Identification of Coronary Thrombus With a IIb/IIIa Platelet Inhibitor Radiopharmaceutical, Technetium-99m DMP-444

A Canine Model

Joseph Mitchel, DO; David Waters, MD; Tianjie Lai, MD; Michael White, CNMT; Tod Alberghini, BS; Ahmad Salloum, MD; David Knibbs, PhD; Dadong Li, MD; Gary V. Heller, MD, PhD

Background—A diagnostic test that could distinguish between stable and unstable coronary atherosclerotic plaques would be useful. We tested the ability of a new glycoprotein IIb/IIIa platelet inhibitor DMP-444, labeled with technetium (Tc)-99 m, to identify platelet-rich thrombus by nuclear imaging in a canine model.

Methods and Results—Combinations of a flow-limiting stenosis and 0 to 15 minutes of endothelial electrical stimulation at a site in the left anterior descending coronary artery were used to induce varying amounts of thrombus formation. In 10 animals with markedly positive nuclear images after the injection of Tc-99m DMP-444, the presence of platelet-rich thrombus was confirmed postmortem by gross appearance, high nuclear counts, and abundant platelets on electron microscopy. The 10 animals with negative images had lower counts, smaller thrombus weights ($P<0.05$ for each), and fewer platelets by electron microscopy.

Conclusions—Activated platelets participating in acute thrombus formation can be accurately detected by nuclear imaging using Tc-99 m DMP-444. (Circulation. 2000;101:1643-1646.)

Key Words: diagnosis ■ imaging ■ platelet aggregation inhibitors ■ thrombosis

Unstable angina and myocardial infarction are initiated by plaque rupture or erosion, with overlying platelet thrombus deposition severe enough to impede coronary flow and to induce ischemia. The diagnosis of acute coronary syndromes is based on secondary phenomena such as symptoms, electrocardiographic evidence, and serum markers of myocyte injury. These diagnostic tools are imperfect, resulting in the inappropriate admission or discharge of patients with acute coronary syndromes, as well as delays in treatment. A diagnostic test that could reliably detect the underlying acute coronary lesion would be useful.

A variety of approaches to imaging arterial thrombi have been developed, but none has been successful enough to be adopted into clinical practice. Fibrin, platelets, and fibrinolytic molecules have been targets for labeling, but cumbersome technology, poor target-to-blood pool ratios, and low sensitivity were encountered. Labeling an inhibitor of the platelet glycoprotein (GP) IIb/IIIa receptor could theoretically overcome some of these difficulties. DMP-444 is a cyclic peptide, protease-resistant, selective GP IIb/IIIa receptor inhibitor that has been labeled with technetium (Tc)-99 m. Tc-99m DMP-444 can be detected in arterial and venous thrombi by nuclear imaging as early as 15 minutes after injection. This study was performed to evaluate the feasibility of Tc-99m DMP-444 imaging in the identification of activated platelets at the site of a coronary lesion using a canine model of endothelial injury.

Methods

Procedure

A canine model was chosen because of the species-specificity of DMP-444. Surgery was performed with electrocardiographic and arterial pressure monitoring under general anesthesia in accordance with Association for Accreditation and Assessment of Laboratory Animal Care laboratory standards. A 7F left coronary bypass catheter (Cordis) was positioned via a right carotid approach. Baseline angiography was performed to precisely define the anatomy.
The left anterior descending coronary artery was dissected free over a 1- to 2-cm interval. A Transonic flow probe (Crystal Biotech Inc) was placed on the vessel to continuously monitor blood flow. A hydraulic-occluder was placed on the artery, and a stenosis severe enough to cause a 30% reduction in baseline blood flow was created. Angiography was again performed to evaluate the stenosis.

A 0.014-inch steerable standard wire connected to a 9 V DC power source was advanced through the catheter to the stenosis. The endothelial surface was stimulated using 150 µA for 0, 5, 10, or 15 minutes, as previously described. Six groups (with or without stenosis and with 0 to 15 minutes of electrical stimulation) were studied; they are shown in Table 1. Tc-99m DMP-444 (DuPont Pharmaceuticals), 1 mCi/kg IV was administered after the procedure was completed. Serial planar images were acquired using a single-head single photon emission computed tomography (SPECT) γ-camera at 5- to 10-minute intervals for 2 hours. In all groups, a final angiogram was performed at the completion of nuclear imaging to confirm the severity of the stenosis.

Assessment of Coronary Lesions

All nuclear images were interpreted by 3 independent readers who had no knowledge of the study group. A Tc-99m marker was placed over the heart to provide readers with the appropriate orientation of the heart in the first 2 animals. All remaining images were interpreted without the use of the marker. The presence of activity was judged on a 5-point scale (0, no activity; 1, possibly present; 2, probably present; 3, definitely present; and 4, intense activity).

Platelet aggregation was measured at the start of the operative procedure, after stimulation, and at the end of the nuclear imaging protocol using a lumiaggregometer (Chrono-log). Blood samples (9 minutes) were collected at the conclusion of each stimulation period to be used for aggregation studies. Blood samples were assayed for aggregation by the lumiaggregometer. Platelet aggregation curves did not change from baseline to

### Table 1. Treatment Groups and Imaging Results

<table>
<thead>
<tr>
<th>Group</th>
<th>Stim Time, min</th>
<th>30% Flow ↓</th>
<th>Positive Images</th>
<th>Nuclear Activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>Yes</td>
<td>6/6</td>
<td>4+/4+ +/4+/4+</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>Yes</td>
<td>3/3</td>
<td>4+/4+ +/4+ +</td>
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<tr>
<td>3</td>
<td>5</td>
<td>Yes</td>
<td>1/3</td>
<td>0/0/0/0/0/0/0/0/0</td>
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<tr>
<td>4</td>
<td>0</td>
<td>Yes</td>
<td>0/3</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>No</td>
<td>0/3</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>No</td>
<td>0/2</td>
<td>0</td>
</tr>
</tbody>
</table>

Stim time indicates length of time of electrical stimulation; 30% flow ↓, presence of a 30% flow-limiting stenosis distal to the site of electrical stimulation.

P = 0.029 for groups 1–3 vs 4–6.

*Nuclear activity was grading using the 5-point scale described in the text.

### Table 2. Nuclear Count Activity and Clot Weight Comparisons Between Nuclear Positive and Negative Images

<table>
<thead>
<tr>
<th>Image Activity Result</th>
<th>No. of Animals</th>
<th>Nuclear Count Activity, cpm</th>
<th>CW, mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>10</td>
<td>88 600 ± 63 800</td>
<td>5.90 ± 6.63</td>
</tr>
<tr>
<td>Negative</td>
<td>10</td>
<td>9300 ± 8300</td>
<td>0.002 ± 0.006</td>
</tr>
</tbody>
</table>

P = 0.01

CW indicates clot weight; cpm, counts per minute.

### Results

The results of the study are shown in Tables 1 and 2. All animals (groups 1 and 2) with a stenosis and 10 or 15 minutes of electrical stimulation developed highly positive nuclear images 20 to 60 minutes after the injection of Tc-99m DMP-444. In each of these cases, gross inspection, high nuclear counts, and large concentrations of platelets on electron microscopy confirmed the presence of a platelet-rich thrombus. All images were negative for thrombus in the 2 groups without stenosis and in the group with stenosis but without endothelial injury. One of 3 animals in the group with a stenosis and 5 minutes of stimulation had a positive image and a small thrombus. As shown in Table 1, a significant difference was seen in positive images between the 3 groups receiving stimulation with a flow reduction compared with all remaining groups. In all cases, blood pool activity in both ventricles was present, but in no case did the blood pool intensity obscure the interpretation of the image. Further, as thrombus activity increased, blood pool activity diminished.

The Figure shows 3 animals with positive images and corresponding platelet-rich electron microscopy photographs and 1 negative image with no electron microscopic evidence of platelets. In Table 2, the 10 animals with positive images are compared with the 10 animals with negative images. Nuclear counts per minute and thrombus weight were both significantly higher in the group with positive images. Platelet aggregation curves did not change from baseline to completion of imaging in all groups.

### Discussion

This study demonstrates that a platelet GP IIb/IIIa inhibitor labeled with Tc-99m can reliably detect acute platelet-rich thrombi in a canine model. Positive nuclear images were associated with high nuclear counts, visible thrombus, and abundant platelets by electron microscopy postmortem in the arterial segment containing the lesion. In animals with negative nuclear images, significant thrombus was not detected by these methods in the postmortem specimens.

Tc-99m is the preferred radionuclide because of its excellent imaging characteristics, intermediate energy, widespread availability, and short half-life.7 The GP IIb/IIIa receptor is expressed in small numbers on the external surface of circulating quiescent platelets, but >50 000 binding sites per platelet can occur during stimulation.7 This yields an excellent target-to-blood ratio. Tc-99m DMP-444 possesses ideal features for imaging in acute coronary syndromes.

### Limitations of the Study

In this study, Tc-99m DMP-444 was injected at a uniformly short interval after the creation of the thrombotic lesion.

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Among patients presenting with acute coronary syndromes, the culprit lesion may have formed hours to days earlier. How this would affect the result of the nuclear imaging is not known, although large numbers of GP IIb/IIIa receptors should still be available for binding. Thrombi in this experimental model were platelet-rich, and the intensity of a positive image probably depends on the number of platelets within the lesion. The relative platelet content and the amount of thrombus in culprit lesions from living patients with acute coronary syndromes seem to be within the range of lesions evaluated in this study. However, the sensitivity of this technique in detecting culprit lesions in patients with acute coronary syndromes remains to be assessed. The injected dose of Tc-99m used in this study was based on previous observations in canines; however, images acquired in humans have used a lower dose of 25 to 30 mCi/patient.

Previous Studies
To date, no studies have been performed in humans with unstable angina or acute myocardial infarction to evaluate the usefulness of Tc-99m DMP-444. Several other approaches to imaging arterial thrombi produced promising findings in animal experiments but were not successful in clinical practice.

Clinical Implications
A nuclear imaging agent that could accurately identify unstable coronary plaques might prove useful in risk-stratifying patients presenting with acute chest pain. Such an agent could also be used in conjunction with coronary angiography to identify the culprit lesion of patients with multiple coronary stenoses. Tc-99m DMP-444 might be useful in assessing the adequacy of platelet GP IIb/IIIa inhibitor therapy with other drugs of its class. Serial imaging studies might reveal when an active lesion becomes quiescent and, thus, when the risk of an acute thrombotic complication decreases. On the basis of the range of thrombi detectable in this study, we think that the clot formed during unstable coronary syndromes in humans would be within the resolution threshold for detection using SPECT imaging. We are currently evaluating Tc-99m DMP-444 in patients with acute coronary syndromes.
Acknowledgments
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References
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