs with thrombin-induced thrombi received I\textsuperscript{125} rabbit antibody to dog fibrinogen...
and a day later were given antiserum to the rabbit gamma globulin. Scintillation scanning techniques successfully detected the site of thrombosis. Excision of the thrombi and surrounding blood vessels demonstrated deposition of radioactivity in the lesion. The immunologic removal of the gamma globulin from the circulating blood increased the difference between the radioactivity deposited in the lesion and that present in the blood. This accentuates the lesion and offers potential diagnostic advantage.

References

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