serious impairment or breakdown of the circulatory system (fig. 1). None of the patients studied revealed any deleterious circulatory responses.

It was therefore concluded that given the proper clinical circumstances in which the potent vasodilator diazoxide might be administered, previous propranolol administration would not be a contraindication.

References

Noninvasive Detection of Intracardiac Thrombosis
131-I Fibrinogen Cardiac Survey

JAMES H. FRISBIE, M.D., DONALD E. TOW, M.D., ARTHUR A. SASAHARA, M.D., ERNEST M. BARSAMIAN, M.D., and ALFRED F. PARISI, M.D.

SUMMARY Cardiac survey following administration of 131-I autologous fibrinogen is a noninvasive technique for the detection of intracardiac thrombosis. Fibrinogen is isolated from plasma by a rapid salting-out method with ammonium sulfate and is iodinated with chloramine T. The purity of 131-I fibrinogen, expressed as clottable radioactivity, is greater than 90%. Cardiac survey consisting of serial gamma camera imaging or rectilinear scanning after intravenous administration of 131-I fibrinogen was conducted in dogs with freshly induced thrombosis of the left atrial appendage. An accumulation of radioactivity was detectable in the area of the left atrium and confirmed in each of nine dogs sacrificed. Similarly, 20 patients with heart disease predisposing to intracardiac thrombosis were surveyed. Eight of nine patients with positive studies and 11 of 11 with negative studies were confirmed subsequently at surgery or autopsy. Cardiac survey with 131-I fibrinogen is a simple and noninvasive method of detecting intracardiac thrombosis.

IN THE COURSE OF CLINICAL PRACTICE intracardiac thrombosis (ICT) is usually recognized subsequent to a peripheral embolic event. Such retrospective diagnosis is not necessarily helpful to a patient, particularly if he has sustained a major stroke or had to undergo emergency surgery for an ischemic limb. Empirically, anticoagulation can be employed to prevent ICT and embolic events in high risk groups such as patients with mitral stenosis, myocardial infarction, and ventricular aneurysm. However, considerable uncertainty exists in the management of individual patients, many of whom may be unnecessarily exposed to the risk of bleeding with anticoagulation. In this light a rapid noninvasive method of documenting ICT would provide an objective basis for instituting prophylactic anticoagulation.

Contrast angiography is the current method for documenting an intracardiac thrombus. This method requires cardiac catheterization, carries some risk, is not easily repeated, and may not be entirely reliable. While radioisotopic cardiac angiography is a more convenient procedure and has been reported for the detection of intracardiac myxoma, it has not been used for the detection of intracardiac thrombosis, presumably due to its lack of necessary resolution.

This report outlines the use of tracer 131-I fibrinogen for the detection of intracardiac thrombosis by labeling the thrombotic process. This radiopharmaceutical, used as an autologous preparation, is produced with reasonable simplicity in one hour, has a minimum purity of 90% clottable radioactivity and a normal in vivo survival time in normal subjects.

Methods

Preparation of Radioactive Autologous Fibrinogen

This technique, recently described in detail, is summarized below. The entire procedure is conducted at room temperature. Twenty ml of blood are collected in Vacutainers (Becton, Dickinson Corp., Lincoln, Neb.) containing ethylenediaminetetraacetic acid. Plasma is separated
by centrifugation at 10,000 × G for 5 min. Fibrinogen is isolated from 6 ml plasma by the addition of 18 ml of 30% saturated ammonium sulfate (at room temperature) buffered with sodium phosphate to pH 7.0 so that the final ammonium sulfate concentration is 22.5% saturated. The precipitate is collected by centrifugation at 300 × G for 3 min, dissolved in 0.1 M sodium phosphate pH 7.4, and fibrinogen salted out again in the same manner. Fibrinogen is iodinated by a modification of the Greenwood, Hunter, Glover method11 so that the chloramine T: fibrinogen molar ratio is 12:1. The iodinated fibrinogen is separated from unbound iodide ion by a repeated salting-out step. This final precipitate is dissolved in 3 ml 0.1 M sodium phosphate pH 7.4 and sterilized by filtration. The radioactivity of the final solution is measured by an isotope dose calibrator and the appropriate volume of solution, 1–3 ml, are administered to the study subject.

Detection of Intracardiac Thrombosis in Dogs

Mongrel dogs, weighing 15–30 kg, were used as experimental models for the detection of intracardiac thrombosis. The dogs were anesthetized with pentobarbital, the heart was exposed by mid-sternal thoracotomy under sterile conditions, and a thrombus was induced by injection of sodium mormhuate into an isolated left atrial appendage as described by Kramer et al.12 131-I human fibrinogen in a dose of 2–20 μCi per kg body weight and a volume of 1–3 ml was injected intravenously before or within three hours of the sodium mormhuate injection of the left atrial appendage. Gamma camera images were obtained immediately and at varying intervals after injection of 131-I fibrinogen. A gamma camera with a half inch thick iodide crystal and a high energy, parallel hole collimator was used to accumulate 100,000 counts. Alternately a dual-probe rectilinear scanner with five-inch iodide crystals and high energy collimators was used to record 300 counts per linear inch. After follow-up cardiac survey, the animals were sacrificed with intravenous pentobarbital, the left atrial appendage removed and dissected, and the radioactivity of the atrial thrombus measured in a deep well scintillation counter for comparison with that of whole blood.

Detection of Intracardiac Thrombosis in Patients

Patients who had heart disease with a high probability for intracardiac thrombosis (mitral stenosis, ventricular aneurysm, prosthetic cardiac valve) and who were scheduled for cardiac surgery were selected for cardiac survey. Thus it was possible to compare the detection of intracardiac thrombosis by cardiac survey with findings at cardiac surgery. Patients were studied at random, without regard for a history of thromboembolic events. Informed consent was obtained, 250 mg potassium iodide were administered orally daily for 1 week from the start of each study, and 5–10 μCi 131-I autologous fibrinogen per kg body weight in a volume of 1–3 ml were administered intravenously. Survey for intracardiac thrombosis was conducted by imaging or rectilinear scanning at intervals of 5 minutes, 3 hours, 24 hours and 2–6 days after radioiodinated fibrinogen administration. Imaging with a gamma camera and accumulation of 300,000 counts was the technique used most often. Subsequent cardiac surgery was performed on each patient studied except for one subject who died and was studied at autopsy.

Results

Detection of Intracardiac Thrombosis in Dogs

A thrombus was induced in the left atrial appendage and externally detected by photoscanning in ten of the ten dogs studied. A thrombus was found in the left atrial appendage in each of nine dogs sacrificed. An example of intracardiac thrombus in the experimental animal is illustrated in figure 1.

The atrial thrombi weighed 1.2 ± 0.8 g (mean ± 1 std; N = 9) and the ratio of thrombus radioactivity to that of blood was 9.8 ± 6.7 (N = 8). Table 1 shows the thrombus: blood ratio generated in each experiment according to the duration of labeling. It can be appreciated that the thrombus to blood ratio increased with time and that the greatest increment in this ratio occurred during the first 24 hours of 131-fibrinogen labeling.

<table>
<thead>
<tr>
<th>No. of dogs</th>
<th>Thrombus labeling time*</th>
<th>Thrombus : Blood†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>24</td>
<td>9 (5, 12)</td>
</tr>
<tr>
<td>1</td>
<td>36</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td>48</td>
<td>10 (5, 14)</td>
</tr>
<tr>
<td>1</td>
<td>96</td>
<td>22</td>
</tr>
</tbody>
</table>

*Expressed in hours exposure to 131-I fibrinogen.
†Radioactivity ratio.

Figure 1. A rectilinear scan, lateral view, of the experimental dog 36 hours after thrombus induction in the left atrial appendage. An autoradiograph of the 0.6 g thrombus removed the same day from the left atrial appendage is at the bottom. This specimen has been flattened for autoradiography and the photograph is 1.5 × actual thrombus size.
Detection of Intracardiac Thrombosis in Patients

Twenty patients were studied. Mitral stenosis was found in 13; ventricular aneurysm in five; and prosthetic aortic valve in two. All patients were male; the mean age was 52.0 ± 4.3 years. One of the patients with ventricular aneurysm had had a stroke two months prior to cardiac survey and two patients with mitral stenosis had probable embolic events two to four years prior to cardiac survey. The presence of fibrin deposit by cardiac survey was compared with the presence of thrombosis at cardiac surgery in 19 patients and at autopsy in one patient.

The results of cardiac survey and the subsequent findings are summarized in table 2. Nineteen of the 20 surveys correctly predicted the presence or absence of thrombi confirmed at surgery or autopsy, yielding an overall diagnostic accuracy of 95%. The smallest thrombus detected weighed 1.1 grams.

The interval between fibrinogen administration and cardiac survey varied from three hours to five days. Of the eight confirmed positive studies, six had a cardiac survey within 24 hours of fibrinogen administration and detectable fibrin deposit was observed at that time. The mean interval between cardiac survey and confirmation was four days (range 1–21 days).

Detection of intracardiac thrombosis with 131-I fibrinogen is shown in figure 2. An autoradiograph of a section of thrombus removed at surgery is shown in figure 3. Radioactivity is present, in varying concentrations, throughout the section of the thrombus illustrated.

The appearance of a positive cardiac survey varied from a thin line of radioactivity concentrated along a border of the cardiac silhouette to a thick band or massive intracardiac accumulation of radioactivity. The overall size of a thrombus was not precisely predictable from its scintiphoto-

table 2. Detection of Intracardiac Thrombosis in Human Studies

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No.</th>
<th>Positive survey—confirmed</th>
<th>Negative survey—confirmed</th>
<th>False positive</th>
<th>False negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitral stenosis</td>
<td>13</td>
<td>2</td>
<td>10</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Ventricular aneurysm</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Prosthetic aortic valve</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Totals</td>
<td>20</td>
<td>8</td>
<td>11</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Fibrinogen, as a plasma protein which is incorporated (as fibrin) into any thrombus, can be labeled and utilized as a blood-borne tracer to detect thrombotic processes. Fibrinogen isolated by ammonium sulfate precipitation and radioiodinated by chloramine T appears to function normally. Denaturation of fibrinogen, iodinated by a chloramine T method when µg amounts of fibrinogen are used, has been reported. In our procedure mg amounts of fibrinogen are used for labeling with radioactive iodine at a low chloramine T-to-fibrinogen molar ratio (12:1). This tracer compares favorably with fibrinogen iodinated with iodine monochloride, a labeling agent used extensively in fibrinogen metabolic studies.

Kramer et al. and Spar et al. have successfully used 131-I antifibrinogen antibody for detection of intracardiac thrombosis. Two of four intracardiac thrombi were detectable in the first study, three of four in the second, and eight of eight in our present study. One false positive survey occurred in this study and the study by Spar et al. The antifibrinogen

FIGURE 2. Patient 4. Ventricular aneurysm. The control scintiphotograph (left) shows the blood pool immediately after administration of 131-I fibrinogen. The 96 hour scintiphotograph (right) shows accumulation of radioactivity at the cardiac apex. At surgery 24 hours after this scintiphotograph a left ventricular aneurysm containing a 12.5 g thrombus was found and removed.

FIGURE 3. A section of the thrombus (top) found in the ventricular aneurysm of patient 4, removed the day after the final scintiphotograph was obtained, and the autoradiograph of that section (bottom). Histologically, the thrombus was unorganized. The thrombus to blood ratio was 22 ± 8 for five sections of thrombus.
antibody technique has not been adopted for general clinical application, possibly because of the use of a foreign protein (rabbit antibody), the skill required to produce the antibody, and the impurity of the antibody preparation.\textsuperscript{14}

The technique used in this study for detection of ICT appears to be quite sensitive. The smallest active thrombus detected was 1.1 g (attached to a larger, organized thrombus, which did not accumulate radioactivity). While a large thrombus might be detected as a filling defect by radioisotopic cardiac angiography, it is unlikely that small thrombi would be discovered by this means.

The optimal timing of cardiac views for visualization of a thrombus is probably dependent on the growth characteristics of the thrombus. A rapidly growing thrombus quickly accumulates fibrin and may be visualized early in the cardiac survey while a slowly growing thrombus may be visualized more clearly after several days of thrombus labeling. In practice cardiac survey at 3, 24, and 72 hours appears workable.

The resolution obtained by imaging with an accumulation of only 300,000 counts was a compromise against the imaging time needed. Potential for improvement of resolution exists in the use of a shorter lived and lower energy radioisotope to permit finer collimation and higher counts per camera time. Another possibility lies in the use of radioiodinated fibrin which has a short in vivo survival time, and thus might enhance thrombus to blood ratios by the early clearance of background radioactivity.\textsuperscript{17}

The 131-I autologous fibrinogen cardiac survey for the detection of ICT has proven to be practical. No untoward reactions have been noted. This method has various potential clinical applications. These include 1) a screening procedure in patients at high risk for ICT, thus providing an objective basis for prophylactic anticoagulation; 2) documentation of a cardiac source in instances of systemic embolism; 3) a screening procedure prior to cardiac surgery or electrical conversion of an arrhythmia.\textsuperscript{18}

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