Scintigraphic Detection of Carotid Atherosclerosis with Indium-111-labeled Autologous Platelets

HARMON H. DAVIS II, M.D., BARRY A. SIEGEL, M.D., LAURENCE A. SHERMAN, M.D.,
W. ANDREW HEATON, M.D., THOMAS P. NAIDICH, M.D., J. HEINRICH JOIST, M.D., PH.D.,
AND MICHAEL J. WELCH, PH.D.

SUMMARY Using autologous platelets labeled with indium-111-oxine, we studied the localization of platelets on arterial lesions by radionuclide scintigraphy in 34 patients with suspected cerebrovascular disease. The imaging results were compared with the findings of contrast angiography in 23 patients, 16 of whom were receiving antplatelet and/or anticoagulant drugs during the platelet imaging study. Angiography demonstrated atherosclerotic lesions at 33 sites in the extracranial arteries of 16 of these patients. There was accumulation of 111In-platelets at 20 of these sites (61%) and at three other sites without definite angiographic abnormalities. Lesions with stenoses < 50% were slightly more frequent than those with greater stenosis (68% vs 45%). The frequency of true-positive scintigraphic results was slightly higher in patients not treated with antithrombotic agents than in those on such drugs (70% vs 57%). Our results suggest that imaging with 111In-labeled autologous platelets may be useful for evaluating the pathophysiologic characteristics of atherosclerotic lesions in patients with cerebrovascular disease.

A METHOD for labeling of platelets using indium-111 complexed with 8-hydroxyquinoline (oxine) has been described.1 This technique is relatively simple and labels the platelets harvested from a small volume of blood with high efficiency. Indium-111-labeled platelets remain in vitro function, as assessed by their aggregation response to adenosine diphosphate and collagen,1-3 and survive normally in vivo.1, 2-5 The in vivo distribution of 111In-labeled platelets can be evaluated readily by radionuclide imaging because of the high gamma photon yield of 111In. This is an advantage over other radionuclides (e.g., 51Cr) that have been used for platelet labeling. We and others have demonstrated that 111In-labeled platelets accumulate at sites of thrombosis, endothelial injury, and atherosclerosis, permitting scintigraphic detection of these lesions in experimental animals1, 6-10 and in man.11, 12 In the present study we performed scintigraphy with 111In-labeled autologous platelets in patients with cerebrovascular disease. We determined the frequency of abnormal images in these patients and correlated the scintigraphic findings with angiographic evidence of vascular disease.

Methods

Patient Population

Platelet imaging studies were performed in 34 adult patients, 22 men and 12 women, with suspected cerebrovascular disease. The patients ranged in age from 39-77 years (mean 58 years). Thirty-three patients had recent symptoms or signs of cerebrovascular disease (transient ischemic attacks [TIAs] in 27 and completed cerebral infarction in six). One patient was asymptomatic but had a unilateral carotid bruit that was shown by angiography to be caused by fibromuscular hyperplasia. Before the study each patient gave written informed consent.

Platelet Labeling

Autologous platelets were labeled under aseptic conditions in a laminar flow hood. In eight patients, the platelets were labeled as described by Davis et al.11 With this method, after platelets were isolated by differential centrifugation, they were resuspended in a modified Tyrode's solution during labeling and subsequent washes. In 26 patients, the platelets were labeled as described by Heaton et al.13 using Squibb modified acid-citrate-dextrose solution (ACD) mixed with normal saline (1:6 vol/vol adjusted to pH 6.5) as the labeling solution and using ACD: plasma (1:6 vol/vol) as the wash solution to remove any free or loosely bound 111In. We prefer the latter technique for platelet labeling because the uptake of 111In-oxine by platelets is less variable with this method.

Imaging Studies

Labeled platelets were administered intravenously in doses of 100-550 μCi. Scintigraphic images of the head, neck, trunk and thighs were obtained immediately, at 4 hours and at 18-24 hours after tracer administration. Imaging was performed with a large-field-of-view scintillation camera fitted with a medium-energy collimator; dual 20% spectrometer settings were centered over both photopeaks of 111In.

The images were initially interpreted by a single observer who had full knowledge of the clinical findings but not the angiographic results. These scintigraphic diagnoses are referred to as the initial interpretations.
To assess the reproducibility of the scintigraphic interpretations, the scans of the study population (along with scans obtained in patients with diagnoses other than cerebrovascular disease) subsequently were interpreted independently by two additional observers who had no knowledge of the clinical findings. The scintigraphic diagnosis at each site was based on agreement of at least two of the three observers and is referred to as the multiobserver interpretation.

Twenty-three patients had selective carotid arteriograms and/or arch aortograms, permitting correlation of the anatomic findings at angiography with the scintigraphic diagnoses. The contrast angiograms were evaluated by a single observer (who did not know the scintigraphic findings) for the presence or absence of atherosclerotic changes in each major extracranial artery. Each lesion was characterized by: 1) the degree of stenosis; 2) the presence or absence of ulceration; and 3) the presence or absence of intraluminal thrombus. Sites of minimal intimal roughening were classified as normal.

Platelet Survival

Sequential blood samples were obtained in 32 patients over 5–7 days to estimate platelet survival. Whole blood samples and standards prepared from an aliquot of each platelet injectate were counted in a NaI crystal-well scintillation detector. The percentage recovery of tracer in the circulating blood was estimated from the activity of each sample and the patient’s predicted blood volume. The initial platelet recovery and mean platelet life span were computed from the weighted mean of the linear and semilogarithmic least-squares estimates. Using the $t$ test, the platelet survival results in each subgroup were compared with each other and with the results obtained previously in 10 control subjects.

Results

Imaging Studies

Based on the initial interpretations, the scintigraphic images showed one or more focal areas of $^{111}$In-platelet accumulation in the extracranial arteries (figs. 1–3) of 17 of the 34 patients (12 of 27 with TIAs, four of six with completed cerebral infarction, and the patient with fibromuscular hyperplasia). Some foci were seen on immediate images, but most became more obvious on delayed images at 4 or 24 hours. Intracranial foci of increased activity were not noted. In addition, the images often demonstrated focal accumulation of labeled platelets at femoral arteriotomy sites (fig. 4) in patients who underwent transfemoral arteriography just before or during the platelet imaging study. In contrast to foci in the neck vessels, increased activity at arteriotomy sites was usually present on early images and tended to fade with time.

Contrast angiography was performed in 23 patients (21 with TIAs and one each with completed cerebral infarction and fibromuscular hyperplasia). Based on the initial interpretations, the scintigrams showed abnormal foci in the neck vessels of 13 patients, 12 of whom had angiographic evidence of extracranial cerebrovascular disease (table 1). The scans were normal in 10 patients and in six of these the angiograms were normal. Thus, when the scans were evaluated only for the presence or absence of focal abnormalities and when the angiograms were analyzed similarly (irrespective of site-by-site correspondence of findings), there was a statistically significant correlation between the results of the two techniques (Yates corrected $\chi^2 = 5.04, p = 0.025$). The sensitivity of $^{111}$In-platelet imaging was 75% (12 of 16 patients) and the apparent false-positive rate was 14% (one of seven patients).

Twenty-three foci of $^{111}$In-platelet accumulation were identified in the neck vessels of the 13 patients with abnormal scintigrams. A site-by-site comparison with the contrast angiograms showed that 20 of these foci corresponded to definite arterial lesions (87%). In the 16 patients with abnormal angiograms, 33 sites of disease were identified; 20 of these corresponded to foci of labeled platelet uptake on the scintigrams (overall sensitivity of 61%). Table 2 is a comparison of the scintigraphic findings and the angiographic assessment of lesion severity. Lesions that resulted in less than 50% stenosis (both ulcerated and nonulcerated) were detected with slightly but not significantly greater frequency than lesions with higher degrees of stenosis. Intraluminal thrombus was present on angiography in two lesions, one of which was detected scintigraphically.

### Table 1. Comparison of Scintigraphic and Angiographic Findings

<table>
<thead>
<tr>
<th>Angiogram abnormal at one or more sites</th>
<th>Angiogram normal</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scan abnormal at one or more sites</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>Scan normal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>7</td>
<td>23</td>
</tr>
</tbody>
</table>

Numbers represent number of patients.

### Table 2. Comparison of Lesion Severity and Scintigraphic Findings

<table>
<thead>
<tr>
<th>Angiographic findings</th>
<th>Number of lesions</th>
<th>Number with abnormal scan</th>
<th>Percent abnormal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stenosis &lt; 50%</td>
<td>22</td>
<td>15</td>
<td>68%</td>
</tr>
<tr>
<td>Without ulceration</td>
<td>13</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>With ulceration</td>
<td>9</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Stenosis &gt; 50%</td>
<td>7</td>
<td>3</td>
<td>43%</td>
</tr>
<tr>
<td>Occlusion</td>
<td>4</td>
<td>2</td>
<td>50%</td>
</tr>
<tr>
<td>Total</td>
<td>33</td>
<td>20</td>
<td>61%</td>
</tr>
</tbody>
</table>
Twenty-eight of the 33 lesions detected by angiography were located at the bifurcations of the common carotid arteries. The common carotid bifurcations were fully visualized by angiography in all 23 patients, permitting an evaluation of the apparent sensitivity and specificity of $^{111}$In-platelet imaging for identification of disease in these regions (table 3). The correlation between scintigraphic and angiographic findings was highly significant (Yates corrected $\chi^2 = 13.35, p = 0.0003$).

When the multiobserver interpretations of the scans were compared with the overall angiographic findings, the sensitivity of the scintigrams was reduced. These interpretations correctly identified 12 of the 33 lesions shown by angiography compared with 20 of 33 correctly identified on the initial readings.

In the 23 patients who underwent angiography, 16 were taking anticoagulant and/or antiplatelet drugs at the time the $^{111}$In-platelet imaging study was performed (aspirin and dipyridamole in eight, heparin in

<table>
<thead>
<tr>
<th>Number of sites</th>
<th>Angiogram abnormal</th>
<th>Angiogram normal</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scan abnormal</td>
<td>16</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>Scan normal</td>
<td>12</td>
<td>18</td>
<td>30</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
<td>18</td>
<td>46</td>
</tr>
</tbody>
</table>

Sensitivity—16 of 28 (57%) Specificity—18 of 18 (100%)
CAROTID ATHEROSCLEROSIS DETECTION BY $^{111}$In/Davis et al.

Figure 1. A 59-year-old man with two prior myocardial infarctions. Six months after coronary artery bypass graft surgery, he developed amaurosis fugax with intermittent episodes of loss of vision in the right eye that lasted as long as 30 minutes. A) Sequential anterior images of the head and neck obtained after administration of $^{111}$In-labeled autologous platelets demonstrate progressively increasing activity at the level of both common carotid bifurcations. At the time of this study, the patient was taking aspirin (300 mg three times daily) and dipyridamole (50 mg three times daily). The activity in the nasopharyngeal region is a normal finding. B) Right carotid angiogram (lateral projection) demonstrates a plaque resulting in less than 50% stenosis of the distal common carotid and proximal internal carotid arteries (curved arrows). There is a central ulceration (arrow). C) Left carotid angiogram (lateral projection) demonstrates a small plaque of the distal common carotid artery (arrow). The anteroposterior projection (not shown) demonstrated a small ulcer in this plaque.

six, aspirin in one and heparin and dipyridamole in one). The frequency of true-positive results in the seven patients not on such drugs (seven of 10 lesions [70%] detected) was slightly but not significantly higher than that in 16 drug-treated patients (13 of 23 lesions [57%] detected).

Platelet Survival

In the 32 patients for whom platelet kinetics could be evaluated, the initial recovery of labeled platelets in the circulating blood was $74 \pm 28\%$ (mean $\pm$ SD) of the administered dose. Mean platelet life span was $7.0 \pm 1.8$ days. These values are not significantly different from those in a group of 10 normal volunteers (recovery $76 \pm 13\%$; life span $7.2 \pm 1.4$ days). There were no significant differences in platelet recoveries or survivals when the results for the following subgroups were compared with each other and with the results in normal subjects: 1) patients with transient cerebral ischemia vs those with completed cerebral infarction; 2) patients with normal vs abnormal scintigrams; 3) patients with normal vs abnormal arteriograms; or 4) patients on anticoagulant and/or antiplatelet drugs vs those not on such drugs.

Discussion

We have shown that autologous platelets labeled with $^{111}$In-oxine accumulate on atherosclerotic lesions in the carotid arteries of patients with cerebrovascular disease. This observation suggests two applications of this technique: 1) its use as a noninvasive test to detect carotid arterial lesions; and 2) its use to evaluate the role of platelets in the pathogenesis of carotid atherosclerosis and cerebral ischemia.

Our results suggest that $^{111}$In-platelet imaging has somewhat limited sensitivity compared with contrast angiography for detection of carotid atherosclerotic lesions. The scintigrams correctly identified 61% of all angiographically defined lesions and 57% of those at the carotid bifurcations, the most frequent site of disease in patients with cerebral ischemia. At the carotid bifurcations, the specificity was 100%, although apparent false-positive foci were noted three times on the scintigrams at other sites. However, several limitations must be considered in evaluating these results. First, the adherence and aggregation of platelets at atherosclerotic sites or other arterial lesions is a pathophysiologic process that depends primarily on loss of integrity of the arterial endothelial lining. Contrast angiography demonstrates only the anatomic alterations associated with an arterial lesion, but it cannot assess the dynamics of the thrombotic process at a given site. Thus, our false-negative scintigraphic results may reflect re-endothelialization and relative inactivity of certain arterial lesions,
Figure 2. A 50-year-old man with type IV hyperlipoproteinemia. Six months before admission he had transient left facial numbness and right-sided monocular blindness. Admission was prompted by a 2-hour episode of aphasia of mixed type. A bruit was heard over the right carotid artery. Computed tomography demonstrated changes consistent with a left frontal lobe infarction. A cerebral radionuclide angiogram was normal. A) The anterior image of the head and neck 28 hours after administration of 111In-platelets shows increased accumulation of tracer at the level of both common carotid bifurcations, more prominent on the right. B) Right carotid angiogram (lateral projection) obtained 1 month after the platelet imaging study shows approximately 70% stenosis at the origin of the internal carotid artery as well as an ulcer (arrow). C) The left carotid angiogram (obtained at the same time as panel B) shows complete occlusion of the internal carotid artery at its origin.

Whereas the false-positive images may represent early endothelial lesions not yet producing an angiographically detectable anatomic alteration.

A second concern in assessing our results is the large proportion of our patients treated with anticoagulant or antiplatelet drugs. Untreated patients had a slightly higher frequency of true-positive scan results than did the patients on such drugs, but this difference was not statistically significant. Evaluation of the effect of antiplatelet or anticoagulant drugs on the accumulation of 111In-labeled platelets on arterial lesions will require studies in a larger number of pa-
tients and in appropriate experimental animal models.

Despite these problems, our results are encouraging. Two-thirds of lesions in which the degree of stenosis was assessed to be less than 50% by angiography were detected. Although such lesions are generally considered to be hemodynamically insignificant, they are clinically important as sources of platelet-fibrin or atheromatous emboli. Further, the degree of stenosis at such sites tends to increase with time. Both of these processes may lead to TIAs or cerebral infarction.22-24 Several noninvasive screening techniques have been used to evaluate carotid arterial disease (e.g., phonoangiography, Doppler ultrasonography and ophthalmodynamometry). However, these techniques are insensitive in detecting nonstenotic lesions or those with low grades of stenosis.22, 28 Thus, 111In-platelet imaging may be useful for the noninvasive detection of some lesions that hitherto could be demonstrated only by contrast angiography.

The lower sensitivity of the multiobserver interpretations reflected interobserver disagreement in the interpretation of mildly abnormal foci on the 111In-platelet images. In addition, two of three observers interpreted the images without access to clinical data that usually would be available during the evaluation of scintigraphic studies. Quantitative analysis of images using computer-assisted methods may improve the overall sensitivity of this technique and increase the objectivity of interpretations.

The survival of 111In-labeled platelets was not significantly shorter in our patient population compared with that measured in normal subjects. Reduced survival of 51Cr-labeled platelets has been observed in some but not all patients with arterial thrombotic disease, including those with TIAs and completed cerebral infarction.24 Our results are difficult to interpret because of the relatively small number of patients evaluated and the large proportion of patients taking antiplatelet and/or anticoagulant drugs, which improve shortened platelet survivals in some patients with cerebrovascular disease.24

Our results raise several potentially important questions. First, do atherosclerotic lesions that accumulate 111In-labeled platelets have a different natural history and prognosis from those that do not, or are such lesions simply at different developmental stages? Second, does an abnormal scan indicate an arterial lesion that is more likely to respond to effective antiplatelet therapy (i.e., can this test identify a subset of patients most likely to benefit from such drugs)? Third, does the detection of certain arterial lesions in spite of concurrent antiplatelet and/or anticoagulant therapy imply that conventional therapy with such drugs is not optimally antithrombocytic? These questions suggest goals for further studies using the 111In-platelet imaging technique in both patients and experimental models.

In conclusion, we have obtained promising clinical results with 111In-labeled platelets, a new agent for detecting thrombi and arterial endothelial lesions.
This method is a means of detecting and potentially quantitating the accumulation of platelets at sites of arterial injury. This is important because of the substantial evidence that platelet deposition at such sites plays a central role in arterial thrombosis and atherogenesis. In the past, the only means for evaluating this process in patients has been by measurements of platelet turnover or of the plasma concentrations of releasable, platelet-specific proteins (e.g., β-thromboglobulin and platelet factor 4). However, these tests provide only indirect information concerning the magnitude of platelet–blood vessel interactions throughout the entire body. In contrast, the technique we have described permits evaluation of localized platelet deposition on individual vascular lesions.

Acknowledgment

We thank Carla J. Mathias and Daniel Callahan for their assistance with platelet labeling, Mary T. Clarke for her help in performing the imaging studies, Drs. David A. Goodwin and Roger H. Secker-Walker for their efforts in interpreting the scintigrams, and Drs. Marcus E. Raichle and Philip W. Majerus for their critical review of the manuscript.

References

Scintigraphic detection of carotid atherosclerosis with indium-111-labeled autologous platelets.

H H Davis, 2nd, B A Siegel, L A Sherman, W A Heaton, T P Naidich, J H Joist and M J Welch

Circulation. 1980;61:982-988
doi: 10.1161/01.CIR.61.5.982

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/61/5/982