Imaging the Inflammatory Response to Acute Myocardial Infarction in Man Using Indium-111-labeled Autologous Platelets

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SUMMARY The feasibility of imaging the inflammatory response to acute transmural myocardial infarction in man using biologically active indium-111 (\textsuperscript{111}In)-labeled autologous leukocytes was assessed in 36 patients. Indium-111 leukocytes (approximately 500 \textmu Ci) were injected i.v. 18–112 hours after the onset of chest pain. Cardiac imaging was performed 24 hours later with a mobile gamma camera. Twenty-one patients had positive images and 15 had negative images. The percent of positive images increased as the interval between infarction and \textsuperscript{111}In-leukocyte injection shortened; all patients injected within 24 hours of infarction had positive images. Patients with positive images were injected with \textsuperscript{111}In leukocytes earlier after infarction (mean ± sem, 43 ± 4 vs 63 ± 7 hours; \(p < 0.05\)) and were younger (53 ± 2 vs 65 ± 3 years; \(p < 0.05\)) than those with negative images. Several other parameters that could possibly have affected the imaging results were examined and were not significantly different in patients with positive and negative images. These included peak serum creatine kinase, location of infarction, incidence of pericarditis, use of antiinflammatory drugs (aspirin and indomethacin) or membrane-active antiarrhythmic drugs (lidocaine and procainamide), peripheral leukocyte count and cell labeling efficiency. The function of the labeled cells was similar in patients with positive and negative images. Six patients with acute infarction serving as controls and given free \textsuperscript{111}In oxine and six patients with stable coronary artery disease given \textsuperscript{111}In leukocytes all had negative cardiac images.

INfiltration of leukocytes into the region of acute myocardial infarction in man is present histologically within 24 hours and is maximal 4–5 days after infarction.\textsuperscript{1} Weiss et al. first imaged the inflammatory response to acute myocardial infarction in dogs using indium-111 (\textsuperscript{111}In)-labeled leukocytes.\textsuperscript{2} Thakur et al. recently extended these observations and demonstrated that discrete uptake of radioactivity could be imaged in animals 1–4 days after infarction, but not after 5 days. Furthermore, uptake was maximal in the lowest flow zones and occurred 24 hours after infarction in the epicardium and 72 hours after infarction in the endocardium.\textsuperscript{3}

The physical properties of \textsuperscript{111}In, with a half-life of 2.8 days and gamma photopeaks of 173 and 247 keV, make it suitable for imaging with conventional gamma cameras. Indium-111 oxine is lipid soluble and is incorporated intracellularly when added to separated leukocytes.\textsuperscript{4} The labeled cells maintain their viability, functional capacity and morphologic integrity.\textsuperscript{5} The half-life of circulating labeled leukocytes is approximately 7.5 hours\textsuperscript{6} and blood pool radioactivity clears by 24 hours, allowing cardiac imaging at that time.\textsuperscript{7} This study was undertaken to evaluate the feasibility of imaging the inflammatory response to acute myocardial infarction in man using autologous \textsuperscript{111}In leukocytes and not to define its accuracy, sensitivity or specificity as a new diagnostic test. In addition, we assessed factors that might affect the cellular uptake.

Methods

Preparation of \textsuperscript{111}In Leukocytes

Autologous leukocytes were isolated and labeled with \textsuperscript{111}In using minor modifications of the technique of Thakur et al.\textsuperscript{8} Briefly, using sterile techniques, 30 ml of venous blood were drawn into a disposable plastic syringe containing 200 IU preservative-free heparin (Abbott Laboratories). The blood settled by gravity for 1 hour at room temperature in a laminar flow hood. The plasma supernatant containing leukocytes and platelets was transferred into one or two 15-ml round bottom polyethylene test tubes (Falcon) and spun at 450 g for 5 minutes in a lead-shielded centrifuge at room temperature. The platelet-rich plasma was removed with a Pasteur pipette and stored in a 15-ml polyethylene test tube. The leukocyte pellet was resuspended in 5 ml of normal saline (Viaflex-Travenol, pH 6.5) to remove trapped plasma, and centrifuged at 450 g for 5 minutes. The supernatant was discarded and the cells were resuspended in 5 ml of saline. Indium-111 oxine, 500–700 \textmu Ci, was added dropwise and the suspension was incubated at room
temperature. Commercial $^{111}$In oxine (1 mCi $^{111}$In and 50 µg oxine in 50 µl ethanol, from Diagnostic Isotopes) diluted fourfold with saline, was used in 31 patients. Indium-111 oxine, prepared by chelation of 111In chloride to 8-hydroxyquinoline in the laboratory, was used for five patients. After 15 minutes of incubation, 5 ml of plasma were added and the cell suspension was centrifuged at 450 g for 5 minutes. The supernatant containing unincorporated isotope was removed. Radioactivity in the leukocyte button and in the supernatant was measured in a dose calibrator (Capintec). The percent cell labeling efficiency was determined (activity in leukocyte button/activity in original suspension × 100). The leukocyte button was resuspended in 10 ml of the original plasma and the appropriate volume was drawn up in a 10-ml plastic syringe to give 500–600 µCi labeled leukocytes. This was reinjected intravenously into the patient as soon as possible. The remaining labeled leukocyte suspension was saved for tests of erythrocyte contamination (41 studies); yeast phagocytic ability (15 studies); and ability to adhere to nylon wool (13 studies) in randomly selected patients.

Assessment of $^{111}$In Leukocyte Function

Percentage erythrocyte contamination of the labeled leukocyte preparation was performed by counting 100 successive cells on an air-dried Wright's stained smear of the cell suspension using oil-immersion microscopy. Morphology of the labeled leukocytes also was examined.

Leukocyte phagocytic ability was assessed after incubation of two drops of cell suspension with one drop of nitroblue tetrazolium (NBT) and one drop of yeast suspension (10⁷ organisms/ml) for 20 minutes at 37°C. This was stained and smeared as above. One hundred leukocytes were examined by oil-immersion microscopy and the percentage of cells that engulfed NBT-stained yeast particles was noted.

The ability of labeled leukocytes to adhere to a surface was determined by passing the cell suspension through nylon wool packed into a Pasteur pipette. The nylon wool was washed with saline, and the percentage of the original radioactivity retained on the column was noted. Retention of normal donor leukocytes on a nylon wool filter has been reported to vary from 34–100%.

To determine that the $^{111}$In activity remained associated with the leukocytes and did not leak out the cells when reinjected, 3 ml of heparinized blood were drawn from six patients from 1–24 hours after $^{111}$In-leukocyte injection. The blood was centrifuged at 1800 g for 10 minutes and the percent of cell-bound radioactivity was determined (activity in cell button [counts/min]/activity in blood sample [counts/min] × 100).

Patient Population

The study group consisted of 36 consecutive patients, ages 32–82 years, with acute transmural myocardial infarction who were imaged after receiving $^{111}$In leukocytes (table 1). Acute transmural myocardial infarction was defined by ischemic chest pain that lasted longer than 30 minutes, typical evolutionary ST-segment and T-wave changes with the development of new pathologic Q waves, and a rise of serum creatine kinase to at least double the upper limit of normal. Anterior and inferior infarction were defined electrocardiographically by conventional criteria. One patient was electrically cardioverted before $^{111}$In-leukocyte injection. Six patients had insulin-dependent diabetes mellitus. The interval between the onset of chest pain and $^{111}$In-leukocyte injection ranged from 18–112 hours. The interval was selected based only upon patient and physician willingness to participate, clinical stability and pharmaceutical availability. The mean delay between venipuncture and injection of the autologous $^{111}$In leukocytes was 197 minutes (range 155–265 minutes). The peripheral leukocyte count was determined using a Coulter counter, either at the time of blood withdrawal for cell labeling or within 24 hours.

Special record was kept of the clinical use of the membrane active antiarrhythmic drugs (lidocaine and procainamide) and the antiinflammatory drugs (aspirin and indomethacin) because of their known effect.

| TABLE 1. Clinical Comparisons of Patients with Acute Myocardial Infarction With Positive and Negative Cardiac Images After Indium-111-leukocyte Injection |
|---|---|---|---|
| Number | Positive image | Negative image | Statistical comparison |
| Age (years), mean ± SEM | 53 ± 2 | 65 ± 3 | p < 0.05 |
| Interval (hours) from chest pain to $^{111}$In-leukocyte injection | 43 ± 4 | 63 ± 7 | p < 0.05 |
| Location of AMI | 14 ant, 7 inf | 5 ant, 10 inf | NS |
| Peak creatine kinase (IU/l) | 1050 ± 121 | 725 ± 87 | NS |
| Pericarditis | 12/21 | 2/15 | NS |
| Lidocaine, procainamide | 15/21 | 7/15 | NS |
| Aspirin, indomethacin | 14/21 | 4/15 | NS |

Abbreviation: AMI = acute myocardial infarction; ant = anterior; inf = inferior.
on leukocyte function and the inflammatory response.11 12 Patients were considered to have received these drugs if administered from 6 hours before cell labeling to the time of imaging. Lidocaine (1–4 mg/min) was administered to 22 patients, procainamide (1–4 mg/min) to six patients, aspirin (600 mg every 4–6 hours) to 17 patients and indomethacin (25–50 mg every 8 hours) to seven patients. One patient also received ibuprofen. No patient received corticosteroids. Pericarditis, defined by typical pericardial pain and/or a friction rub, was present in 14 patients.

Two control groups of patients also were studied. One group consisted of six patients with acute myocardial infarction, ages 30–79 years, who were given 500 μCi of free 111In oxine 15–62 hours after infarction. These patients were clinically indistinguishable from the study group. The other control group consisted of six patients, ages 60–76 years, with stable coronary artery disease given 111In leukocytes. The latter group was admitted electively for cardiac catheterization. None had sustained acute myocardial infarction within 2 months; all had angiographically documented coronary artery disease.

Cardiac Imaging

Bedside cardiac imaging was performed 24 hours after the injection of 111In leukocytes or free 111In oxine. This interval was chosen on the basis of data from experimental animals demonstrating clearance of most of the circulating blood pool radioactivity by 24 hours, permitting detection of myocardial uptake distinct from blood pool background.3, 7 Persistent blood pool radioactivity was seen 24 hours after 111In-leukocyte injection in three of 36 study patients. Imaging in these patients was repeated again 24 hours later (48 hours after injection). Circulating blood pool radioactivity took longer to clear after the injection of free 111In oxine because of binding to plasma transfusion14 and imaging was repeated 48 hours after injection in four of these six control patients. Cardiac imaging was performed with a mobile scintillation camera, interfaced with a small dedicated computer.

The camera was equipped with a custom-made, high-resolution, medium-energy, parallel-hole collimator. Simultaneous dual-peak imaging was performed using 20% windows around both photo peaks (173 and 247 keV) of 111In. Both unprocessed analog and computer digital images were collected on Polaroid film. One-hundred-thousand-count images were collected in the anterior and 45° left anterior oblique positions and occasionally in the left lateral position.

An image was defined as positive for myocardial uptake of 111In-labeled leukocytes if there was activity in the region of the heart distinctly above background, in a pattern not resembling blood pool activity. Focal uptake was defined as a discrete zone of radioactivity distinctly greater than background, localized to one region of the left ventricle. Nonfocal uptake was defined as visualized activity in the region of the heart distinctly above background activity and different in appearance from blood pool activity, which could not be localized to one region of the left ventricle. Localization of 111In leukocyte in the heart was based upon the position of the uptake relative to the position of splenic, hepatic and sternal uptake. The images were interpreted by four observers and a consensus was reached.

Statistical Analysis

Data are expressed as mean ± SEM. Statistical difference between the means of two groups were tested using a t test. Differences between ratios of patients within each of two groups were analyzed by chi-square with Yates correction for small group size. A p value < 0.05 was considered significant.

Results

Cardiac Imaging

Twenty-four hours after the injection of 111In leukocytes, there was intense splenic uptake, moderately intense hepatic uptake, modest sternal and vertebral uptake and lesser rib activity. None of the patients in the two control groups (figs. 1 and 2) had identifiable cardiac uptake of 111In. Patients who were given 111In ox-
had more liver uptake and less spleen and skeletal uptake.

Twenty-one of the 36 patients with acute myocardial infarction had positive \(^{111}\)In-leukocyte cardiac images (table 1). Of the 21 positive images, 12 had focal uptake (figs. 3 and 4) and nine had nonfocal uptake. Nine of the focal images demonstrated discrete anterior wall uptake, eight in patients with anterior myocardial infarction and one in a patient with inferior infarction. Three patients had focal inferior cardiac \(^{111}\)In activity, all of whom had inferior infarction. Nonfocal images occurred in six patients with anterior infarction and in three patients with inferior infarction. In five patients with positive focal images, uptake was in a "doughnut" pattern. Skeletal uptake of radioactivity was qualitatively similar in patients with positive and negative cardiac images.

Among patients with acute myocardial infarction who received \(^{111}\)In leukocytes, the interval between the onset of chest pain and the injection of labeled cells was shorter in patients with positive images (43 \pm 4 hours) than those with negative images (63 \pm 7 hours, \(p < 0.05\)) (table 1). All six patients injected with \(^{111}\)In leukocytes within 24 hours of infarction (actually 18-24 hours of infarction) had positive cardiac images. The percentage of positive images appeared to decrease as the interval between the onset of chest pain and the injection of the labeled cells increased (table 2). Also, patients with positive images were younger (mean 53 \pm 2 years) than patients with negative images (65 \pm 3 years, \(p < 0.05\)). Other clinical factors that did not affect the imaging results included location of infarction, peak serum creatine kinase, presence of pericarditis and use of antiinflammatory or antiarrhythmic drugs (table 1). Likewise, these same clinical factors were not different in patients receiving \(^{111}\)In leukocytes early (<24 hours) or late (>24 hours). The one patient who was cardioverted had a negative \(^{111}\)In-leukocyte image. Among the six patients with diabetes mellitus, two had positive \(^{111}\)In-leukocyte images and four had negative images.

Indium-111-leukocyte Function Studies

The overall NBT-yeast phagocytic ability of the labeled cells was good, 79 \pm 3\%, and the overall ability of the labeled leukocytes to adhere to nylon wool was adequate at 46 \pm 4\%. In the six patients (three positive and three negative images) studied 1-24 hours after the injection of \(^{111}\)In leukocytes, the amount of cell-bound radioactivity varied from 80-97\% of the total radioactivity remaining in the
Table 2. Interval from Chest Pain to Indium-111-leukocyte Injection

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>No. of pts</th>
<th>No. of positive images</th>
<th>% positive images</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;24</td>
<td>6</td>
<td>6</td>
<td>100%</td>
</tr>
<tr>
<td>25-48</td>
<td>12</td>
<td>8</td>
<td>67%</td>
</tr>
<tr>
<td>49-72</td>
<td>12</td>
<td>6</td>
<td>50%</td>
</tr>
<tr>
<td>73-96</td>
<td>4</td>
<td>1</td>
<td>25%</td>
</tr>
<tr>
<td>97-112</td>
<td>2</td>
<td>0</td>
<td>0%</td>
</tr>
</tbody>
</table>

blood sample. The leukocyte count, dose of $^{111}$In oxine injected, preparation of $^{111}$In oxine, cell labeling efficiency, cell suspension erythrocyte contamination, NBT-yeast phagocytic ability and surface adherent ability were similar in patients with positive and negative $^{111}$In-leukocyte images (table 3) and in patients with stable coronary artery disease and negative $^{111}$In-leukocyte images.

Discussion

This study demonstrates that the inflammatory response to acute myocardial infarction in man can be imaged using autologous $^{111}$In leukocytes, which may provide a means of studying additional aspects of the pathophysiology of acute infarction. However, it is clear from these preliminary data and the technical complexity of tracer preparation that this imaging approach should not be considered a sensitive diagnostic procedure capable of competing with or replacing currently available radionuclide imaging modalities in defining acute myocardial infarction. Pathologic studies in dogs and in man have shown that polymorphonuclear leukocytes infiltrate the area of ischemic injury beginning within the first 24 hours. This process is apparently maximal in 4–5 days and disappears within about 2 weeks. Histologic studies demonstrate the number of leukocytes that have accumulated in the tissue since the onset of infarction, but do not reflect the temporal sequence and rate of this process. In contrast, cardiac imaging 24 hours after the injection of labeled cells reflects only $^{111}$Induced leukocyte infiltration that occurred during that time interval. As demonstrated by the control patients and the previously reported animal controls, injection of free $^{111}$In oxine at comparable intervals does not result in positive images. These data suggest that the radioactive label remains incorporated in leukocytes at the time of myocardial deposition. However, the possibility of transfer of the radioactive label to other compounds or cells within the infarct zone cannot be excluded. Previous work in this laboratory showed that all animals with experimental infarctions who received $^{111}$In leukocytes 2–72 hours after infarction had positive images, but all animals injected with labeled cells 96 hours after infarction had negative images.

In the present study in man, the relationship between image positivity and the temporal imaging sequence was not as distinct. In addition, image patterns were frequently nonfocal in distribution. Nevertheless, patients with positive images were injected with labeled cells earlier after infarction than those with negative images (table 1). All patients injected with $^{111}$In leukocytes within 24 hours of acute myocardial infarction had positive cardiac images. The percentage of positive images increased as the interval between chest pain and the injection of labeled cells shortened (table 2).

Patients with positive images also were younger than those with negative images. This was independent of the imaging time sequence, infarct site or peak serum creatine kinase. One explanation for this observation may be based on the effects of patient age upon leukocyte function, although reports that leukocyte function declines with advancing age have been variable. In addition, abnormalities in leukocyte

Table 3. Comparisons of Indium-111-Leukocyte Preparation in Patients with Acute Myocardial Infarction and Positive or Negative Cardiac Images After Indium-111-leukocyte Injection

<table>
<thead>
<tr>
<th></th>
<th>Positive image</th>
<th>Negative image</th>
<th>Statistical comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocyte count ($\times 10^9$/dl)</td>
<td>11.3 ± 0.9</td>
<td>10.6 ± 0.4</td>
<td>NS</td>
</tr>
<tr>
<td>Amount of $^{111}$In (µCi)</td>
<td>538 ± 25</td>
<td>520 ± 40</td>
<td>NS</td>
</tr>
<tr>
<td>Interval from venipuncture to reinjection of $^{111}$In leukocytes (minutes)</td>
<td>198 ± 7</td>
<td>198 ± 6</td>
<td>NS</td>
</tr>
<tr>
<td>Cell labeling efficiency (%)</td>
<td>79 ± 4</td>
<td>79 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>Preparation of $^{111}$In oxine (commercial/total)</td>
<td>18/21</td>
<td>13/15</td>
<td>NS</td>
</tr>
<tr>
<td>Erythrocyte contamination (%)</td>
<td>25 ± 3</td>
<td>25 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>NBT-yeast phagocytosis (%)</td>
<td>78 ± 5 (n = 6)</td>
<td>93 ± 3 (n = 3)</td>
<td>NS</td>
</tr>
<tr>
<td>Adherence to nylon wool (%)</td>
<td>42 ± 7 (n = 4)</td>
<td>58 ± 11 (n = 3)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean ± sem.

Abbreviation: NBT = nitroblue tetrazolium.
function in elderly patients may be secondary to age-related diseases that may affect inflammatory and immune responses. For example, polymorphonuclear leukocytes from patients with diabetes mellitus demonstrate decreased migration with chemoattractant stimuli. But in the present study the presence of diabetes did not appear to affect the imaging results.

Specific biologic or pharmacologic factors in patients with acute infarction also may affect leukocyte function and thereby alter $^{111}$In-leukocyte images in individual instances. Procaine, a local anesthetic, decreases the survival of certain irradiated mammalian cells, and aspirin and lidocaine reduce granulocyte adherence. However, in the present study, the use of the antiarrhythmic membrane-active drugs (lidocaine and procainamide) and the antiinflammatory drugs (aspirin and indomethacin) was comparable in patients with positive and negative cardiac images. The labeled cells potentially could be injured by trauma, excess radiation or excess concentrations of oxine or ethanol. However, in vitro studies have shown that the labeled cells are morphologically and functionally intact. Previous in vivo clinical studies using $^{111}$In leukocytes for abcess imaging also have demonstrated that the labeled cells function normally biologically. Furthermore, in the present study, leukocyte preparation and function were comparable in patients with positive and negative images (table 3).

Cardiac images after the injection of $^{111}$In leukocytes revealed more skeletal uptake than after free $^{111}$In-oxine administration. The reason for skeletal uptake after $^{111}$In-leukocyte injection is not known precisely, but could be due to bone marrow uptake of polymorphonuclear leukocytes. Lymphocytes, which are known to recirculate between the blood, lymphoid tissue and bone marrow, are not separated from the polymorphonuclear leukocytes by our centrifugation technique and are labeled with $^{111}$In. This also could be responsible for some of the bone activity.

Before $^{111}$In leukocytes can be used to assess infarction on a broader investigational scale, further insights into leukocyte uptake in infarcted tissue must be obtained. The inflammatory response may influence the degree of tissue injury that occurs as a result of an ischemic insult. Proteolytic enzymes released by granulocytes may increase the extent of ischemic injury. In experimental models, reducing the degree of inflammation with pharmacologic agents working by different mechanisms can reduce apparent infarct size and the extent of ischemic necrosis.

Imaging with $^{111}$In leukocytes clearly is not meant to replace already available clinical radionuclide means of detecting acute infarction but should be viewed as an investigational method which in the future may be suitable for studying the pathophysiology of inflammation in acute infarction. However, the insensitivity of the technique and its frequent poor localization make it difficult to use in patients. Technically, $^{111}$In-leukocyte uptake in some inferior infarctions could have been obscured due to the proximity of intense liver and spleen activity to the inferior wall of the heart. Based on the relatively low proportion of patients with focally positive images in this study, it is unlikely that this imaging technique can be applied to the study of groups of patients receiving drug interventions during the early hours of acute infarction. However, although this initial study has demonstrated a relative insensitivity of the technique, these observations may be modified in the future when additional studies specifically investigate the variety of biologic factors generally affecting leukocyte function in the setting of acute myocardial necrosis. Furthermore, additional work is necessary in defining means of augmenting radiopharmaceutical delivery to the infarct zone without either affecting cellular function or resulting in an excessive radiation burden to the patient.

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