Noninvasive Radioisotopic Technique for Detection of Platelet Deposition in Coronary Artery Bypass Grafts in Dogs and Its Reduction with Platelet Inhibitors

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SUMMARY At 8 and 32 hours after saphenous vein bypass graft surgery in six dogs, in vivo images of the graft were obtained with a gamma camera after intravenous injection, 2 hours postoperatively, of autologous platelets labeled with indium-111. Platelet deposition in the grafts could be imaged in vivo from the scintiphotos. In vitro images of the excised heart showed saphenous vein graft uptake confirming the in vivo image. In vitro determination of radioactivity in the graft averaged 17 ± 14 times greater than in blood and 33 ± 26 times greater than in the lung.

Under identical conditions, in eight dogs treated with dipyridamole (55 mg/day) plus aspirin (325 mg/day), the grafts appeared to have considerably less platelet deposition as estimated by imaging. In vitro determination of radioactivity in the graft averaged 5 ± 2 times greater than in blood and 8 ± 4 times greater than in the lung (p < 0.01).

This noninvasive technique may be a promising tool for a better understanding of the role played by platelets in the process of occlusion of saphenous vein bypass grafts in man and its prevention with platelet inhibitors.

AORTOCORONARY BYPASS SURGERY with autologous saphenous vein grafts1 is used widely in patients with coronary artery disease. A major limitation of this procedure is the high frequency of occlusion of the vein graft.2-4 Secondary to thrombosis or intimal proliferation or both.4-6 There is now suggestive clinical and experimental evidence that platelets may play an important role in the pathogenesis of the occlusion.7-8 and it has been suggested that the use of platelet inhibitory drugs might interrupt this process. However, no in vivo technique is available for direct demonstration of this process of platelet deposition in the vein grafts and its possible reduction with platelet inhibitory drugs.

Labeling of platelets with indium-111 (111In) and its applications in thrombosis research have been described by Thakur and associates9 and Scheffel and associates.10 Recently, by means of 111In-labeled platelets and imaging with a gamma camera, we have been able to detect in vivo platelet deposition in saphenous vein bypass grafts in dogs.11 Since our initial report, we have expanded our study, and this report presents a comparison between the in vivo platelet deposition and in vitro quantification in coronary artery bypass grafts in a larger group of normal control dogs and in another group treated with platelet-inhibitory drugs.

Methods

Aortocoronary Bypass Grafting

With the use of cardiopulmonary bypass, 14 mongrel dogs weighing 18-26 kg underwent aorta-left anterior descending coronary artery bypass grafting carried out through a thoracotomy in the fourth intercostal space. The unoperated, undistended, reversed right femoral vein was used for the bypass graft. In all dogs, the distal anastomosis, which was carried out with the use of systemic hypothermia to 25°C and cross-clamping of the aorta, was to the anterior descending coronary artery just distal to the first diagonal branch and was performed with a running suture of 7-0 Prolene interrupted at two points. After completing this distal anastomosis, a bulldog clamp was placed across the saphenous vein near the vein-coronary artery anastomosis, the aorta was unclamped, and rewarmin was begun.

A side-biting clamp was then placed on the ascending aorta, and the proximal anastomosis of vein to aorta was carried out with a running 6-0 Prolene stitch interrupted at two points. Before removing the bulldog clamp on the distal portion of the vein, air was vented from the vein graft by aspiration through a 25-gauge needle. Each animal was defibrillated with a single shock, and when suitable cardiac action had been reestablished, bypass was discontinued. The effect of heparin was reversed with protamine, a chest tube was placed, and the chest was closed in the routine fashion. During the operative procedure and the immediate postoperative period, arterial and venous pressures were monitored through the left internal mammary artery and left external jugular vein. When spontaneous respiration was adequate in the postoperative period, the chest tube and the monitoring catheters were removed, and the dogs were returned to their cages.

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Medication

The dogs were distributed into two groups. Six dogs underwent bypass grafting and received no medication other than systemic antibiotics during surgery. Eight were given oral dipyridamole 55 mg/day for 2 days preoperatively and then dipyridamole 55 mg plus aspirin 325 mg postoperatively at 1 hour and again at 24 hours after surgery.

Angiographic Studies

About 24 hours after surgery, four untreated and three treated dogs were subjected to catheterization under light sodium pentobarbital so we could visualize the bypass graft. The dogs were oriented in a right anterior oblique position, and a #7F catheter was introduced through the previously ligated left common carotid artery and guided under fluoroscopic vision to the area of the aorta-vein anastomosis. At this point, an automated delivery system injected a constant volume of indocyanine green (Renografin), and a spot film was taken to demonstrate the anatomic location and the patency of the bypass graft. When suitable angiograms had been obtained, the catheter was removed, the artery was ligated, and the dogs were returned to their cages.

Preparation of 111In-labeled Platelets, Imaging and Tissue Distribution

Indium-111-labeled 8-hydroxyquinoline (oxine), prepared according to the method of Thakur and associates, was supplied by Diagnostic Isotopes (Upper Saddle River, New Jersey). Each vial contained 1 mCi of 111In and 50 μg of oxine in 50 μl of absolute alcohol. The labeling of the dog platelets was performed during surgery based on the procedure of Scheffel et al. as we have applied it to dogs. Dog blood was collected 1 hour before surgical intervention and platelet harvesting, labeling, washing, and separation of microaggregates were completed in about a 2.5-3-hour period. About 2 hours after the operation, the dogs were injected with 300-500 μCi of autologous 111In-labeled platelets in 4-8 ml of acid-citrate-dextrose plasma solution. At 6 and 30 hours after intravenous administration of labeled platelets, dogs anesthetized with sodium pentobarbital were imaged with a gamma camera (Searle, large field of view) fitted with a medium-energy, parallel-hole collimator. The camera spectrometer was adjusted to cover both the >174-keV and <247-keV peaks in two vein doses of the 111In radioisotope. The relative distribution of 111In-labeled platelets in the thorax and abdomen was then obtained. One hundred thousand counts were accumulated at the anterior, left and right lateral, and left and right anterior oblique positions. Pinhole views of the graft with a Searle camera (Pho-Gamma 37 GP) were also obtained simultaneously with both peaks in one window.

Within 1 hour of the in vivo imaging (about 31 hours after intravenous administration of labeled platelets), the dogs were killed with an overdose of sodium pentobarbital and the heart, lungs, liver, spleen, kidneys, blood and tissue samples from skeletal muscle, bone, and marrow were obtained and weighed: from this the distribution of radioactivity in the organs and in the whole body was determined with a dose calibrator (Capintec) and an automatic gamma well counter (Beckman). Finally, chambers of the heart were washed free of blood and postmortem clots with isotonic saline, the interventricular septum was removed, and the right and left ventricles were spread flat and imaged in the same orientation as used for the in vivo imaging. The graft, along with the two sites of anastomosis, was removed from the aorta and myocardium; the proximal and distal sites of anastomosis, 2-3 mm away from each end of the graft, were cut and saved for measurement of radioactivity. The graft was opened by a midline incision, rinsed with saline, and divided into three equal sections 1 cm long: proximal, middle, and distal. Each section was weighed with a microbalance and the radioactivity per unit weight of the graft sections and surrounding right ventricle, left ventricle, right atrium, and interventricular septum was determined. Then the radioactivity in the graft was compared with that in blood, lung, cardiac muscle and skeletal muscle.

Results

111In-labeled Platelet Imaging and Angiography

In the untreated dogs, at 8 and 32 hours after surgery and 6 and 30 hours after intravenous administration of radioactive platelets, scintiphotos of the distribution of 111In-labeled platelets clearly delineated the bypass grafts (fig. 1). The intensity of radioactivity in the bypass graft as visualized by imaging was not as great as that found in the liver and spleen but was greater than that in the nontarget tissues — blood, lung, cardiac muscle and skeletal muscle. The values for the ratio of radioactivity in the graft to that in blood, lung, cardiac muscle or skeletal muscle increased considerably after 30 hours compared with those at 6 hours as a result of clearance from the blood and platelet deposition. The coronary angiograms of the bypass grafts showed patent grafts in all dogs. (fig. 2).

For confirmation of the results of external imaging, the isolated heart without the interventricular septum was imaged in the orientation of the in vivo imaging, and this again demonstrated the graft as the major site of platelet deposition in the heart. Other small hot spots indicated platelet deposition at the sites of cannulation for the surgical procedure. Figure 3 shows the scintiscan and the photograph of the isolated saphenous vein graft specimen.

In the treated as compared with the untreated dogs, scintiphotos of the distribution of 111In-labeled platelets showed less intense radioactivity or no evidence of increased radioactivity in the bypass graft (fig. 4). The intensity of radioactivity in the nontarget tissues in the treated dogs, however, did not appear appreciably different from that in the untreated dogs.
The coronary angiograms of the bypass grafts showed patency of the grafts in all dogs studied.

**Biodistribution of $^{111}$In-labeled Platelets**

In the untreated dogs, the postmortem studies showed organ distribution of the injected radioactivity decreased in the following order: liver, spleen, blood, skeletal muscle, lungs, kidneys and in the bypass graft (table 1). The percentage of injected dose per gram of liver and spleen was 0.061 and 0.493, respectively. Because $^{111}$In ion binds strongly with intracellular proteins, no significant radioactivity (less than 1%) was found in bone marrow or excreted in urine and feces. The mean values and standard deviations of the relative distribution of $^{111}$In-labeled platelets in the proximal, middle, and distal sections of the grafts compared with the nontarget tissues (blood and lung) at 30 hours after injection are shown in table 2. Radioactivity in the graft averaged $17 \pm 14$ times greater than in blood and $33 \pm 26$ times greater than in the lung. Both sites of anastomosis also had higher radioactivity than the nontarget tissues. The graft-to-

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**TABLE 1. Biodistribution* of $^{111}$In-labeled Platelets† in Six Untreated Dogs and Eight Dogs Treated With Dipyridamole and Aspirin (mean = SD)**

<table>
<thead>
<tr>
<th></th>
<th>Untreated</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>38.23 ± 8.27</td>
<td>37.69 ± 7.86</td>
</tr>
<tr>
<td>Spleen</td>
<td>28.27 ± 9.14</td>
<td>26.43 ± 10.29</td>
</tr>
<tr>
<td>Blood</td>
<td>22.69 ± 11.21</td>
<td>26.12 ± 9.31</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>6.47 ± 4.93</td>
<td>5.79 ± 3.86</td>
</tr>
<tr>
<td>Lungs</td>
<td>4.52 ± 2.35</td>
<td>2.23 ± 1.31</td>
</tr>
<tr>
<td>Kidneys</td>
<td>1.45 ± 0.41</td>
<td>1.26 ± 0.46</td>
</tr>
<tr>
<td>ACBG</td>
<td>0.343 ± 0.24</td>
<td>0.082 ± 0.032</td>
</tr>
</tbody>
</table>

*Percentage of injected dose.
†Thirty-one hours after intravenous administration of labeled platelets.
Abbreviation: ACBG = aortocoronary bypass graft.

**TABLE 2. Relative Distribution of $^{111}$In-labeled Platelets* in Six Untreated Dogs (mean = SD)**

<table>
<thead>
<tr>
<th>Graft section</th>
<th>Graft/blood</th>
<th>Graft/lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal</td>
<td>15.17 ± 8.98</td>
<td>32.58 ± 24.72</td>
</tr>
<tr>
<td>Middle</td>
<td>15.42 ± 10.64</td>
<td>31.90 ± 25.76</td>
</tr>
<tr>
<td>Distal</td>
<td>20.45 ± 25.17</td>
<td>36.20 ± 36.60</td>
</tr>
</tbody>
</table>

*Thirty hours after intravenous administration of labeled platelets.
blood and graft-to-lung ratios of radioactivity are the determining factors for external imaging. The favorable ratios account for the imaging of the grafts.

The postmortem studies indicated that distribution of radioactive platelets in the organs was not statistically different in the treated dogs than in the untreated dogs (table 1). The relative distribution of $^{111}$In-labeled platelets in the proximal, middle and distal sections of the grafts compared with the nontarget tissues, however, was significantly lower ($p < 0.01$) in the treated dogs (tables 2 and 3). Thus, radioactivity in the overall graft averaged $5 \pm 2$ times greater than in blood and $8 \pm 4$ times greater than in the lung.

**Discussion**

The method of $^{111}$In labeling of platelets developed by Thakur and associates is a new tool for the study by external imaging of the process of platelet deposition in vivo. Thus, the half-life of 2.8 days along with essentially 186% yield of gamma emissions of two gamma ray energies of $> 174$ and $< 247$ keV makes it an excellent platelet marker for in vivo imaging.

We were able to image the deposition of $^{111}$In-labeled platelets within the grafts. Good experimental evidence reveals that platelets tend to adhere to damaged endothelial vascular surfaces. Our recent finding that the saphenous vein graft becomes diffusely de-endothelialized during the surgical procedure most likely explains the deposition of platelets as visualized by imaging and confirmed morphologically (Josa M, Kaye MP, Fuster V: unpublished data).

**Table 3.** Relative Distribution of $^{111}$In-labeled Platelets* in Eight Dogs Treated With Dipyridamole and Aspirin (mean ± SD)

<table>
<thead>
<tr>
<th>Graft section</th>
<th>Graft/blood</th>
<th>Graft/lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal</td>
<td>3.92 ± 1.62</td>
<td>6.82 ± 4.05</td>
</tr>
<tr>
<td>Middle</td>
<td>5.55 ± 1.64</td>
<td>9.70 ± 4.49</td>
</tr>
<tr>
<td>Distal</td>
<td>4.25 ± 2.11</td>
<td>7.89 ± 6.00</td>
</tr>
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</table>

*Thirty hours after intravenous administration of labeled platelets.
The detection by in vivo imaging of an extensive platelet deposition in the coronary artery bypass graft in dogs may be extremely important because current clinical and experimental evidence indicates that platelets may play a major role in the pathogenesis of the highly frequent graft occlusion in man. Although the usefulness of the external imaging of platelet deposition in the bypass graft is evident in dogs, its suitability in studying the same phenomenon in man needs to be cautiously evaluated for three reasons. First, we do not know whether this early deposition by platelets with angiographic evidence of graft patency is only a transient response to graft endothelial injury or is a continuous process that may lead to the occlusion of the graft. Second, we do not know whether the small hot spots detected in dogs indicating platelet deposition at the sites of cannulation might lead, in future studies in man, to a false-positive interpretation of platelet deposition in the coronary graft. Third, because blood flow is impaired in occluded bypass grafts, we do not know whether this will prevent the deposition of 111In-labeled platelets at the occlusion site and therefore will be a limitation for the diagnosis of graft occlusion by this noninvasive method.

In our study in dogs pretreated with the regimen dipyridamole plus aspirin at dosages comparable to those used in humans, the radioactivity ratio in the graft was reduced to approximately one-fourth compared with the untreated dogs. Good experimental morphologic evidence indicates that these agents may interrupt the process of platelet deposition on damaged arterial endothelial surfaces and in coronary artery saphenous vein bypass grafts. The fact that the deposition of platelets as assessed by our technique was reduced only at the graft and cannulation sites, but not in the other nontarget tissues, is important. This indicates that these agents only inhibited the deposition of platelets at the damaged vascular sites and did not affect the circulation and accumulation of platelets in the other organs. This is not surprising, because nontarget tissue distribution probably represents physiologic pooling or normal senescence of platelets and not the thrombogenic deposition likely to be affected by platelet inhibitors. The most current information suggests that a low dose of aspirin, as used in our experimental model, affects the platelet by preventing its generation of the intraplatelet proaggregating thromboxane A2 and the dose does not appear to be sufficient to have an anti-inflammatory action.

The finding with this new technique that platelet deposition in the grafts was reduced by treatment with dipyridamole and aspirin is important. It may be a promising tool for better and rapid assessment of potential platelet-inhibitory drugs that can be used in future preventive studies in man. We realize, however, that a serious limitation of the present external imaging method is that it provides only qualitative information and not quantitative data as we obtain with postmortem radioisotope counting of the different tissues.

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References
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