Kinetics and Imaging of Indium-111-labeled Autologous Platelets in Experimental Myocardial Infarction

KENNETH H. LAWS, M.D., JEFFREY A. CLANTON, R.P.H., VAUGHN A. STARNES, M.D., FLAVIAN M. LUPINETTI, M.D., JERRY C. COLLINS, PH.D., JOHN A. OATES, M.D., AND JOHN W. HAMMON, JR., M.D.

SUMMARY The kinetics of accumulation and the external imaging patterns of indium-111-labeled platelets infused in a dog model of left anterior descending coronary artery occlusion with reperfusion were studied. The effects of infarct age and regional residual myocardial blood flow upon platelet accumulation were quantitated, and the capacity of indium-111 platelets to image the experimental infarct was evaluated qualitatively. The endocardial accumulation of indium-111 platelets occurred primarily in infarct zones with residual blood flow < 0.6 times normal and was maximal (24.98 ± 2.76 times normal) in the lowest blood flow zone (< 0.1 times normal). Indium-111 platelet accumulation in the epicardium occurred in the regions with blood flow < 0.6 times normal and was maximal (17.83 ± 1.20 times normal) in the lowest blood flow zone (< 0.1 times normal). The maximal endocardial and epicardial platelet accumulation occurred 24 hours after reperfusion and was significantly decreased at 48 hours. In vivo cardiac images revealed discrete areas of increased myocardial radioactivity uptake in the anterior wall of dogs 24 hours after reperfusion. All images 48 hours after reperfusion were negative. Thus, in the experimental setting, indium-111 platelets allow quantification of platelet accumulation after myocardial infarction at a tissue level and provide a noninvasive means of in vivo imaging of reperfused infarcted myocardium. PLATELET accumulation in areas of myocardial infarction has been demonstrated histologically and with chromium-51 (51Cr)-labeled platelets.1,2 Indium-111 (111In)-labeled platelets have been used to identify left ventricular thrombi,3 coronary bypass graft thrombi4 and acute coronary artery thrombi.5 The ability to image the inflammatory response to acute myocardial infarction in dogs and man using 111In-labeled leukocytes has been demonstrated.6,7 Recent studies also suggest the crucial role that platelet aggregate microemboli may play in infarct extension2,8 and fatal ventricular arrhythmias.9 Definition of the kinetics of platelet accumulation into acute infarct zones is important because platelet inhibitors may limit the degree of ischemic necrosis10 and may prevent fatal ventricular arrhythmias resulting from acute myocardial infarction.11 The development of the technique for 111In-labeling of platelets provides a direct means for assess-

From the Departments of Cardiac and Thoracic Surgery, Biomedical Engineering, Nuclear Medicine, and Pharmacology, Vanderbilt University, Nashville, Tennessee. Supported by American Heart Association grant 79885.

Address for correspondence: John W. Hammon, Jr., M.D., Department of Cardiac and Thoracic Surgery, 1211 21st Avenue South, #338, Nashville, Tennessee 37212.

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ing platelet kinetics. Indium-111-labeled platelets retain the label intracellularly,12 maintain aggregatability13, 14 and have already been used in the study of intravascular thrombosis. The half-life of circulating labeled platelets is approximately 45 hours;15 blood pool activity clears by 20 hours, allowing cardiac imaging at that time. This study was undertaken to evaluate the potential of 111In platelets as a means of in vivo imaging of reperfusion myocardial infarctions, and to determine the effect of infarct age and residual regional myocardial blood flow upon the kinetics of platelet accumulation.

Methods

In vivo and ex vivo imaging and postmortem tissue distribution studies involving autologous 111In-labeled platelets were performed in dogs subjected to acute anterior wall myocardial infarction.

Preparation of 111In Platelets

Autologous platelets in each dog were isolated and labeled with 111In according to a modification of the method of Thakur et al.12 One hundred milliliters of venous blood were withdrawn in two disposable 60-ml plastic syringes that each contained 5.0 ml of Ware citrate buffer pH 5.0 (3.24 g Na citrate H2O and 1.05 g citric acid in 150 ml H2O). The blood was transferred into two 50-ml conical plastic test tubes and centrifuged at 180 g at room temperature for 25 minutes. The platelet-rich plasma (PRP) was removed from the packed cells and combined in a single 50-ml conical plastic tube. The PRP was centrifuged at 800 g for 6 minutes and the platelet-poor plasma (PPP) was removed, leaving 5 ml of the PPP above the platelet button. The platelets were then gently resuspended to begin labeling. The red and white cell contamination in these preparations was 0.1%. Indium-111-oxine was prepared using a method modified from Thakur et al.12 Indium-111 CI, was added to a glass centrifuge tube that contained 50 mg of 8-hydroxyquinoline (oxine) dissolved in 50 ml of 95% ethanol. This mixture was allowed to incubate at room temperature for at least 5 minutes. After incubation, acetate buffer (pH 5.5) was added volume for volume immediately before use. After addition of acetate buffer, the desired quantity of 111In-oxine was added to the platelets suspended in 5 ml of plasma and allowed to incubate at room temperature for 30 minutes. After incubation, 15 ml of PPP was added to the platelet-oxine mixture, gently agitated and centrifuged for 6 minutes. The PPP was then pulled off to the top of the platelet button and the platelets were gently resuspended in 10 ml of autologous PPP. Platelets were confirmed to be viable after labeling by testing for aggregability by the method of Born using 10 μM of ADP as a stimulus.16 Only platelets that aggregated normally after labeling, thus retaining biologic function, were infused.

Instrumentation

Healthy mongrel dogs of either sex that weighed 15–25 kg underwent a left thoracotomy under general anesthesia (sodium pentobarbital 25–35 mg/kg i.v.) and two balloon occluders were placed around the left anterior descending coronary artery (LAD) just proximal to the first diagonal (1.5–2.5 cm from the aorta). Heparin saline fluid–filled plastic catheters were placed in the left atrium and common carotid artery. The distal ends of the catheters and balloon occluder were placed in subcutaneous pockets in the chest and neck.

Experiments

Three to 5 days later, experiments were performed on dogs lightly sedated with i.v. sodium pentobarbital (15 mg/kg). A Swan-Ganz thermodilution catheter was inserted percutaneously through the external jugular vein into the pulmonary artery. Lead II of the ECG, pulmonary arterial pressure, mean left atrial pressure and aortic pressures (using Statham P23b transducers) were recorded continuously on a multichannel recorder. The dogs were premedicated with i.v. lidocaine (1.5 mg/kg). Five minutes later, the balloon occluder was inflated to occlude totally the LAD (table 1). At 1 hour after occlusion, myocardial blood flow was measured by standard techniques using strontium-85 (85Sr)-labeled microspheres 12–15 μm in diameter (3M Company) with polysorbate-80 added as a surfactant. After mechanical agitation, approximately 2 million microspheres were injected into the left atrium and flushed with 5 ml of saline solution. Reference arterial blood samples were withdrawn at a constant rate of 7.64 ml/min on a calibrated Harvard pump, starting 30 seconds before injection of microspheres and continuing for 2 minutes thereafter. At 1 hour after occlusion, the 111In platelet preparation was infused intravenously (200 ± 40 μCi). Further lidocaine boluses of 10–30 mg were given to suppress postocclusion arrhythmias. Four dogs died of ventricular fibrillation within 20 minutes after occlusion and two died of ventricular fibrillation after reperfusion. After 2 hours of occlusion, the surviving dogs were randomly allocated into four time-interval groups; either no reperfusion with sacrifice before release of the balloon occluder or sacrifice after 3, 24 or 48 hours of reperfusion. Dogs that were randomized to the 24-hour group underwent in vivo cardiac imaging 24 hours after 111In platelet administration and all dogs randomized to the 48-hour group underwent in vivo cardiac imaging at 24 and 48 hours. The delay from the time of injection to imaging was necessary to allow clearance of 111In platelets from the body.

TABLE 1. Protocol

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Left anterior descending coronary artery occlusion</td>
</tr>
<tr>
<td>1</td>
<td>Infusion of 111In-labeled platelets; myocardial blood flow determination with 85Sr</td>
</tr>
<tr>
<td>2</td>
<td>No-reperfusion group killed; left anterior descending coronary artery reperfusion</td>
</tr>
<tr>
<td>3</td>
<td>3-hour reperfusion group killed</td>
</tr>
<tr>
<td>26</td>
<td>24-hour reperfusion group killed</td>
</tr>
<tr>
<td>50</td>
<td>48-hour reperfusion group killed</td>
</tr>
</tbody>
</table>
the blood pool. Images obtained earlier primarily reflect blood pool rather than myocardial activity. For imaging, the dogs were lightly sedated with sodium pentobarbital and positioned beneath a scintillation camera (Searle Scintiview). To increase the effectiveness of the \(^{111}\)In-platelet infarct scan, the liver and spleen were shielded using a lead apron. Left anterior oblique, right anterior oblique and anterior-posterior images were obtained in each dog. One-hundred-thousand-count images were obtained using a 20% window about the 173 keV and 247 keV photo peaks of \(^{111}\)In.

At the appropriate interval, dogs were killed with an overdose of sodium pentobarbital. The hearts were quickly removed and washed free of blood. The left ventricle was dissected free from the right ventricle, atria and connective and fatty tissue, and weighed. The entire left ventricle was cut into transverse sections (1–1.5 cm) from the base to the apex. The layers were placed in a nitroblue tetrazolium (NBT) solution (720 ml of distilled H\(_2\)O, 80 ml of 0.1% potassium phosphate buffer, 250 mg of NBT (Sigma), which stains intracellular lactate dehydrogenase. After NBT staining, the transverse sections were imaged by a scintillation camera (Searle LFOV). The entire left ventricle was sectioned into 1–2-g endocardial and epicardial sections. Sections were identified as ischemic zone, border zone or normal myocardium by the lack of NBT staining of ischemic tissue. All samples were placed in preweighed glass vials and counted for radioactivity together with the reference blood sample in a well-type gamma scintillation counter (Packard Instruments) with three energy windows adjusted to the peak emission \(^{85}\)Sr (514 keV) and the two peaks for \(^{111}\)In (173 and 247 keV). After subtraction of background counts, the activity for each radionuclide was calculated as counts per minute per gram of tissue. Tissue accumulation of \(^{111}\)In (reflecting platelet accumulation) were then expressed as the ratio of counts in the infarct sample divided by the mean activity from the normal myocardium samples. Tissue accumulations of \(^{85}\)Sr were used to determine regional myocardial blood flows using a modification of the method described by Domenech et al. Relative regional myocardial blood flows were obtained by comparing flow in the infarct sample to the mean flow in the normal myocardium samples. These ratios were established for endocardial and epicardial samples in each dog. In this way, relationships could be established between platelet accumulation and relative regional myocardial blood flow with infarcts of various ages and sizes.

Statistical Methods

Data were expressed for each group as mean ± sd. Comparison of the differences of \(^{111}\)In uptake ratios in myocardial samples of different blood flow at different times after infarction was made by analysis of covariance and group t tests.

For control purposes, in two dogs, a sham infarct procedure was performed with prior thoracotomy and catheter and balloon placement, but without infarct production. The dogs received \(^{111}\)In-labeled platelets and were killed 24 hours later, and imaging and tissue distribution studies were performed.

Results

Cardiac Imaging

In vivo cardiac imaging at 24 hours after infarction revealed regions of increased anterior wall myocardial

![Figure 1](http://circ.ahajournals.org/) Cardiac scintillation camera images from one dog taken in the anterior-posterior position. The anatomic orientation is such that the upper portion of each image is cephalad and the lower portion caudad. The first image demonstrates the technetium blood pool for orientation and the second image myocardial activity at 24 hours after infarction seen above the activity in the liver. The third image demonstrates the decreased myocardial indium-111 (\(^{111}\)In) activity at 48 hours after infarction, with only hepatic uptake remaining.
\[ \textit{In vivo} \] accumulation in four of five dogs in the 24-hour reperfusion group and three of five dogs in the 48-hour reperfusion group (figs. 1 and 2). All five of the dogs imaged at 48 hours after infarction, however, had negative images (fig. 1). Images before 20 hours were not useful because of an interfering blood pool of \textit{In} platelets. The abnormal myocardial uptake was identi-

\[ \textit{In vivo} \] imaging correlated with the ventricle and the hepatic activity. The \textit{in vivo} cardiac scintillation image of a dog was taken in the right anterior oblique position. The anatomic orientation is such that the upper portion of the image is cephalad, the lower portion caudal, the right portion anterior and the left portion posterior. The \textit{in vivo} image demonstrates the myocardial activity present above the hepatic activity. The \textit{in vitro} study was performed with the left ventricle opened flat on the scanner. The area of \textit{in vitro} uptake correlates with the area of infarct obtained with nitroblue tetrazolium staining.

\[ \textit{In vitro} \] images

**FIGURE 2.** \textit{In vivo} (top) and \textit{in vitro} (bottom) indium-111 imaging studies from a dog 24 hours after infarction. The \textit{in vivo} cardiac scintillation image of a dog was taken in the right anterior oblique position. The anatomic orientation is such that the upper portion of the image is cephalad, the lower portion caudal, the right portion anterior and the left portion posterior. The \textit{in vivo} image demonstrates the myocardial activity present above the hepatic activity. The \textit{in vitro} study was performed with the left ventricle opened flat on the scanner. The area of \textit{in vitro} uptake correlates with the area of infarct obtained with nitroblue tetrazolium staining.

Tissue Distribution Studies

Comparison of tissue uptake of \textit{In} and \textit{Sr} microspheres in multiple myocardial samples allowed determination of the effects of regional myocardial blood flow and infarct age upon regional platelet deposition (table 2, figs 4–6). At 24 hours, the endocardial accumulation of \textit{In} platelets was significantly different from control (\( p < 0.05 \)) in infarct zones with residual blood flow < 0.6 times normal (fig. 4). These same trends were present at 5 and 48 hours after infarction. Endocardial accumulation was maximal (24.98 ± 2.76 times normal) in the lowest flow zones (< 0.1 times normal) at 24 hours after infarction. Endocardial uptake in this lowest-flow zone was significantly different (\( p < 0.05 \)) from that at earlier and later periods in regions with the same flow decrement.

Likewise, significant epicardial accumulation of platelets (\( p < 0.05 \)) compared with control occurred predominantly in regions with residual myocardial blood flow < 0.6 times normal (fig. 5). Maximal epicardial deposition (17.83 ± 1.20 times normal) occurred in the lowest flow regions (< 0.1 times nor-

**FIGURE 3.** Relationship between scintillation images of transverse slices of the left ventricle and pathologic zones of infarction in a dog 24 hours after infarction. The zones of infarction as determined by nitroblue tetrazolium staining are represented by the darkened areas. The zones of abnormal indium-111 platelet accumulation are evident in the images of the tissue slices. Each tissue slice is approximately 1 cm thick.
TABLE 2. Infarct/Normal Myocardium Indium-111 Radioactivity Ratios As a Function of Relative Blood Flow

<table>
<thead>
<tr>
<th>Hours after infarction‡</th>
<th>No. of dogs</th>
<th>0.0–0.1§</th>
<th>0.1–0.2</th>
<th>0.2–0.4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Endo</td>
<td>Epi</td>
<td>Endo</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>0.76 ± 0.12</td>
<td>1.01 ± 0.09</td>
<td>0.87 ± 0.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(7)</td>
<td>(3)</td>
<td>(3)</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>4.42 ± 0.43*</td>
<td>4.75 ± 0.78*</td>
<td>3.70 ± 0.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(15)</td>
<td>(2)</td>
<td>(12)</td>
</tr>
<tr>
<td>24</td>
<td>5</td>
<td>24.98 ± 2.76*</td>
<td>17.83 ± 1.20*</td>
<td>14.59 ± 2.02*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(13)</td>
<td>(7)</td>
<td>(8)</td>
</tr>
<tr>
<td>48</td>
<td>5</td>
<td>4.38 ± 0.26*</td>
<td>4.89 ± 0.30*</td>
<td>3.53 ± 0.72</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(4)</td>
<td>(4)</td>
<td>(3)</td>
</tr>
<tr>
<td>Control/sham infarct</td>
<td>2</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Values are mean ± SD.
*p < 0.05 compared with mean of control samples (either endocardial or epicardial) obtained during the same time period.
†p < 0.05 compared between time intervals.
§Refers to time of sacrifice.
¶Relative regional myocardial blood flow as assessed by 85Sr microspheres with 1.0 = 100% control activity.
Abbreviations: Endo = endocardium; Epi = epicardium.

Discussion

This study demonstrates in a reperfusion model of myocardial ischemia the kinetics of platelet uptake and the ability of 111In-labeled platelets to image the area of infarction both in vivo and ex vivo. The ex vivo images confirmed that the infarction area, as determined by NBT staining, was the major site of 111In-labeled platelet uptake. Since it has been shown by others that unbound 111In-oxyne is not concentrated to any significant extent in the thrombotic process, the marked 111In activity in the infarct area represents intense platelet deposition. Images before 20 hours represent 111In platelet blood pool and are not useful in infarct visualization. At 24 hours, however, infarct visualization was possible. The positive infarct images at 24 hours were uniformly negative at 48 hours.

The kinetic data from this study show that platelet accumulation in the ischemic regions is inversely proportional to blood flow and maximal in the lowest-flow regions. The platelet uptake is negligible before reperfusion, increases at 3 hours after reperfusion, maximizes at 24 hours after reperfusion and then signifi-
stantly falls at 48 hours after reperfusion. Injury to the intima of a blood vessel results in endothelial disruption and exposure of collagen. At the site of injury, platelet aggregates form with the release of the vasoconstrictor and platelet aggregator thromboxane, which causes increased platelet aggregation. The platelet accumulation becomes surrounded by fibrin, and the platelet mass degenerates as more fibrin is laid down. By 48 hours, intact platelets are no longer recognizable, as the thrombus consists primarily of fibrin. This sequence of events, based on pathologic studies, is compatible with our imaging results and kinetic data. These data do not rule out the possibility that the platelet accumulation is entirely secondary to a hemorrhagic process upon reperfusion, but in that case, the highest \(^{111}\text{In} \) intake should occur in the 3-hour reperfusion group. This experimental protocol studied the reperfusion type of myocardial infarction because of recent evidence that total coronary occlusion spontaneously decreases in frequency during the initial 24 hours of a transmural infarction, and because of recent interest in early surgical revascularization and streptokinase coronary thrombolysis all resulting in infarction reperfusion. Reperfusion does not increase the quantity of ischemic tissue that becomes necrotic, and the resulting hemorrhagic reaction has been shown to be confined to the most ischemic areas. This study verifies that the greatest platelet uptake upon reperfusion occurs in the low-blood-flow zones. This study does not indicate that external imaging is possible in a no-reperfusion myocardial infarction model, for the platelet uptake ratios in the no reperfusion group are low. Thakur et al. suggested that the \(^{111}\text{In} \) label remains incorporated in platelets at the time of deposition. However, the possibility of transfer of the radioactive label to other cells or tissue within the infarct zone cannot be excluded.

The study of platelet kinetics during myocardial infarction has been stimulated by recent reports that demonstrate the effect that platelet microaggregate emboli may play in infarct extension and fatal arrhythmias. Platelet accumulation in myocardial infarctions in baboons was demonstrated using \(^{51}\text{Cr} \)-labeled platelets by McNamara and colleagues. Indium-111-labeled platelets have also been used to image left ventricular thrombi, occlusion of coronary bypass grafts, and experimental coronary thrombosis. The optimal time for imaging stable venous thrombus in humans is 24 hours after platelet administration. Studies attempting to prevent platelet accumulation have shown a small decrease in infarct border zone platelet accumulation with aspirin administration. Ribeiro et al. demonstrated reduction of infarct size using prostaclin, a potent platelet inhibitor. The mechanism of this protective effect has not been demonstrated, but may be secondary to decreased platelet aggregation and

![Figure 5. Infarct/normal indium-111 platelet (\(^{111}\text{In PLT} \)) epicardial accumulation expressed as a function of both infarct age and relative regional myocardial blood flow. The format is identical to that in figure 4. Statistical analyses of the data are in table 2 and in the text.](image-url)
microcapillary occlusion. The use of 111In-labeled platelets appears ideal for further detailed experimental studies involving platelet-inhibitor drugs to determine effect on platelet kinetics during acute myocardial infarction.

The physical characteristics of 111In are well suited for cell-labeling studies, for its half-life of 67 hours ensures that sufficient activity will remain at the time of imaging 24 hours after injection. This delay is required for platelets to clear from the circulation blood pool before imaging. Although this method does not replace clinical infarct imaging agents (technetium-99m pyrophosphate and thallium-201), our preliminary studies suggest that 111In-labeled platelet scintigraphy can be useful for evaluating the extent of acute myocardial infarction when administered at the onset of clinical symptoms during the time of presumed rapid platelet deposition. More important, this method can be useful for evaluating the effect of platelet-inhibitor drugs on platelet kinetics during acute myocardial infarction.

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References

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