Effect of Coronary Thrombus Age on Fibrinogen Uptake

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SUMMARY A study was carried out to define the time limits during which an experimental coronary thrombus remains capable of incorporating fibrinogen. 125I-fibrinogen was given to intact anesthetized dogs at different time intervals, up to 67 hours, following the formation of a coronary thrombus by catheter-electrode. Radioactivity of the recovered thrombi was analyzed. In some dogs, the ratio of radioactivity of the thrombus to the blood was determined following variable time intervals of exposure to circulating fibrinogen and was expressed as thrombus/blood ratio. The results indicate that coronary thrombi formed in a normal coronary vessel remain capable of incorporating fibrinogen for at least eighteen hours, with no significant differences in the segmental distribution of radioactivity. These findings do not support the view that the recovery of isotopic fibrinogen, which was given after the onset of coronary symptoms, in thrombi from patients with myocardial infarction establishes that the thrombus was initiated after the ischemic process.

RADIOIODINATED FIBRINOGEN remains at the present time the most commonly used agent for pathophysiological studies of the thrombotic process and for noninvasive diagnosis of a formed thrombus. Although data are available on the incorporation of fibrinogen in human and experimental venous thrombi, information regarding the length of time during which a thrombus formed in the coronary artery remains actively capable of incorporating fibrinogen is scanty. Regional determinants of the thrombotic process might be quite different. A systemic vein is a part of a low pressure system whereas a systemic artery is exposed to high pressures with pulsatile flow. In addition, the coronary arteries constitute the location with the highest incidence of atherosclerosis and thrombosis in the adult human subject. Therefore, extrapolating to coronary arteries data obtained from observations on systemic vessels regarding the initiation, duration and organization of the thrombotic process is not warranted.

The objective of the present study has been to expand our initial observations to define the time limits during which a coronary thrombus, once formed, remains capable of incorporating fibrinogen. Such information would be useful for studies concerned with the pathogenetic role of coronary artery thrombosis in myocardial infarction as well as the development of techniques for its detection.

Methods

Intact male mongrel dogs weighing 22–26 kg and fasting for 18 hours were anesthetized with morphine (3 mg/kg) and nembutal (20 mg/kg). Coronary thrombus was induced by a catheter-electrode as described previously. Hist-
logically, the structure of the induced thrombus is similar to that formed spontaneously in the human arteries.5,10 Formation of an occluding thrombus was signaled by the appearance of ST elevation in the continuously monitored electrocardiogram as well as by the occurrence of ventricular extrasystoles. Shortly after ST-segment elevation was noted, the wire-electrode was removed gently from the catheter and then the catheter itself was removed from the coronary artery into the arch of the aorta. Since the objective of the study was assessment of the length of time during which experimentally induced coronary thrombi were capable of incorporating fibrinogen, the animals were treated liberally with antiarrhythmic drugs to reduce the high mortality characterizing this model.11 Considering the appearance of ST elevation as the starting point (zero time) at which an occlusive thrombus was formed, the animals were randomly divided into groups on the basis of the age of thrombus, which was determined as the time elapsed between ST elevation and infusion of 131I-tagged fibrinogen into the animal. Forty-five animals out of a total of 70 survived the formation of an occlusive coronary thrombus. However, five animals had to be excluded from the study because of inadvertent omissions on the protocol. The remaining 40 animals were divided into six groups, each with a thrombus of different age varying from one hour to 67 hours after ST elevation (table 1). The animals in each group received radioactive fibrinogen within the same two hour period, except one animal of the last group (table 1) in which fibrinogen was given 67 hours following the formation of thrombus. Canine fibrinogen with a 94% clottability (ImCo Corporation, Stockholm, Sweden) was tagged commercially with 131I (Abbott Radiopharmaceuticals, Chicago) with a resulting thrombin clottability above 80%. The various batches of tagged fibrinogen had an approximate specific activity of 400 to 500 μCi/mg of fibrinogen. At the specified intervals following ST elevation discussed above, 75-100 μCi of labelled fibrinogen was given intravenously through a catheter previously placed in the right atrium. Following a waiting period ranging from one hour to 33 hours the animals were sacrificed by intravenous injection of KC1, the heart excised, the coronary artery was opened lengthwise and the thrombus removed. This interval or incubation time was used to determine the effects of the length of exposure to the radioactive marker on the incorporation of fibrinogen into thrombus. Just before sacrifice a blood sample was obtained from the animal and the radioactivity, as counts per minute (cpm) per ml of blood, was determined. Following careful removal of the thrombus from the dissected coronary artery, we were able to divide it in 29 animals into three easily discernible sections. The middle section was the most adherent part of the thrombus overlying the local brownin of the intima which had resulted from its exposure to the wire-electrode. The proximal and distal portions of the thrombus were then separated and all three parts weighed and counted for ten minutes in a well-type scintillation counter, expressing the radioactivity as cpm/gm of thrombus. The radioactivity of the remaining eleven thrombi was determined as a whole. In twelve animals the counting of thrombus was made before and after washing with saline in order to determine whether there was contamination from surrounding plasma or blood. In addition, samples were taken from the infarcted as well as noninfarcted myocardium and also from the adjacent coronary artery containing the thrombus as well as the contralateral normal coronary artery to determine isotopic fibrinogen. In each animal the following radioactivity ratios were determined: thrombus to blood, thrombus to normal and infarcted myocardium and thrombus to occluded and nonoccluded coronary artery. Tabulation of counts was made a) on the basis of incubation time, namely the time elapsed between administering fibrinogen and recovering the thrombus in order to determine optimal time for the highest thrombus to blood radioactivity ratios (fig. 1); and b) on the basis of segmental distribution of thrombus to blood radioactivity ratios namely at the proximal, middle and distal parts of the thrombus (fig. 2). Statistical evaluation of the results was made by using the Student's t-test for paired or nonpaired observations where applicable.

**Results**

The weight of thrombus varied widely from animal to animal and older thrombi tended to be smaller than those of shorter duration (table 1). Radioactivity of thrombi indicating intensity of 131I-fibrinogen incorporation, declined significantly with increasing age of thrombus (table 1). The thrombus to blood radioactivity ratios in all forty animals are shown in figure 1. The 20 dogs with thrombi up to four hours old had a mean ratio of 8.65 ± 1.56. Seventeen of the 20 animals had ratios greater than three. Increasing the

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**Table 1. Weight of Thrombi and Radioactivity in Six Groups of Animals**

<table>
<thead>
<tr>
<th>Number of Animals</th>
<th>Age of Thrombus (hr)</th>
<th>Weight (mean and ss) (mg)</th>
<th>Radioactivity (X 10^9) cpm/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>1-2</td>
<td>57.1 ± 15.5</td>
<td>519 ± 225</td>
</tr>
<tr>
<td>14</td>
<td>3-4</td>
<td>71.9 ± 13.5</td>
<td>582 ± 260</td>
</tr>
<tr>
<td>5</td>
<td>6-8</td>
<td>47.4 ± 10.9</td>
<td>461 ± 132</td>
</tr>
<tr>
<td>5</td>
<td>16-18</td>
<td>69.0 ± 10.8</td>
<td>169 ± 63</td>
</tr>
<tr>
<td>7</td>
<td>22-24</td>
<td>37.0 ± 9.8</td>
<td>83.5 ± 24</td>
</tr>
<tr>
<td>3</td>
<td>43-67</td>
<td>41.4 ± 20.0</td>
<td>46 ± 40</td>
</tr>
</tbody>
</table>

**Figure 1. Mean ratios for the group with thrombi up to four hours old and the group with thrombi 6–18 hours old were significantly higher than those for the group with older thrombi (P < 0.001 and < 0.05, respectively).**
\[^{131}\text{I}}-\text{fibrinogen incubation time resulted generally in increased radioactivity ratios. In the group with thrombi ranging from 6–18 hours old, seven out of ten had ratios greater than three including all those with longer exposure to \[^{131}\text{I}}-\text{fibrinogen. The mean ratio for this group was } 6.96 \pm 1.90. Finally, in the group with thrombi more than 22 hours old, the radioactivity ratios were less than three in nine out of ten animals, with no enhanced incorporation after comparable exposure to \[^{131}\text{I}}-\text{fibrinogen. The mean ratio in the last group was } 2.38 \pm 1.00, \text{ significantly lower than the other groups (fig. 1). Comparing radioactivities of 12 thrombi obtained before and after washing with saline we found a } 12.3 \pm 2.36\% \text{ decrease in counts in the washed thrombi.}

In the 29 animals in which proximal, middle and distal portions of the thrombus were sampled, the length of the thrombus varied from 2 to 5 cm. The middle and proximal segments were always occlusive, whereas the distal segment was more frequently only partially occlusive. Segmental radioactivity in 29 animals (fig. 2) was found to be somewhat differently distributed in the three sections of the thrombus with lower readings in the distal part, although the differences among the three segments were not statistically significant. The radioactivity ratios of thrombus to the occluded and nonoccluded coronary artery were 14.1 \pm 3.1 and 36.2 \pm 10.1, respectively, and thrombus to ischemic and nonischemic myocardial tissue, 38.9 \pm 11.3 and 55.2 \pm 14.9, respectively.

\section*{Discussion}

The results obtained with this animal model indicate that coronary thrombi formed in a normal coronary vessel remain capable of incorporating fibrinogen for at least 18 hours. This was quantitatively sufficient to give thrombus to blood ratios of more than three. This amount of radioactivity has been shown to suffice for demonstrating a thrombus scintigram \textit{in vivo} in the experimental animal.\textsuperscript{7}

While younger thrombi gave generally higher ratios even with shorter incubation time, older thrombi appeared to give satisfactory ratios when the time of exposure to \[^{131}\text{I}}-\text{fibrinogen was increased. Such increased ratios obtained with longer incubation times could also be, at least in part, a function of declining blood radioactivity levels. The low ratio observed in the group with thrombi of 22 to 67 hours was significantly less than short term thrombi presumably due to a substantial reduction in fibrinogen uptake with thrombus maturation (table 1).

The distribution of radioactivity was not significantly different among the three segments of the thrombus. The somewhat lower counts in the distal part of the thrombus may have been due to its shorter exposure to the circulating radioactive tracer since the distal portion was presumably the last formed. Nonetheless, retrograde flow into the anterior descending artery via collaterals resulted in relatively high radioactivity in the distal segment of thrombus. The varied weight of the thrombi appeared to depend, at least partly, on the apparent size of the occluded coronary artery although the possibility of partial detachment of thrombus at the time of the catheter removal cannot be excluded. Smaller thrombi with increased age of thrombus observed in this study are in agreement with findings of other studies.\textsuperscript{6} Isotopic fibrinogen was also found in samples of heart muscles but at considerably lower levels than in thrombus.

After washing thrombi with saline \textit{in vitro} there was an approximately 12\% reduction of labelled fibrinogen counts. This did not appear to differ in thrombi of different duration and may in part be due to trapped plasma. However, fibrinogen in the superficial layers of the thrombus may also be eluted by the washing procedure.

The temporal relationship between thrombus formation in the coronary artery and myocardial infarction has been a point of controversy in recent years.\textsuperscript{10} The studies of Erhardt et al.\textsuperscript{5} led to the impression that the incorporation of radioactively-tagged fibrinogen given to a coronary patient several hours after the onset of his symptoms was compatible with thrombus formation secondary to myocardial infarction. The data obtained in this study disagree with this conclusion. It appears that a thrombus formed in the coronary artery can incorporate fibrinogen for many hours after it has been formed, which is in agreement with previous observations on the pathophysiology of thrombus formation obtained in other models of study.\textsuperscript{13,14} Therefore, the argument that radioactivity found in thrombi recovered from patients who were given radioactively-tagged fibrinogen several hours after the onset of coronary symptoms, was indicative of formation of thrombus secondary to myocardial infarction does not appear valid.

Finally, the formed thrombi in the coronary artery incorporated less radioactive fibrinogen compared to venous thrombi. The latter had high incorporation rates for longer periods than for coronary thrombi.\textsuperscript{5,6} It is possible that the longer time of exposure of venous thrombi to radioiodinated fibrinogen in those studies might account, at least in part, for this difference.

\section*{Acknowledgment}

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\section*{References}

Noninvasive Assessment of Mitral Insufficiency by Transcutaneous Doppler Ultrasound

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SUMMARY Instantaneous aortic arch blood velocity was recorded transcutaneously from the suprasternal notch, using a 2.2 MHz Doppler ultrasound unit, in 18 normals and 16 patients undergoing cardiac catheterization who had murmurs of mitral regurgitation. In normals aortic blood velocity rose rapidly in early systole to a midystolic peak then fell to zero velocity. These roughly parabolic patterns had area ratios beneath the first and second halves of the curves measuring 52.48 ± 3 (SD). With increasingly severe mitral regurgitation the pattern became skewed leftward such that the percent in the first half of systole ranged from 53–79%. From the angiograms of our sixteen patients an estimate of true percent regurgitation was made using the Fick cardiac output and ventricular volume measurements. When compared with the area under the first half of the velocity curve a strong correlation was found (r = 0.84) indicating that this Doppler technique can be used to evaluate mitral insufficiency.

ABNORMAL AORTIC FLOW PATTERNS in mitral insufficiency have been demonstrated by several authors. The usefulness of these flow changes in assessing mitral regurgitation has not been fully evaluated since those flow studies were limited to small numbers of patients and selected situations, due to the complexity of the techniques used.

In their study, Elkins et al. used an electromagnetic cuff type flowmeter to measure aortic blood flow in two groups with mitral regurgitation: nine dogs with surgically induced regurgitation, and five patients with severe mitral insufficiency, at the time of mitral valve replacement. In both situations the aortic flow pattern was altered, showing a proportionately greater flow in the first half of systole than normal. Kendall et al. obtained similar results in five patients with mitral insufficiency using the pressure gradient technique.

A simple noninvasive technique, using transcutaneous Doppler ultrasound, has been described by Light and Joyner et al. for recording instantaneous aortic blood velocity. This report illustrates the changes that can be recorded using that method in patients with mitral regurgitation and outlines the potential value of the technique in assessing the severity of the mitral valve lesion.

Method

Aortic blood velocity patterns were obtained using a battery operated 2.2 MHz directional Doppler ultrasound unit. The ultrasound probe was a 13 mm diameter lead zirconate-titanate piezoelectric disc cut in two and mounted in the “double D” configuration. The two elements (transmitter-receiver) were set to focus at a depth of 10 cm, at which point the beam width was approximately 13 mm high by 7 mm wide (manufacturer’s specifications). The acoustic power output was 10 mW/cm². The system was a zero crossing unit with an adjustable Schmidt trigger level to a minimum of 10 mV and had a noise figure of approximately 6 decibels. To eliminate the low frequency components of the returning signal (i.e., the heart sounds) a filter was installed which produced a band width of 300 Hz to 3.0 kHz at –3 decibels. This meant that the unit was sensitive to velocities from about 5 cm/sec to 100 cm/sec. Each study was done with the patient supine and the transducer hand held in the suprasternal notch. When aimed downward and posteriorly toward the tip of the left scapula the beam crossed the descending aortic arch at an angle approximately parallel to blood flow (fig. 1). In this area of the arch the transluminal velocity profile was relatively flat, i.e.,...
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